STABLE FORMULATIONS OF A HYALURONAN-DEGRADING ENZYME RELATED APPLICATIONS

Benefit of priority is claimed to U.S. Provisional Application Serial No. 61/520,962, entitled "STABLE CO-COFORMULATIONS OF A HYALURONAN-DEGRADING ENZYME AND AN INSULIN," filed June 17, 2011.

This application is related to U.S. Application Serial No. (Attorney Docket No. 33320.03085.US01/3085), filed the same day herewith, entitled "STABLE FORMULATIONS OF A HYALURONAN-DEGRADING ENZYME," which claims priority to U.S. Provisional Application Serial No. 61/520,962. This application also is related to U.S. Application Serial No. (Attorney Docket No. 33320.03085.US02/3085B), filed the same day herewith, entitled "STABLE FORMULATIONS OF A HYALURONAN-DEGRADING ENZYME," which claims priority to U.S. Provisional Application Serial No. 61/520,962. The subject matter of the above-noted related application is incorporated by reference in its entirety.

This application also is related to U.S. Provisional Application No. 61/520,940 filed June 17, 2011, U.S. Provisional Application No. 61/628,389 filed October 27, 2011, and U.S. Provisional Application No. 61/657,606 filed June 08, 2012, each entitled "Continuous Subcutaneous Insulin Infusion Methods with a Hyaluronan-Degrading Enzyme." This application also is related to U.S. Application Serial No. (Attorney Docket No. 33320.03097.US03/3097), filed the same day herewith, entitled "Continuous Subcutaneous Insulin Infusion Methods with a Hyaluronan-Degrading Enzyme," which claims priority to U.S. Provisional Application Serial No. 61/520,940, 61/628,389 and 61/657,606. This application also is related to International PCT Application No. (Attorney Docket No. 33320.03097.WO01/3097PC), filed the same day herewith, entitled "Continuous Subcutaneous Insulin Infusion Methods with a Hyaluronan-Degrading Enzyme," which claims priority to U.S. Provisional Application Serial No. 61/520,940, 61/628,389 and 61/657,606.

This application also is related to U.S. Application Ser. No. 12/387,225, published as U.S. publication No. US20090304665, to Inventors Gregory Frost, Igor Blinsky, Daniel Vaughn and Barry Sugarman, entitled "Super Fast-Acting Insulin

Compositions," filed April 28, 2009, which claims priority to U.S. Provisional Application No. 61/125,835, filed April 28, 2008.

The subject matter of the above-referenced applications is incorporated by reference in its entirety.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING PROVIDED ELECTRONICALLY

An electronic version of the Sequence Listing is filed herewith, the contents of which are incorporated by reference in their entirety. The electronic file is 860 kilobytes in size, and titled 3085seqPC1.txt.

FIELD OF INVENTION

Provided are compositions that are stable formulations of a hyaluronan-degrading enzyme or are stable co-formulations of a fast-acting insulin and a hyaluronan degrading enzyme, including a recombinant human PH20 (rHuPH20).

BACKGROUND

Diabetes results in chronic hyperglycemia due to the inability of the pancreas to produce adequate amounts of insulin or due to the inability of cells to synthesize and release the insulin appropriately. Hyperglycemia also can be experienced by critically ill patients, resulting in increased mortality and morbidity. Insulin has been administered as a therapeutic to treat patients having diabetes, including, for example, type 1 diabetes, type 2 diabetes and gestational diabetes, in order to mimic the endogenous insulin response that occurs in normal individuals. Insulin also has been administered to critically ill patients with hyperglycemia to control blood glucose level.

Typically, fast-acting insulins are administered to such subjects in response to hyperglycemia or in anticipation of hyperglycemia, such as following consumption of a meal, which can result in glycemic control. However, current fast-acting forms of insulins have a delay in absorption and action, and therefore do not approximate the rapid endogenous insulin action. Thus, such formulations do not act quickly enough to shut off hepatic glucose production that occurs shortly after this first phase of insulin release. Due to the delay in pharmacological action, the fast-acting insulin preparations should be administered in advance of meals in order to achieve the desired glycemic control. Further, the doses that must be administered lead to an

extended duration of action that contributes to hypoglycemia, and in many cases, obesity.

Hyaluronan-degrading enzymes are enzymes that exhibit therapeutic activity alone and/or are co-administered without therapeutic agents, such as insulin. For example, super-fast acting insulin compositions have been developed containing a hyaluronan-degrading enzyme and a fast acting insulin (*e.g.* rapid acting insulin analog) that results in a composition that, when administered to a subject, more closely mimics the endogenous (*i.e.*, natural) post-prandial insulin release of a nondiabetic subject compared to the fast-acting insulin alone (*see e.g.* U.S. Pub No. US20090304665). There is a need for improved formulations and co-formulations of hyaluronan-degrading enzymes. There also exists a need for improved stable insulin formulations for treating subjects, for example, to control blood glucose levels in diabetic subjects.

SUMMARY

Provided herein are stable co-formulation compositions containing a therapeutically effective amount of a fast-acting insulin and a hyaluronan-degrading enzyme in an amount sufficient to render the composition super-fast acting. The provided stable co-formulations are formulated for multiple drug injection (MDI) or are formulated for continuous subcutaneous insulin infusion (CSII), each with differing requirements for stability. In particular, co-formulations for CSII are formulated to be stable at elevated temperatures and under agitation, while co-formulations for MDI are formulated to be stable when stored at refrigerated or ambient temperatures.

Provided herein are stable co-formulation compositions containing a therapeutically effective amount of a fast-acting insulin, a hyaluronan-degrading enzyme in an amount sufficient to render the composition super-fast acting, NaCl at a concentration between or about between 50 mM to 200 mM, an anti-microbial effective amount of a preservative or mixture of preservatives and a stabilizing agent or agents. The provided co-formulations have a pH of between or about between 6.8 to 7.8 and are formulated such that the compositions are stable for at least 6 months at a temperature from or from about 2 °C to 8 °C and/or for at least 14 days at a temperature from or from about 20 °C to 30 °C.

In some examples, the hyaluronan-degrading enzyme in the stable co-formulation provided herein retains at least 50 % of the initial hyaluronidase activity for at least 6 months at a temperature from or from about 2 °C to 8 °C and/or for at least 14 days at a temperature from or from about 20 °C to 30 °C and/or the insulin retains at least 90 % potency or recovery of the initial level of insulin in the composition for at least 6 months at a temperature from or from about 2 °C to 8 °C and/or for at least 14 days at a temperature from or from about 20 °C to 30 °C and/or the insulin retains at least 90 % of the initial insulin purity for at least 6 months at a temperature from or from about 2 °C to 8 °C and/or for at least 14 days at a temperature from or from about 20 °C to 30 °C and/or the insulin retains less than 2 % high molecular weight (HMWt) insulin species for at least 6 months at a temperature from or from about 2 °C to 8 °C and/or for at least 14 days at a temperature from or from about 20 °C to 30 °C. For example, the hyaluronan-degrading enzyme in the stable co-formulation retains at least 55 %, 60 %, 65 %, 70 %, 80 %, 85 %, 90 %, 95 % or more of the initial hyaluronidase activity for at least 6 months at a temperature from or from about 2 °C to 8 °C and/or for at least 14 days at a temperature from or from about 20 °C to 30 °C and the purity or potency of insulin is at least 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or more for at least 6 months at a temperature from or from about 2 °C to 8 °C and/or for at least 14 days at a temperature from or from about 20 °C to 30 °C.

In some embodiments, the pH of the stable co-formulation compositions provided herein is between or about between 7.0 to 7.6. For example, the pH of the stable co-formulation is or is about 6.8 ± 0.2, 6.9 ± 0.2, 7.0 ± 0.2, 7.1 ± 0.2, 7.2 ± 0.2, 7.3 ± 0.2, 7.4 ± 0.2, 7.5 ± 0.2, 7.6 ± 0.2, 7.7 ± 0.2 or 7.8 ± 0.2. The NaCl concentration in the stable co-formulation compositions provided herein is between or about between 80 mM to 200 mM, 80 mM to 140 mM, 50 mM to 100 mM, 80 mM to 100 mM, 50 mM to 80 mM, 100 mM to 140 mM or 120 mM to 140 mM. For example, the NaCl concentration of the stable co-formulation is or is about or at least 50 mM, 55 mM, 60 mM, 65 mM, 70 mM, 75 mM, 80 mM, 85 mM, 90 mM, 95 mM, 100 mM, 120 mM, 125 mM, 130 mM, 135 mM, 140 mM, 145 mM, 150 mM, 155 mM, 160 mM, 165 mM, 170 mM, 175 mM, 180 mM, 185 mM, 190 mM, 195 mM or 200 mM. In such examples, the upper amount of NaCl in compositions of less than

100 mM is up to 110 mM, 120 mM, 130 mM, 140 mM, 150 mM, 160 mM, 170 mM, 180 mM, 190 mM or 200 mM. In some examples, the stable co-formulations provided herein contain a sufficient amount of a buffering agent to maintain a pH range of between or about between 6.8 to 7.8.

In one embodiment, the stable co-formulations provided herein are stable at a temperature from or from about 2 °C to 8 °C, inclusive, for at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 20 months, 21 months, 22 months, 23 months, 24 months, 25 months, 26 months, 27 months, 28 months, 29 months or 30 months. For example, the co-formulations are stable at a temperature from or from about 2 °C to 8 °C, inclusive, for at least 18 months or at least 24 months. In another embodiment, the stable co-formulations provided herein are stable at a temperature from or from about 20 °C to 30 °C, inclusive, for at least 15 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 35 days, 40 days, 45 days or 50 days. For example, the co-formulations are stable at a temperature from or from about 20 °C to 30 °C, inclusive, for at least a month.

Also provided herein are stable co-formulation compositions containing a therapeutically effective amount of a fast-acting insulin, a hyaluronan-degrading enzyme in an amount sufficient to render the composition super-fast acting, NaCl at a concentration between or about between 120 mM to 200 mM, an anti-microbial effective amount of a preservative or mixture of preservatives, and a stabilizing agent or agents. The provided co-formulations have a pH of between or about between 6.5 to 7.5 and the compositions are stable for at least 3 days at a temperature from or from about 32 °C to 40 °C or are stable for at least 3 hours under agitation.

In some examples, the stable co-formulation further contains an effective amount of a hyaluronidase inhibitor, such as, but not limited to, proteins, glycosaminoglycans (GAG), polysaccharides, fatty acids, lanostanoids, antibiotics, anti-nematodes, synthetic organic compounds and/or a plant-derived bioactive component. Exemplary of a plant-derived bioactive component is an alkaloid, antioxidant, polyphenol, flavonoids, terpenoids and/or anti-inflammatory drugs. In some example, the hyaluronidase inhibitor in the stable co-formulations provided

herein does not form covalent complexes with the hyaluronan-degrading enzyme or insulin. Exemplary hyaluronidase inhibitors are, but are not limited to, a serum hyaluronidase inhibitor, *Withania somnifera* glycoprotein (WSG), heparin, heparin sulfate, dermatan sulfate, chitosans, β-(1,4)-galacto-oligosaccharides, sulphated verbascose, sulphated planteose, pectin, poly(styrene-4-sulfonate), dextran sulfate, sodium alginate, polysaccharide from *Undaria pinnatifida*, mandelic acid condensation polymer, eicosatrienoic acid, nervonic acid, oleanolic acid, aristolochic acid, ajmaline, reserpine, flavone, desmethoxycentauredine, quercetin, apigenin, kaempferol, silybin, luteolin, luteolin-7-glucoside, phloretin, apiin, hesperidin, sulphonated hesperidin, calycosin-7-O-β-D-glucopyranoside, sodium flavone-7-sulphate, flavone 7-fluoro-4'-hydroxyflavone, 4'-chloro-4,6-dimethoxychalcone, sodium 5-hydroxyflavone 7-sulphate, myricetin, rutin, morin, glycyrrhizin, vitamin C, D-isoascorbic acid, D-saccharic 1,4-lactone, L-ascorbic acid-6-hexadecanoate (Vcpal), 6-O-acylated vitamin C, catechin, nordihydroguaiaretic acid, curcumin, N-propyl gallate, tannic acid, ellagic acid, gallic acid, phlorofucofuroeckol A, dieckol, 8,8'-bieckol, procyanidine, gossypol, celecoxib, nimesulide, dexamethasone, indomethcin, fenoprofen, phenylbutazone, oxyphenbutazone, salicylates, disodium cromoglycate, sodium aurothiomalate, transilist, traxanox, ivermectin, lincomycin and spectinomycin, sulfamethoxazole and trimerthoprim, neomycin sulphate, 3α-acetylpolyporenic acid A, (25S)-(+)-12α-hydroxy-3α-methylcarboxyacetate-24-methyllanosta-8,24(31)-diene-26-oic acid, lanostanoid, polyporenic acid C, PS53 (hydroquinone-sulfonic acid-formaldehyde polymer), polymer of poly(styrene-4-sulfonate), VERSA-TL 502, 1-tetradecane sulfonic acid, mandelic acid condensation polymer (SAMMA), 1,3-diacetylbenzimidazole-2-thione, N-monoacylated benzimidazol-2-thione, N,N'-diacylated benzimidazol-2-thione, alkyl-2-phenylindole derivate, 3-propanoylbenzoxazole-2-thione, N-alkylated indole derivative, N-acylated indole derivate, benzothiazole derivative, N-substituted indole-2- and 3-carboxamide derivatives, halogenated analogs (chloro and fluoro) of N-substituted indole-2- and 3-carboxamide derivatives, 2-(4-hydroxyphenyl)-3-phenylindole, indole carboxamides, indole acetamides, 3-benzolyl-1-methyl-4-phenyl-4-piperidinol, benzoyl phenyl benzoate derivative, l-arginine derivative, guanidium HCL, L-NAME, HCN,

linamarin, amygdalin, hederagenin, aescin, CIS-hinokiresinol and/or 1,3-di-p-hydroxyphenyl-4-penten-1-one.

In some embodiments, the stable co-formulations provided herein contain a hyaluronidase inhibitor that is a hyaluronan (HA) oligosaccharide at a concentration of between or about between 1 mg/mL to 20 mg/mL. In some examples, the HA oligosaccharide is a disaccharide or a tetrasaccharide. In other examples, the HA oligosaccharide has a reacted reducing end.

The stable co-formulations provided herein are formulated such that the hyaluronan-degrading enzyme in the co-formulation retains at least 50 % of the initial hyaluronidase activity for at least 3 days at a temperature from or from about 32 °C to 40 °C or is stable for at least 3 hours under agitation, and/or the insulin in the composition retains at least 90 % potency or recovery of the initial level of insulin in the composition for at least 3 days at a temperature from or from about 32 °C to 40 °C or is stable for at least 3 hours under agitation, and/or retains at least 90 % of the initial insulin purity for at least 3 days at a temperature from or from about 32 °C to 40 °C or is stable for at least 3 hours under agitation, and/or retains less than 2 % high molecular weight (HMWt) insulin species for at least 3 days at a temperature from or from about 32 °C to 40 °C or is stable for at least 3 hours under agitation. In one example, the stable co-formulations are formulated such that the hyaluronan-degrading enzyme in the composition retains at least 55 %, 60 %, 65 %, 70 %, 80 %, 85 %, 90 %, 95 % or more of the initial hyaluronidase activity for at least 3 days at a temperature from or from about 32 °C to 40 °C or is stable for at least 3 hours under agitation. In another example, the stable co-formulations are formulated such that the purity or potency of insulin is at least 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98%, 99 % or more for at least 3 days at a temperature from or from about 32 °C to 40 °C or is stable for at least 3 hours under agitation.

In some embodiments, the pH of the stable co-formulations provided herein is or is about 6.3 ± 0.2, 6.4 ± 0.2, 6.5 ± 0.2, 6.6 ± 0.2, 6.7 ± 0.2, 6.8 ± 0.2, 6.9 ± 0.2, 7.0 ± 0.2, 7.1 ± 0.2, 7.2 ± 0.2, 7.3 ± 0.2, 7.4 ± 0.2 or 7.5 ± 0.2. In other embodiments, the NaCl concentration of the stable co-formulation compositions is between or about between 150 mM to 200 mM or 160 mM to 180 mM. For example, the NaCl concentration is or is about 120 mM, 130 mM, 140 mM, 150 mM, 155 mM, 160 mM,

165 mM, 170 mM, 175 mM, 180 mM, 185 mM, 190 mM, 195 mM or 200 mM. In some embodiments, the stable co-formulation is stable at or about 37 °C for at least 3 days. In other embodiments, the stable co-formulation is stable for at least 4 days, at least 5 days or at least 6 days. In some embodiments, the stable co-formulations contain a sufficient amount of a buffering agent to maintain the pH range of between or about between 6.5 to 7.5.

Hyaluronan degrading enzymes contained in the stable co-formulation compositions provided herein include, for example, hyaluronidases, such as animal, including human, hyaluronidases, particularly soluble forms thereof, and/or chondroitinases. Exemplary hyaluronan degrading enzymes are hyaluronidases and/or chondroitinases. In some embodiments, the hyaluronan-degrading enzyme is a hyaluronidase that is active at neutral pH. In other embodiments, the hyaluronan-degrading enzyme lacks a glycosylphosphatidylinositol (GPI) anchor or is not membrane-associated when expressed from a cell. For example, the hyaluronan-degrading enzyme contains C-terminal truncations of one or more amino acid residues to remove all or part of a GPI anchor. In some examples, the hyaluronan-degrading enzyme in the stable co-formulations provided herein is a hyaluronidase that is a PH20. Exemplary of PH20 hyaluronidases are non-human or human PH20 hyaluronidases. Included are PH20 hyaluronidases that have a sequence of amino acids that contains at least amino acids 36-464 of SEQ ID NO:1, or have a sequence of amino acids that has at least 85 % sequence identity to a sequence of amino acids that contains at least amino acids 36-464 of SEQ ID NO:1 and retains hyaluronidase activity. For example, the PH20 has at least 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 % or 99 % sequence identity to a sequence of amino acids that contains at least amino acids 36-464 of SEQ ID NO:1 and retains hyaluronidase activity. Included are PH20 polypeptides that have a sequence of amino acids that contains a C-terminal truncation after amino acid position 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499 or 500 of the sequence of amino acids set forth in SEQ ID NO:1. Variants include PH20 polypeptides that that exhibit at least 85 % sequence identity to a sequence of amino acids that contains a C-terminal truncation after amino acid position 465, 466, 467,

468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499 or 500 of the sequence of amino acids set forth in SEQ ID NO:1 and retain hyaluronidase activity. In some examples, the PH20 in the stable co-formulations provided herein has at least 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 % or 99 % sequence identity to a sequence of amino acids that contains that contains a C-terminal truncation after amino acid position 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499 or 500 of the sequence of amino acids set forth in SEQ ID NO:1 and retains hyaluronidase activity. In some embodiments, the hyaluronan-degrading enzyme is a truncated PH20 that is a C-terminal truncated PH20 polypeptide that includes any from among polypeptides having a sequence of amino acids set forth in any of SEQ ID NOS:4-9, or allelic variants and other variants thereof.

In some embodiments, the amount of PH20 in the stable co-formulations provided herein is between or about between 0.1 μg/mL to 100 μg/mL, 1 μg/mL to 50 μg/mL or 1 μg/mL to 20 μg/mL. For example, the amount of PH20 is or is about 5 μg/mL. In other embodiments, the specific activity of the PH20 is or is between 75 Units (U)/μg to 120 U/ μg or is at least, about or is 75 Units (U)/μg, 80 U/ μg, 85 U/ μg, 90 U/ μg, 100 U/ μg, 110 U/ μg or 120 U/ μg. The amount of a hyaluronan-degrading enzyme in the stable co-formulations provided herein is between or about between 10 U/mL to 5000 U/mL, 50 U/mL to 4000 U/mL, 100 U/mL to 2000 U/mL, 300 U/mL to 2000 U/mL, 600 U/mL to 2000 U/mL, or 100 U/mL to 1000 U/mL. For example, the amount of a hyaluronan-degrading enzyme is at least or is about or is 30 U/mL, 35 U/mL, 40 U/mL, 50 U/mL, 100 U/mL, 150 U/mL, 200 U/mL, 250 U/mL, 300 U/mL, 350 U/mL, 400 U/mL, 450 U/mL, 500 U/mL, 600 U/mL, 700 U/mL, 800 U/mL, 900 U/mL, 1000 U/mL or 2000 U/mL. In an exemplary stable co-formulation, the amount of a hyaluronan-degrading enzyme is or is about 600 U/mL.

The fast-acting insulin can be, for example, monomeric or multimeric, such as dimeric or hexameric. In one embodiment, the fast-acting insulin is a fast-acting human insulin. In another embodiment, the fast-acting insulin is a regular insulin, for example, a human insulin or pig insulin. In one example, the fast-acting insulin is a

regular insulin and the NaCl concentration of the stable co-formulation provided herein is 50 mM to 80 mM. Among the fast-acting insulins are regular insulins, such as, an insulin with an A chain having a sequence of amino acids set forth in SEQ ID NO:103 and a B chain having a sequence of amino acids set forth in SEQ ID NO:104 or an insulin with an A chain with a sequence of amino acids set forth as amino acid residue positions 88-108 of SEQ ID NO:123 and a B chain with a sequence of amino acids set forth as amino acid residue positions 25-54 of SEQ ID NO:123. The insulin can be recombinant insulin or can be synthesized or partially-synthesized or can be isolated from a natural source. The fast-acting insulin can be an insulin analog. Exemplary of insulin analogs is an insulin analog selected from among an insulin having an A chain with a sequence of amino acids set forth in SEQ NOS:103 and a B chain having a sequence of amino acids set forth in any of SEQ NOS:147-149. In some exemplary stable co-formulations provided herein, the fast acting insulin is an insulin aspart having a sequence of amino acids set forth in SEQ NOS:103 (A-chain) and SEQ ID NO:147 (B-chain) and the NaCl concentration is between or is about between 80mM to 160 mM. In other exemplary stable co-formulations provided herein, the fast acting insulin is an insulin glulisine having a sequence of amino acids set forth in SEQ NOS:103 (A-chain) and SEQ ID NO:149 (B-chain) and the NaCl concentration is between or is about between 80 mM to 200 mM. In yet another exemplary stable co-formulations provided herein, the fast acting insulin is an insulin lispro having a sequence of amino acids set forth in SEQ ID NOS: 103 (A-chain) and SEQ ID NO:148 (B-chain) and the NaCl concentration is between or about between 50 mM to 120 mM.

In some embodiments, the insulin in the stable co-formulation provided herein is in amount that is 10 U/mL to 1000 U/mL, 50 U/mL to 500 U/mL, 100 U/mL to 1000 U/mL or 500 U/mL to 1000 U/mL, inclusive. For example, the amount of fast-acting insulin is at least or is about or is 10 U/mL, 20 U/mL, 30 U/mL, 40 U/mL, 50 U/mL, 60 U/mL, 70 U/mL, 80 U/mL, 90 U/mL, 100 U/mL, 150 U/mL, 200 U/mL, 250 U/mL, 300 U/mL, 350 U/mL, 400 U/mL, 500 U/mL or 1000 U/mL. In an exemplary stable co-formulation, the amount of fast-acting insulin is or is about 100 U/mL. In another exemplary stable co-formulation, the fast-acting insulin is an insulin analog and the hyaluronan-degrading enzyme is a PH20.

The stable co-formulations provided herein optionally include a buffering agent, such as, but not limited to, a non-metal binding agent or a metal binding agent. In some examples, the buffering agent is selected from among Tris, histidine, phosphate or citrate. In an exemplary stable co-formulation, the buffering agent is Tris. The concentration of the buffering agent is between or is between about 1 mM to 100 mM, 10 mM to 50 mM or 20 mM to 40 mM. For example, the concentration of the buffering agent is or is about or is at least 1 mM, 5 mM, 10mM, 15 mM, 20 mM, 21 mM, 22 mM, 23 mM, 24 mM, 25 mM, 26 mM, 27 mM, 28 mM, 29 mM, 30 mM, 31 mM, 32 mM, 33 mM, 34 mM, 35 mM, 36 mM, 37 mM, 38 mM, 39 mM, 40 mM, 45 mM, 50 mM, 55 mM, 60 mM, 65 mM, 70 mM, 75 mM or more. In an exemplary stable co-formulation, the concentration of buffering agent is or is about 30 mM.

The stable co-formulation compositions provided herein include an anti-microbial effective amount of the preservative that kills or inhibits the propagation of microbial organisms in a sample of the composition such that at least a 1.0 $\log_{10}$ unit reduction in bacterial organisms occurs at 7 days following inoculation. In some examples, the anti-microbial effective amount of the preservative kills or inhibits the propagation of microbial organisms such that, when tested in an antimicrobial preservative effectiveness test (APET), following inoculation of the composition with a microbial inoculum there is at least a 1.0 $\log_{10}$ unit reduction in bacterial organisms at 7 days following inoculation, at least a 3.0 $\log_{10}$ unit reduction of bacterial organisms at 14 days following inoculation, at least no further increase in bacterial organisms after 28 days following inoculation, and at least no increase in fungal organisms after 7 days following inoculation. In other examples, the anti-microbial effective amount of the preservative kills or inhibits the propagation of microbial organisms such that, when tested in an antimicrobial preservative effectiveness test (APET), following inoculation of the composition with a microbial inoculum there is at least a 1.0 $\log_{10}$ unit reduction of bacterial organisms at 24 hours following inoculation, at least a 3.0 $\log_{10}$ unit reduction of bacterial organisms at 7 days following inoculation, no further increase in bacterial organisms after 28 days following inoculation, at least a 1.0 $\log_{10}$ unit reduction of fungal organisms at 14 days following inoculation, and at least no further increase in fungal organisms after

28 days following inoculation. In yet another example, the anti-microbial effective amount of the preservative kills or inhibits the propagation of microbial organisms such that, when tested in an antimicrobial preservative effectiveness test (APET), following inoculation of the composition with a microbial inoculum there is at least a 2.0 $\log_{10}$ unit reduction of bacterial organisms at 6 hours following inoculation, at least a 3.0 $\log_{10}$ unit reduction of bacterial organisms at 24 hours following inoculation, no recovery of bacterial organisms after 28 days following inoculation of the composition with the microbial inoculum, at least a 2.0 $\log_{10}$ unit reduction of fungal organisms at 7 days following inoculation, and at least no further increase in fungal organisms after 28 days following inoculation.

The preservative(s) in the stable co-formulations can include one or more of a phenolic preservative(s), a non-phenolic preservative(s) or a phenolic preservative(s) and a non-phenolic preservative(s). For example, the preservative(s) is(are) selected from among, but not limited to, phenol, m-cresol, methylparaben, benzyl alcohol, thimerosal, benzalkonium chloride, 4-chloro-1-butanol, chlorhexidine dihydrochloride, chlorhexidine digluconate, L-phenylalanine, EDTA, bronopol, phenylmercuric acetate, glycerol, imidurea, chlorhexidine, sodium dehydroacetate, o-cresol, p-cresol, chlorocresol, cetrimide, benzethonium chloride, ethyl paraben, propylparaben, butylparaben and any combinations thereof. In some examples, the stable co-formulation contains a single preservative. In other examples, the stable co-formulation contains a mixture of preservatives that contains 2, 3 or 4 different preservatives. In some embodiments, the stable co-formulations contain at least one phenolic preservative. In a particular embodiment, the one or more preservative(s) is(are) phenol, m-cresol or phenol and m-cresol.

The total amount of the one or more preservative agents as a percentage (%) of mass concentration (w/v) in the stable co-formulations provided herein is or is between 0.1 % and 0.4 %, 0.1 % to 0.3 %, 0.15 % to 0.325 %, 0.15 % to 0.25 %, 0.1 % to 0.2 %, 0.2 % to 0.3 % or 0.3 % to 0.4 % inclusive. In some examples, the preservatives are phenol and m-cresol and the amount as a % of mass concentration (w/v) in the formulation is between or about between 0.1 % to 0.25 % phenol and between or about between 0.05 % to 0.2 % m-cresol, is between or about between 0.10 % to 0.2 % phenol and between or about between 0.6 % to 01.8 % m-cresol,

between or about between 0.1 % to 0.15 % phenol and 0.8 % t 0.15 % m-cresol, is between or about between 0.10 % to 0.15 % phenol and between or about between 0.06 to 0.09 % m-cresol or is between or about between 0.12 % to 0.18 % phenol and between or about between 0.14 to 0.22 % m-cresol. In exemplary co-formulations, the preservative(s) are phenol and m-cresol and the amount as a % of mass concentration (w/v) in the formulation is or is about 0.1 % phenol and 0.075 % m-cresol, is or is about 0.1 % phenol and 0.15 % m-cresol, is or is about 0.125 % phenol and 0.075 % m-cresol, is or is about 0.13 % phenol and 0.075 % m-cresol, is or is about 0.13 % phenol and 0.08 % m-cresol, is or is about 0.15 % phenol and 0.175 % m-cresol or is or is about 0.17 % phenol and 0.13 % m-cresol.

The stable co-formulations provided herein contain a stabilizing agent that is selected from among, but not limited to, an amino acid, amino acid derivative, amine, sugar, polyols, salt or surfactant. In some examples, the stable co-formulations contain a single stabilizing agent. In other examples, the stable co-formulations contain 2, 3, 4, 5 or 6 different stabilizing agents. In some examples, the stabilizing agent is an amino acid, amino acid derivative or amine that is selected from among L-Arginine, glutamine, glutamic acid, glycine, lysine, methionine, proline, Lys-Lys, Gly-Gly, Trimethylamine oxide (TMAO), betaine or salts thereof. In a particular example, the amino acid is glycine or proline. The concentration of the amino acid is between or between about 0.01 M to 1 M, 0.01 M to 0.1 M, 0.1 M to 0.75 M or 0.2 M to 0.5 M, inclusive. In some examples, the stabilizing agent is a sugar or polyol that is selected from among, but not limited to, glycerol, sorbitol, mannitol, inositol, sucrose and trehalose.

In an exemplary stable co-formulation, the stabilizing agent is a surfactant and the amount of surfactant as a % of mass concentration (w/v) in the formulation is between or about between 0.005 % to 1.0 %, 0.01 % to 0.5 %, 0.01 % to 0.1 %, 0.01 % to 0.05 %, or 0.01 % to 0.02 %. For example, the stabilizing agent is a surfactant and the amount of surfactant as a % of mass concentration (w/v) in the formulation is or is about 0.001 %, 0.005 %, 0.01 %, 0.015 %, 0.02 %, 0.025 %, 0.03 %, 0.035 %, 0.04 %, 0.045 %, 0.05 %, 0.055 %, 0.06 %, 0.065 %, 0.07 %, 0.08 % or 0.9 %. The surfactant in the stable co-formulations provided herein can be selected from among, but not limited to, a polypropylene glycol, polyethylene glycol, glycerin, sorbitol,

poloxamer and polysorbate. For example, the surfactant is selected from among poloxamer 188, polysorbate 20 or polysorbate 80. In an exemplary stable co-formulation, the stabilizing agent is a surfactant that is poloxamer 188 and is provided in an amount as a % of mass concentration (w/v) of between or about between 0.01 % to 0.05 %. In another exemplary stable co-formulation, the stabilizing agent is a surfactant that is polysorbate 20 and is provided in an amount as a % of mass concentration (w/v) of between or about between 0.01 % to 0.05 %. In yet another exemplary stable co-formulation, the stabilizing agent is a surfactant that is polysorbate 80 and is provided in an amount as a % of mass concentration (w/v) of between or about between 0.01 % to 0.05 %.

The stable co-formulations provided herein optionally include a tonicity modifier, that is selected from among, but not limited to, glycerin, salt, amino acids, polyalcohols or trehalose, to maintain the osmolarity of between or about between 245 mOsm/kg to 305 mOsm/kg. In some example, the tonicity modifier maintains the osmolarity of the formulation of about or at 245 mOsm/kg, 250 mOsm/kg, 255 mOsm/kg, 260 mOsm/kg, 265 mOsm/kg, 270 mOsm/kg, 275 mOsm/kg, 280 mOsm/kg, 285 mOsm/kg, 290 mOsm/kg, 300 mOsm/kg or 305 mOsm/kg. In an exemplary stable co-formulation, the tonicity modifier maintains the osmolarity of the formulation of or of about 275 mOsm/kg. In one embodiment, the tonicity modifier is glycerin that is present in the co-formulation at a concentration less than 60 mM, less than 55 mM, less than 50 mM, less than 45 mM, less than 40 mM, less than 35 mM, less than 30 mM, less than 25 mM, less than 20 mM, less than 15 mM, less than 10 mM. In an exemplary stable co-formulation, the fast-acting insulin is an insulin analog that is insulin aspart and the formulation contains glycerin in a concentration between or about between 20 mM to 50 mM, inclusive. In another exemplary stable co-formulation, the fast acting insulin is a regular insulin or is insulin lispro and the formulation comprises glycerin in a concentration between or about between 40 mM to 60 mM, inclusive.

In some embodiments, the stable co-formulations provided herein optionally contain an antioxidant. In other embodiments, the stable co-formulations provided herein optionally contain a surfactant and/or hyaluronan oligosaccharides, and also contain an antioxidant. The antioxidant included in the stable co-formulations

provided herein is selected from among, but not limited to, cysteine, tryptophan and methionine. In an exemplary stable co-formulation, the antioxidant is methionine. The antioxidant in the stable co-formulations is at a concentration from between or from about between 5 mM to 50 mM, 5 mM to 40 mM, 5 mM to 20 mM or 10 mM to 20 mM, inclusive. In an exemplary embodiment, the antioxidant is methionine and the concentration of methionine is between or about between 10 mM to 20 mM.

The stable co-formulations provided herein optionally contain zinc. For example, in one embodiment, the fast-acting insulin is regular insulin, insulin lispro or insulin aspart and the formulation contains zinc. The zinc in the stable co-formulations is selected from among, but not limited to, zinc oxide, zinc acetate or zinc chloride, and is present at a concentration of between or about between 0.001 to 0.1 mg per 100 units of insulin (mg/100U), 0.001 to 0.05 mg/100U or 0.01 to 0.05 mg/100U.

The stable co-formulations provided herein optionally contain a nicotinic compound, that is selected from among, but not limited to, nicotinamide, nicotinic acid, niacin, niacinamide, vitamin B3 and/or salts thereof and/or any combination thereof. The nicotinic compound(s) are present in a concentration from or from about 1 mm to 150 mM, 10 mM to 150 mM, 50 mM to 100 mM or of or of about 80 mM.

The stable co-formulations provided herein optionally contain one or more amino acid(s), selected from among, but not limited to, arginine, glutamic acid, and/or salts thereof and/or combinations thereof. The amino acids are present at a concentration of 1 to 100 mM, 10 to 100 mM, or of or of about 30 mM, 50 mM or 80 mM.

An exemplary stable co-formulation provided herein has a pH of between or about between 7.0 to 7.6, and contains a hyaluronan-degrading enzyme that is a PH20 in an amount between or about between 100 U/mL to 1000 U/mL, inclusive; a fast-acting insulin analog in an amount between or about between 10 U/mL to 1000 U/mL, inclusive; a Tris buffering agent at a concentration of between or about between 10 mM to 50 mM, inclusive; NaCl at a concentration of between or about between 50 to 200 mM, inclusive; methionine at a concentration between or about between 5 mM to 50 mM, inclusive; glycerin at a concentration from between or about between 0 mM to 50 mM, inclusive; a surfactant that is poloxamer 188, polysorbate 20 or polysorbate

80 at a percentage (%) of mass concentration (w/v) of between or about between 0.01 % to 0.5 %; and a preservative(s) that contains phenol at a percentage (%) of mass concentration (w/v) of between or between about between 0.1 % to 0.25 % and m-cresol at a % w/v of between or between about 0.05 % to 0.2 %. In another embodiment, this exemplary stable co-formulation further contains zinc at a concentration of 0.001 to 0.1 mg per 100 units of insulin (mg/100U). In one embodiment, the preservatives in the stable co-formulations are or are about 0.1 % phenol and 0.015 % m-cresol, 0.125 % phenol and 0.075 % m-cresol, 0.13 % phenol and 0.075 % m-cresol, 0.13 % phenol and 0.08 % m-cresol or 0.17 % phenol and 0.13 % m-cresol.

An exemplary stable co-formulation provided herein has a pH between or about between 7.0 to 7.6 and contains a fast-acting insulin that is insulin lispro in an amount between or about between 10 U/mL to 1000 U/mL, inclusive; a hyaluronan-degrading enzyme that is a PH20 in an amount between or about between 100 U/mL to 1000 U/mL, inclusive; a Tris buffering agent at a concentration of between or about between 25 mM to 35 mM, inclusive; NaCl at a concentration of between or about between 50 mM to 120 mM, inclusive; methionine at a concentration between or about between 10 mM to 30 mM, inclusive; glycerin at a concentration from between or about between 40 mM to 60 mM, inclusive; a surfactant that is poloxamer 188, polysorbate 20 or polysorbate 80 at a percentage (%) of mass concentration (w/v) of between or about between 0.01 % to 0.05 %, inclusive; zinc at a concentration of 0.017 to 0.024 mg per 100 units of insulin (mg/100U); and a preservative(s) that contains a percentage (%) of mass concentration(w/v) of between or about between 0.08 % to 0.17 % phenol, inclusive, and between or about between 0.07 % to 0.17 % m-cresol. In one embodiment, the NaCl concentration is between or about between 70 mM to 100 mM. In another embodiment, the pH is or is about 7.1 ± 0.2, 7.2 ± 0.2, 7.3 ± 0.2 or 7.4 ± 0.2. In one embodiment, the preservatives in the stable co-formulations are or are about 0.1 % phenol and 0.015 % m-cresol, 0.125 % phenol and 0.075 % m-cresol, 0.13 % phenol and 0.075 % m-cresol, 0.13 % phenol and 0.08 % m-cresol or 0.17 % phenol and 0.13 % m-cresol.

An exemplary stable co-formulation provided herein has a pH between or about between 7.0 to 7.6 and contains a fast-acting insulin that is insulin aspart in an

amount between or about between 10 U/mL to 1000 U/mL, inclusive; a hyaluronan-degrading enzyme that is a PH20 in an amount between or about between 100 U/mL to 1000 U/mL, inclusive; a Tris buffering agent at a concentration of between or about between 25 mM to 35 mM, inclusive; NaCl at a concentration of between or about between 80 mM to 160 mM, inclusive; methionine at a concentration between or about between 10 mM to 30 mM, inclusive; glycerin at a concentration from between or about between 20 mM to 50 mM, inclusive; a surfactant that is poloxamer 188, polysorbate 20 or polysorbate 80 at a percentage (%) of mass concentration (w/v) of between or about between 0.01 % to 0.05 %, inclusive; zinc at a concentration of 0.017 to 0.024 mg per 100 units of insulin (mg/100U); and a preservative(s) that contains a percentage (%) of mass concentration(w/v) of between or about between 0.08 % to 0.17 % phenol, inclusive, and between or about between 0.07 % to 0.17 % m-cresol. In one embodiment, the NaCl concentration is or is about between 70 mM to 100 mM. In another embodiment, the pH is or is about 7.2 ± 0.2, 7.3 ± 0.2, 7.4 ± 0.2 or 7.5 ± 0.2. In one embodiment, the preservatives in the stable co-formulations are or are about 0.1 % phenol and 0.015 % m-cresol, 0.125 % phenol and 0.075 % m-cresol, 0.13 % phenol and 0.075 % m-cresol, 0.13 % phenol and 0.08 % m-cresol or 0.17 % phenol and 0.13 % m-cresol.

An exemplary stable co-formulation provided herein has a pH between or about between 7.0 to 7.6 and contains a fast-acting insulin that is insulin glulisine in an amount between or about between 10 U/mL to 1000 U/mL, inclusive; a hyaluronan-degrading enzyme that is a PH20 in an amount between or about between 100 U/mL to 1000 U/mL, inclusive; a Tris buffering agent at a concentration of between or about between 25 mM to 35 mM, inclusive; NaCl at a concentration of between or about between 80 mM to 200 mM, inclusive; methionine at a concentration between or about between 10 mM to 30 mM, inclusive; glycerin at a concentration from between or about between 40 mM to 60 mM, inclusive; a surfactant that is poloxamer 188 at a percentage (%) of mass concentration (w/v) of between or about between 0.01 % to 0.05 %, inclusive; and a preservative(s) that has a percentage (%) of mass concentration(w/v) of between or about between 0.08 % to 0.17 % phenol, inclusive, and between or about between 0.07 % to 0.17 % m-cresol. In one embodiment, the NaCl concentration is between or about between 100 mM to

150 mM. In another embodiment, the pH is or is about 7.2 ± 0.2, 7.3 ± 0.2, 7.4 ± 0.2 or 7.5 ± 0.2.

In one embodiment, the PH20 in the stable co-formulations provided herein is a human PH20 that has a sequence of amino acids that contains at least amino acids 36-464 of SEQ ID NO:1, or has a sequence of amino acids that has at least 85 %, 90 % or 95 % sequence identity to a sequence of amino acids that contains at least amino acids 36-464 of SEQ ID NO:1 and retains hyaluronidase activity. For example, the PH20 polypeptide has a sequence of amino acids that contains a C-terminal truncation after amino acid position 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499 or 500 of the sequence of amino acids set forth in SEQ ID NO:1, or is a variant thereof that exhibits at least 85 %, 90 % or 95 % sequence identity to a sequence of amino acids that contains a C-terminal truncation after amino acid position 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499 or 500 of the sequence of amino acids set forth in SEQ ID NO:1 and retains hyaluronidase activity. In another example, the PH20 polypeptide has a sequence of amino acids that contains a C-terminal truncation after amino acid position 482 of the sequence of amino acids set forth in SEQ ID NO:1, or is a variant thereof that exhibits at least 85 %, 90 % or 95 % sequence identity to a sequence of amino acids that contains a C-terminal truncation after amino acid position 482 of the sequence of amino acids set forth in SEQ ID NO:1 and retains hyaluronidase activity. In an exemplary stable co-formulation, the PH20 polypeptide has a sequence of amino acids set forth in any of SEQ ID NOS: 4-9. The hyaluronan-degrading enzyme or PH20 in the stable co-formulations provided herein can be produced and expressed from mammalian cells, for example, Chinese Hamster Ovary (CHO) cells. In a particular example, the PH20 is designated rHuPH20.

The stable co-formulations provided herein can be formulated for multi-dose administration. The volume of the stable co-formulations can be between or about between 0.5 mL to 50 mL, 1 mL to 40 mL, 1 mL to 20 mL, 1 mL to 10 mL, or 3 mL to 10mL, inclusive. The stable co-formulations provided herein can be formulated for delivery using a vial, syringe, pen, reservoir for a pump or a closed loop system. In a

particular example, the stable co-formulations are formulated for delivery using a continuous subcutaneous insulin infusion, that is provided by a closed loop system.

The stable co-formulations provided herein can be provided in syringes or vials, a closed loop system, an insulin pump, and/or an insulin pen.

Provided are methods in which the stable co-formulations are administered. For example, provided herein are methods of treating diabetes by administering to a subject a therapeutically effective amount of a stable co-formulation provided herein. The diabetes to be treated includes type 1 diabetes mellitus, type 2 diabetes mellitus or gestational diabetes. Also provided herein are methods for controlling blood glucose levels in a subject by administering to a subject a therapeutically effective amount of a stable co-formulation provided herein. In practicing the methods herein, the stable co-formulations are administered subcutaneously or intraperitoneally, for example, via a syringe or insulin pen or by continuous subcutaneous infusion. In practicing the methods herein, the stable co-formulations can be administered prior to a meal as prandial insulin therapy. In the methods provided herein, the stable co-formulation can be administered using a delivery method to achieve continuous subcutaneous insulin infusion, such as via an insulin pump or a closed loop system. In some instances, the methods provided herein include administering another anti-diabetic drug, that is selected from among, but not limited to, sulfonylureas, biguanides, meglitinides, thiazolidinediones, alpha-glucosidase inhibitors, peptide analogs, including glucagon-like peptide (GLP) analogs and, gastric inhibitory peptide (GIP) analogs and DPP-4 inhibitors.

Also provided herein are compositions containing a therapeutically effective amount of a hyaluronan-degrading enzyme and lysyl lysine (Lys-Lys) in an amount to render the hyaluronan-degrading enzyme stable. In some examples, the concentration of Lys-Lys is between or about between 5 mM to 120 mM, 10 mM to 100 mM, 10 mM to 50 mM, 30 mM to 110 mM, 30 mM to 80 mM, 50 mM to 100 mM or 100 mM to 120 mM. In other examples, the concentration of Lys-Lys is at least or at least about or is 5 mM, 10 mM, 15 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, 100 mM, 110 mM or 120 mM. Provided herein are compositions containing a therapeutically effective amount of a hyaluronan-degrading enzyme and lysyl lysine (Lys-Lys) in an amount sufficient such that the hyaluronan-degrading

enzyme retains at least 50% of the initial hyaluronidase activity for at least three (3) days at 37°C. In some examples, the hyaluronan-degrading enzyme retains at least 50% of the initial hyaluronidase activity at 37°C for at least 4 days, 5 days, 6 days, one week, two weeks, three weeks, one month, two months, three months four months, five months, six months or more. In a particular example, the hyaluronan-degrading enzyme retains at least 50% of the initial hyaluronidase activity for at least one month at 37°C. In other examples, the hyaluronan-degrading enzyme retains at least 60%, 70%, 75%, 80%, 85%, 90%, 95% or more of the initial hyaluronidase activity.

In some examples of the provided compositions, the pH of the formulation is between or about between 6.5 to 8.0, 6.5 to 7.4, 6.8 to 7.8, 7.0 to 7.6 or 6.8 to 7.2, inclusive. For example, the pH of the formulation is or is about or at least 6.5± 0.2, 6.6± 0.2, 6.7± 0.2, 6.8 ± 0.2, 6.9 ± 0.2, 7.0 ± 0.2, 7.1 ± 0.2, 7.2 ± 0.2, 7.3 ± 0.2, 7.4 ± 0.2, 7.5 ± 0.2, 7.6 ± 0.2, 7.7 ± 0.2 or 7.8 ± 0.2.

Any of the provided compositions containing a therapeutically effective amount of a hyaluronan-degrading enzyme and lysyl lysine (Lys-Lys) can further contain a stabilizing agent. For example, the compositions can contain a stabilizing agent that is selected from among an amino acid, an amino acid derivative, an amine, a sugar, a polyol, a salt and a surfactant. In some examples, the stabilizing agent is a surfactant and the amount of surfactant, as a % of mass concentration (w/v) in the formulation, is between or about between 0.0005% to 1.0%, 0.0005% to 0.005%, 0.001% to 0.01%, 0.01% to 0.5%, 0.01% to 0.1%, 0.01% to 0.05%, or 0.01% to 0.02%, inclusive. In other examples, the stabilizing agent is a surfactant and the amount of surfactant, as a % of mass concentration (w/v) in the formulation, is or is about or at least 0.001%, 0.005%, 0.01%, 0.015%, 0.02%, 0.025%, 0.03%, 0.035%, 0.04%, 0.045%, 0.05%, 0.055%, 0.06%, 0.065%, 0.07%, 0.08% or 0.9%. The surfactant can be selected from among a polypropylene glycol, polyethylene glycol, glycerin, sorbitol, poloxamer and polysorbate. In a particular example, the surfactant is selected from among poloxamer 188, polysorbate 20 and polysorbate 80.

In some examples, the provided compositions containing a therapeutically effective amount of a hyaluronan-degrading enzyme and lysyl lysine (Lys-Lys) also contain an antioxidant. For example, the compositions contain an antioxidant that is

selected from among cysteine, tryptophan and methionine. In a particular example, the antioxidant is methionine. In some examples, the antioxidant is present at a concentration from between or from about between 5 mM to 50 mM, 5 mM to 40 mM, 5 mM to 20 mM or 10 mM to 20 mM, inclusive. In other examples, the antioxidant is present at a concentration that is or is about or is at least 5 mM, 10 mM, 15 mM, 20 mM, 30 mM, 40 mM or 50 mM.

In some examples, the provided compositions containing a therapeutically effective amount of a hyaluronan-degrading enzyme and lysyl lysine (Lys-Lys) also contain a tonicity modifier to maintain the osmolarity of between or about between 245 mOsm/kg to 500 mOsm/kg, inclusive. In some examples, the compositions contain a tonicity modifier to maintain the osmolarity of the formulation of about or at least about or 245 mOsm/kg, 250 mOsm/kg, 255 mOsm/kg, 260 mOsm/kg, 265 mOsm/kg, 270 mOsm/kg, 275 mOsm/kg, 280 mOsm/kg, 285 mOsm/kg, 290 mOsm/kg, 300 mOsm/kg, 350 mOsm/kg, 400 mOsm/kg, 450 mOsm/kg or 500 mOsm/kg. In some examples, the tonicity modifier is selected from among glycerin, NaCl, amino acids, polyalcohols or trehalose. In a particular example, the tonicity modifier is NaCl and the concentration of NaCl is or is about between 20 mM to 200 mM, 40 mM to 160 mM, 80 mM to 120 mM, 20 mM to 80 mM or 50 mM to 150 mM, inclusive. In other examples, the tonicity modifier is NaCl and the concentration if NaCl is 0 mM to 150 mM, 10 mM to 50 mM, 50 mM to 100 mM and 100 mM to 130 mM. In some example, the NaCl is at a concentration of less than 150 mM, less than 140 mM, less than 130 mM, less than 120 mM, less than 110 mM, less than 100 mM, less than 90 mM, less than 80 mM, less than 70 mM, less than 60 mM, less than 50 mM, less than 40 mM, less than 30 mM, less than 20 mM, less than 10 mM, or less.

Any of the provided compositions containing a therapeutically effective amount of a hyaluronan-degrading enzyme and lysyl lysine (Lys-Lys) can also contain a sufficient amount of a buffering agent to maintain the pH range of between or about between 6.5 to 8.0, 6.8 to 7.8, or 7.0 to 7.6, 6.5 to 7.2, 6.8 to 7.4, inclusive. In some examples, the buffering agent is selected from among Tris, histidine, phosphate and citrate. In a particular example, the buffering agent is a phosphate that is sodium phosphate. In another particular example, the buffering agent is Tris. In

some examples, the concentration of the buffering agent in the compositions is between or is between about 1 mM to 100 mM, 10 mM to 80 mM, 5 mM to 50 mM or 20 mM to 40 mM, inclusive.

The hyaluronan-degrading enzyme in any of the provided compositions containing a therapeutically effective amount of a hyaluronan-degrading enzyme and lysyl lysine (Lys-Lys) can be a hyaluronidase or a chondroitinase. In some examples, the hyaluronan-degrading enzyme is a hyaluronidase that is active at neutral pH. In some examples, the hyaluronan-degrading enzyme lacks a glycosylphosphatidylinositol (GPI) anchor or is not membrane-associated when expressed from a cell. In other examples, the hyaluronan-degrading enzyme is a hyaluronan-degrading enzyme that contains C-terminal truncations of one or more amino acid residues to remove all or part of a GPI anchor.

In other examples of the provided compositions, the hyaluronan-degrading enzyme is a hyaluronidase that is a PH20 or a C-terminally truncated fragment thereof. In some examples the hyaluronan-degrading enzyme is a PH20 that is a non-human or a human PH20. In some examples, the PH20 has a sequence of amino acids that contains at least amino acids 36-464 of SEQ ID NO:1, or has a sequence of amino acids that has at least 85% sequence identity to a sequence of amino acids that contains at least amino acids 36-464 of SEQ ID NO:1 and retains hyaluronidase activity. For example, the PH20 has at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to a sequence of amino acids that contains at least amino acids 36-464 of SEQ ID NO:1 and retains hyaluronidase activity. In some examples of the compositions, the hyaluronan-degrading enzyme is a PH20 polypeptide that has a sequence of amino acids that contains a C-terminal truncation after amino acid position 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499 or 500 of the sequence of amino acids set forth in SEQ ID NO:1, or is a variant thereof that exhibits at least 85% sequence identity to a sequence of amino acids that contains a C-terminal truncation after amino acid position 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499 or 500 of the sequence of amino acids set forth

in SEQ ID NO:1 and retains hyaluronidase activity. In particular examples, the hyaluronan-degrading enzyme is a C-terminal truncated PH20 that has a sequence of amino set forth in any of SEQ ID NOS: 4-9.

In some examples of the provided compositions, the amount of a hyaluronan-degrading enzyme is between or about between 10 U/mL to 5000 U/mL, 50 U/mL to 4000 U/mL, 100 U/mL to 2000 U/mL, 300 U/mL to 2000 U/mL, 600 U/mL to 2000 U/mL, 100 U/mL to 1000 U/mL, 200 U/mL to 800 U/mL, 100 U/mL to 500 U/mL, or 150 U/mL to 300 U/ml, inclusive. For example, the amount of a hyaluronan-degrading enzyme is at least or is about or is 30 U/mL, 35 U/mL, 40 U/mL, 45 U/mL, 50 U/mL, 55 U/mL, 60 U/mL, 65 U/mL, 70 U/mL, 75 U/mL, 80 U/mL, 85 U/mL, 90 U/mL, 95 U/mL, 100 U/mL, 105 U/mL, 110 U/mL, 115 U/mL, 120 U/mL, 125 U/mL, 130 U/mL, 135 U/mL, 140 U/mL, 145 U/mL, 150 U/mL, 155 U/mL, 160 U/mL, 170 U/mL, 180 U/mL, 190 U/mL, 200 U/mL, 250 U/mL, 300 U/mL, 350 U/mL, 400 U/mL, 450 U/mL, 500 U/mL, 600 U/mL, 700 U/mL, 800 U/mL, 900 U/mL, 1000 U/mL or 2000 U/mL.

In some examples of the compositions containing a therapeutically effective amount of a hyaluronan-degrading enzyme and lysyl lysine (Lys-Lys), the concentration of Lys-Lys is 5 mM to 50 mM, inclusive. For example, the concentration of Lys-Lys is at least 5 mM, 10 mM, 15 mM, 20 mM, 30 mM or 50 mM; and/or is less than 50 mM, 40 mM, 30 mM, 20 mM or 10 mM.

Provided herein are compositions containing a therapeutically effective amount of a hyaluronan-degrading enzyme and lysyl lysine (Lys-Lys) wherein the pH of the composition is between or about between 6.5 to 7.2 and the composition contains a hyaluronan-degrading enzyme in an amount that is between or about between 100 U/mL to 500 U/mL, inclusive; Lys-Lys at a concentration that is between or about between 5 mM to 30 mM, inclusive; NaCl at a concentration less than 140 mM NaCl; a surfactant that is polysorbate 80 at a percentage (%) of mass concentration (w/v) of between or about between 0.01% to 0.05%, inclusive; methionine at a concentration that is between or about between 5 mM to 20 mM, inclusive; and sodium phosphate at a concentration that is between or about between 5 mM to 50 mM, inclusive. In some examples, the hyaluronan-degrading enzyme is a PH20 or a C-terminally truncated fragment thereof.

In some examples, the volume of the provided compositions is between or about between 0.5 mL to 50 mL, 1 mL to 40 mL, 1 mL to 20 mL, 1 mL to 10 mL, or 3 mL to 10mL, inclusive. The compositions can be formulated for delivery using a vial, syringe, pen, reservoir for a pump or a closed loop system. Also provided herein is a syringe or vial containing any of the compositions provided herein.

Also provided herein are compositions containing a therapeutically effective amount of a hyaluronan-degrading enzyme, lysyl lysine (Lys-Lys) in an amount to render the hyaluronan-degrading enzyme stable and a fast-acting insulin. In some examples, the concentration of Lys-Lys is 30 mM to 120 mM, 50 mM to 105 mM or 80 mM to 100 mM, inclusive. In the provided compositions, the fast-acting insulin can be monomeric, dimeric or hexameric. In some examples, the fast-acting insulin is a fast-acting human insulin. In other examples, the fast-acting insulin is a regular insulin. In a particular example, the fast-acting insulin is a regular insulin that is a human insulin or pig insulin. In some examples, the fast-acting insulin is a regular insulin which is an insulin with an A chain having a sequence of amino acids set forth in SEQ ID NO:103 and a B chain having a sequence of amino acids set forth in SEQ ID NO:104 or an insulin with an A chain with a sequence of amino acids set forth as amino acid residue positions 88-108 of SEQ ID NO:123 and a B chain with a sequence of amino acids set forth as amino acid residue positions 25-54 of SEQ ID NO:123. In yet other examples of the provided compositions, the fast-acting insulin is a recombinant insulin. The fast-acting insulin can be synthesized or partially-synthesized. In some examples, the insulin is isolated.

In other examples of the provided compositions, the fast-acting insulin is an insulin analog. In some examples, the insulin analog is selected from among insulin aspart, insulin lispro and insulin glulisine. For example, the insulin analog is selected from among an insulin having an A chain with a sequence of amino acids set forth in SEQ NOS:103 and a B chain having a sequence of amino acids set forth in any of SEQ NOS:147-149. In any of the provided compositions, the fast-acting insulin can be present in an amount between or about between 10 U/mL to 1000 U/mL, 20 U/mL to 500 U/mL, 50 U/mL to 300 U/mL or 200 U/mL to 800 U/mL, inclusive. For example, the amount of fast-acting insulin is at least or is about or is 10 U/mL, 20 U/mL, 30 U/mL, 40 U/mL, 50 U/mL, 60 U/mL, 70 U/mL, 80 U/mL, 90 U/mL, 100

U/mL, 150 U/mL, 200 U/mL, 250 U/mL, 300 U/mL, 350 U/mL, 400 U/mL, 500 U/mL, 600 U/mL, 700 U/mL, 800 U/mL, 900 U/mL or 1000 U/mL.

In particular examples of the provided compositions containing a therapeutically effective amount of a hyaluronan-degrading enzyme, lysyl lysine (Lys-Lys) in an amount to render the hyaluronan-degrading enzyme stable and a fast-acting insulin, the fast-acting insulin is an insulin analog and the hyaluronan-degrading enzyme is a PH20 or a C-terminally truncated fragment thereof. In a particular example, the fast-acting insulin is an insulin analog that is glulisine and the concentration of Lys-Lys is 50 to 105 mM. In another example, the fast-acting insulin is an insulin analog that is insulin aspart or insulin lispro and the concentration of Lys-Lys is 80 to 100 mM.

Any of the compositions containing a therapeutically effective amount of a hyaluronan-degrading enzyme, lysyl lysine (Lys-Lys) in an amount to render the hyaluronan-degrading enzyme stable and a fast-acting insulin provided herein can be for single dosage administration or for multiple dosage administration. In examples where the composition is for multiple dosage administration, the composition contains an anti-microbially effective amount of a preservative or mixture of preservatives. The preservative(s) in the formulation can contain one or more of a phenolic preservative(s), a non-phenolic preservative(s) or a phenolic preservative(s) and a non-phenolic preservative(s). In some examples, the preservative(s) is(are) selected from among phenol, m-cresol, methylparaben, benzyl alcohol, thimerosal, benzalkonium chloride, 4-chloro-1-butanol, chlorhexidine dihydrochloride, chlorhexidine digluconate, L-phenylalanine, EDTA, bronopol, phenylmercuric acetate, glycerol, imidurea, chlorhexidine, sodium dehydroacetate, o-cresol, p-cresol, chlorocresol, cetrimide, benzethonium chloride, ethyl paraben, propylparaben, butylparaben and any combinations thereof. In some examples of the provided compositions for multiple dosage administration, the formulation contains a single preservative. In other examples of the provided compositions for multiple dosage administration, the formulation contains a mixture of preservatives that contains 2, 3 or 4 different preservatives. In some examples, the compositions contain at least one phenolic preservative. In other examples, the preservative(s) is(are) phenol, m-cresol or phenol and m-cresol.

In some examples of the provided compositions, the total amount of the one or more preservative agents as a percentage (%) of mass concentration (w/v) in the formulation is or is between 0.1% and 0.4%, 0.1% to 0.3%, 0.15% to 0.325%, 0.15% to 0.25%, 0.1% to 0.2%, 0.2% to 0.3% or 0.3% to 0.4%, inclusive. In particular examples of the provided compositions wherein the preservatives are phenol and m-cresol, the amount as a % of mass concentration (w/v) in the formulation is between or about between 0.1% to 0.25% phenol and between or about between 0.05% to 0.2% m-cresol, is between or about between 0.10% to 0.2% phenol and between or about between 0.06% to 0.18% m-cresol, is between or about between 0.1% to 0.15% phenol and 0.08% to 0.15% m-cresol, is between or about between 0.10% to 0.15% phenol and between or about between 0.06 to 0.09% m-cresol or is between or about between 0.12% to 0.18% phenol and between or about between 0.14 to 0.22% m-cresol, inclusive. In other particular examples of the provided compositions wherein the preservatives are phenol and m-cresol, the amount as a % of mass concentration (w/v) in the formulation is or is about 0.1% phenol and 0.075% m-cresol, is or is about 0.1% phenol and 0.15% m-cresol, is or is about 0.125% phenol and 0.075% m-cresol, is or is about 0.13% phenol and 0.075% m-cresol, is or is about 0.13% phenol and 0.08% m-cresol, is or is about 0.15% phenol and 0.175% m-cresol or is or is about 0.17% phenol and 0.13% m-cresol.

Provided herein are compositions containing a therapeutically effective amount of a hyaluronan-degrading enzyme, lysyl lysine (Lys-Lys) in an amount to render the hyaluronan-degrading enzyme stable and a fast-acting insulin wherein the pH of the composition is between or about between 6.8 to 7.4; and the composition contains a hyaluronan-degrading enzyme that is a PH20 in an amount between or about between 100 U/mL to 1000 U/mL, inclusive; a fast-acting insulin analog that is insulin glulisine is an amount between or about between 10 U/mL to 1000 U/mL, inclusive; Lys-Lys at a concentration between or about between 50 mM to 105 mM, inclusive; NaCl at a concentration of less than 100 mM; a surfactant that is polysorbate 20 at a percentage (%) of mass concentration (w/v) of between or about between 0.0005% to 0.005%, inclusive; methionine at a concentration between or about between 5 mM to 20 mM, inclusive; and a preservative(s) that contains phenol

at a percentage (%) of mass concentration (w/v) of between or between about between 0.1% to 0.25% and m-cresol at a % w/v of between or between about 0.05% to 0.2%.

Provided herein are compositions containing a therapeutically effective amount of a hyaluronan-degrading enzyme, lysyl lysine (Lys-Lys) in an amount to render the hyaluronan-degrading enzyme stable and a fast-acting insulin wherein the pH of the composition is between or about between 6.8 to 7.4; and the composition contains a hyaluronan-degrading enzyme that is a PH20 in an amount between or about between 100 U/mL to 1000 U/mL, inclusive; a fast-acting insulin analog that is insulin aspart or insulin lispro is an amount between or about between 10 U/mL to 1000 U/mL, inclusive; Lys-Lys at a concentration between or about between 80 mM to 100 mM, inclusive; NaCl at a concentration of less than 30 mM; a surfactant that is polysorbate 20 at a percentage (%) of mass concentration (w/v) of between or about between 0.0005% to 0.005%, inclusive; methionine at a concentration between or about between 5 mM to 20 mM, inclusive; and a preservative(s) that contains phenol at a percentage (%) of mass concentration (w/v) of between or between about between 0.1% to 0.25% and m-cresol at a % w/v of between or between about 0.05% to 0.2%.

In some examples, the volume of the provided compositions is between or about between 0.5 mL to 50 mL, 1 mL to 40 mL, 1 mL to 20 mL, 1 mL to 10 mL, or 3 mL to 10mL, inclusive. The compositions can be formulated for delivery using a vial, syringe, pen, reservoir for a pump or a closed loop system. In some examples, the compositions are formulated for delivery using a continuous subcutaneous insulin infusion. Also provided herein is a syringe or vial containing any of the compositions provided herein.

Provided herein are compositions containing a therapeutically effective amount of a hyaluronan-degrading enzyme and $MgCl_2$ in a sufficient such that the hyaluronan-degrading enzyme retains at least 50% of the initial hyaluronidase activity for at least three (3) days at 37°C. In some examples, the compositions contain $MgCl_2$ at a concentration that is between or about between 50 mM to 150 mM, 75 mM to 125 mM or 80 mM to 100 mM, inclusive. For example, the compositions contain $MgCl_2$ at a concentration that is at least or is about or is 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, 100 mM, 110 mM, 120 mM, 130 mM, 140 mM or 150 mM. In some examples of the compositions, the hyaluronan-degrading enzyme retains at least 50%

of the initial hyaluronidase activity at 37°C for at least 4 days, 5 days, 6 days, one week, two weeks, three weeks, one month, two months, three months, four months, five months, six months or more. For example, the hyaluronan-degrading enzyme retains at least 50% of the initial hyaluronidase activity for at least one month at 37°C, such as at least 60%, 70%, 75%, 80%, 85%, 90%, 95% or more of the initial hyaluronidase activity.

The provided compositions containing a therapeutically effective amount of a hyaluronan-degrading enzyme and $MgCl_2$ can have a pH that is between or about between 6.5 to 8.0, 6.5 to 7.4, 6.8 to 7.8, 7.0 to 7.6 or 6.8 to 7.2. In some examples, the pH of the composition is or is about or at least 6.5± 0.2, 6.6± 0.2, 6.7± 0.2, 6.8 ± 0.2, 6.9 ± 0.2, 7.0 ± 0.2, 7.1 ± 0.2, 7.2 ± 0.2, 7.3 ± 0.2, 7.4 ± 0.2, 7.5 ± 0.2, 7.6 ± 0.2, 7.7 ± 0.2 or 7.8 ± 0.2. The compositions can further contain a stabilizing agent that is selected from among an amino acid, an amino acid derivative, an amine, a sugar, a polyol, a salt and a surfactant. In some examples, the stabilizing agent is a surfactant and the amount of surfactant, as a % of mass concentration (w/v) in the formulation, is between or about between 0.0005% to 1.0%, 0.0005% to 0.005%, 0.001% to 0.01%, 0.01% to 0.5%, 0.01% to 0.1%, 0.01% to 0.05% or 0.01% to 0.02%, inclusive. For example, the stabilizing agent is a surfactant and the amount of surfactant, as a % of mass concentration (w/v) in the formulation, is or is about or at least 0.001%, 0.005%, 0.01%, 0.015%, 0.02%, 0.025%, 0.03%, 0.035%, 0.04%, 0.045%, 0.05%, 0.055%, 0.06%, 0.065%, 0.07%, 0.08% or 0.9%. In some examples, the surfactant is selected from among a polypropylene glycol, polyethylene glycol, glycerin, sorbitol, poloxamer and polysorbate. In particular examples, the surfactant is selected from among poloxamer 188, polysorbate 20 and polysorbate 80.

In some examples, the provided compositions containing a therapeutically effective amount of a hyaluronan-degrading enzyme and $MgCl_2$ contain an antioxidant that is selected from among cysteine, tryptophan and methionine. In particular examples, the antioxidant is methionine. In some examples, the antioxidant is at a concentration from between or from about between 5 mM to 50 mM, 5 mM to 40 mM, 5 mM to 20 mM or 10 mM to 20 mM, inclusive. For example, the antioxidant is methionine and the concentration is or is about or is at least 5 mM, 10 mM, 15 mM, 20 mM, 30 mM, 40 mM or 50 mM. In some examples, the compositions contain a

sufficient amount of a buffering agent to maintain the pH range of between or about between 6.5 to 8.0, 6.8 to 7.8, 7.0 to 7.6, 6.5 to 7.2 or 6.8 to 7.4. In some examples, the buffering agent is selected from among Tris, histidine, phosphate and citrate. In a particular example, the buffering agent is histidine hydrochloride. The concentration of the buffering agent in the provided compositions can be between or between about 1 mM to 100 mM, 10 mM to 80 mM, 5 mM to 50 mM or 20 mM to 40 mM.

In some examples, the provided compositions containing a therapeutically effective amount of a hyaluronan-degrading enzyme and $MgCl_2$ contain a hyaluronan-degrading enzyme is a hyaluronidase or a chondroitinase. In some examples, the hyaluronan-degrading enzyme is a hyaluronidase that is active at neutral pH. In other examples, the hyaluronan-degrading enzyme lacks a glycosylphosphatidylinositol (GPI) anchor or is not membrane-associated when expressed from a cell. For example, the hyaluronan-degrading enzyme is a hyaluronan-degrading enzyme that contains C-terminal truncations of one or more amino acid residues to remove all or part of a GPI anchor.

In other examples, the hyaluronan-degrading enzyme is a hyaluronidase that is a PH20 or a C-terminally truncated fragment thereof. The PH20 can be a non-human or a human PH20. In some examples of the provided compositions, the PH20 has a sequence of amino acids that contains at least amino acids 36-464 of SEQ ID NO:1, or has a sequence of amino acids that has at least 85% sequence identity to a sequence of amino acids that contains at least amino acids 36-464 of SEQ ID NO:1 and retains hyaluronidase activity. For example, the PH20 has at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to a sequence of amino acids that contains at least amino acids 36-464 of SEQ ID NO:1 and retains hyaluronidase activity. In other examples, the PH20 polypeptide has a sequence of amino acids that contains a C-terminal truncation after amino acid position 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499 or 500 of the sequence of amino acids set forth in SEQ ID NO:1, or is a variant thereof that exhibits at least 85% sequence identity to a sequence of amino acids that contains a C-terminal truncation after amino acid position 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484,

485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499 or 500 of the sequence of amino acids set forth in SEQ ID NO:1 and retains hyaluronidase activity. In particular examples, the hyaluronan-degrading enzyme is a C-terminal truncated PH20 that has a sequence of amino acids set forth in any of SEQ ID NOS: 4-9.

In some examples, the provided compositions containing a therapeutically effective amount of a hyaluronan-degrading enzyme and $MgCl_2$ contain a hyaluronan-degrading enzyme in an amount that is between or about between 10 U/mL to 5000 U/mL, 50 U/mL to 4000 U/mL, 100 U/mL to 2000 U/mL, 300 U/mL to 2000 U/mL, 600 U/mL to 2000 U/mL, 100 U/mL to 1000 U/mL, 200 U/mL to 800 U/mL, 100 U/mL to 500 U/mL, or 150 U/mL to 300 U/ml, inclusive. For example, the compositions contain a hyaluronan-degrading enzyme in an amount that is at least or is about or is 30 U/mL, 35 U/mL, 40 U/mL, 45 U/mL, 50 U/mL, 55 U/mL, 60 U/mL, 65 U/mL, 70 U/mL, 75 U/mL, 80 U/mL, 85 U/mL, 90 U/mL, 95 U/mL, 100 U/mL, 105 U/mL, 110 U/mL, 115 U/mL, 120 U/mL, 125 U/mL, 130 U/mL, 135 U/mL, 140 U/mL, 145 U/mL, 150 U/mL, 155 U/mL, 160 U/mL, 170 U/mL, 180 U/mL, 190 U/mL, 200 U/mL, 250 U/mL, 300 U/mL, 350 U/mL, 400 U/mL, 450 U/mL, 500 U/mL, 600 U/mL, 700 U/mL, 800 U/mL, 900 U/mL, 1000 U/mL or 2000 U/mL.

Provided herein are compositions containing a therapeutically effective amount of a hyaluronan-degrading enzyme and $MgCl_2$ wherein the pH of the composition is between or about between 6.5 to 7.2 and the composition contains a hyaluronan-degrading enzyme in an amount that is between or about between 100 U/mL to 500 U/mL, inclusive; $MgCl_2$ at a concentration that is between or about between 50 mM to 150 mM, inclusive; a surfactant that is polysorbate 80 at a percentage (%) of mass concentration (w/v) of between or about between 0.01% to 0.05%, inclusive; methionine at a concentration that is between or about between 5 mM to 20 mM, inclusive; and histidine/HCl at a concentration that is between or about between 5 mM to 50 mM, inclusive.

In some examples, the volume of the provided compositions is between or about between 0.5 mL to 50 mL, 1 mL to 40 mL, 1 mL to 20 mL, 1 mL to 10 mL, or 3 mL to 10mL, inclusive. The compositions can be formulated for delivery using a vial,

syringe, pen, reservoir for a pump or a closed loop system. Also provided herein is a syringe or vial containing any of the compositions provided herein.

The compositions provided herein containing an insulin, such as a regular insulin or a rapid acting insulin analog (e.g. aspart, lispro or glulisine or other insulin analog), can be used in methods and uses for treating diabetes. For example, provided herein are methods of treating diabetes by administering to a subject a therapeutically effective amount of a any of the above stable composition provided herein. The diabetes to be treated includes type 1 diabetes mellitus, type 2 diabetes mellitus or gestational diabetes. Also provided herein are methods for controlling blood glucose levels in a subject by administering to a subject a therapeutically effective amount of a stable composition provided herein containing a fast-acting insulin. In practicing the methods herein, the stable compositions are administered subcutaneously or intraperitoneally, for example, via a syringe or insulin pen or by continuous subcutaneous infusion. In practicing the methods herein, the stable compositions can be administered prior to a meal as prandial insulin therapy. In the methods provided herein, the stable compositions can be administered using a delivery method to achieve continuous subcutaneous insulin infusion, such as via an insulin pump or a closed loop system. In some instances, the methods provided herein include administering another anti-diabetic drug, that is selected from among, but not limited to, sulfonylureas, biguanides, meglitinides, thiazolidinediones, alpha-glucosidase inhibitors, peptide analogs, including glucagon-like peptide (GLP) analogs and, gastric inhibitory peptide (GIP) analogs and DPP-4 inhibitors.

DETAILED DESCRIPTION

A. Definitions

B. HYALURONAN DEGRADING ENZYME FORMULATIONS AND GENERATING INSULIN CO-FORMULATIONS

1. Hyaluronan-Degrading Enzyme Formulations
2. Fast-Acting Insulin Formulations
3. Hyaluronan-Degrading Enzyme and Insulin Co-formulations
   a. Opposing requirements for stability
      i. Preservatives
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   b. Compatible Co-Formulation

C. HYALURONAN DEGRADING ENZYMES

1. Hyaluronidases
   a. Mammalian-type hyaluronidases
      PH20
   b. Bacterial hyaluronidases
   c. Hyaluronidases from leeches, other parasites and crustaceans

2. Other hyaluronan degrading enzymes
3. Truncated hyaluronan degrading enzymes or other soluble forms
a. C-terminal Truncated Human PH20
b. rHuPH20
4. Glycosylation of hyaluronan degrading enzymes
5. Modifications of hyaluronan degrading enzymes to improve their pharmacokinetic properties
D. STABLE HYALURONAN-DEGRADING ENZYME FORMULATIONS
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3. pH and Buffer
4. Surfactant
5. Anti-Oxidation Agent
6. Tonicity Modifier
7. Other Agents or Excipients
8. Exemplary Stable Hyaluronan-Degrading Enzyme Formulations
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F. STABLE CO-FORMULATIONS OF INSULIN AND HYALURONAN-DEGRADING ENZYME
1. Components of Stable Co-Formulations
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c. Preservative
d. NaCl
e. pH
f. Buffer
g. Lys-Lys
h. Additional Exemplary Excipients or Stablizers
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ii. Tonicity Modifier
iii. Glycerin
iv. Antioxidants
v. Zinc
vi. Amino acid stabilizer
vii. Hyaluronidase Inhibitor
viii. Nicotinic Compound
ix. Other Excipients or Agents
2. Exemplary Stable Co-formulations
a. Exemplary Multi-Dose Injection (MDI) Co-Formulations
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c. Exemplary Lys-Lys Co-Formulations
G. DOSAGE AND ADMINISTRATION
Mode of administration
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b. Insulin pen
c. Insulin pumps and other insulin delivery devices
d. Continuous Infusion Pump Systems
i. Open loop systems
ii. Closed loop systems

H. METHODS OF PRODUCING NUCLEIC ACIDS ENCODING AN INSULIN OR HYALURONAN DEGRADING ENZYME AND POLYPEPTIDES THEREOF
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b. Yeast Cells
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I. METHODS OF ASSESSING STABILITY AND ACTIVITY
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2. Hyaluronan degrading enzymes
J. THERAPEUTIC USES
1. Diabetes Mellitus
a. Type 1 diabetes
b. Type 2 diabetes
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2. Insulin therapy for critically ill patients
K. COMBINATION THERAPIES
L. ARTICLES OF MANUFACTURE AND KITS
M. EXAMPLES

A. DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the invention(s) belong. All Patents, Pat. applications, published applications and publications, GENBANK sequences, websites and other published materials referred to throughout the entire disclosure herein, unless noted otherwise, are incorporated by reference in their entirety. In the event that there is a plurality of definitions for terms herein, those in this section prevail. Where reference is made to a URL or other such identifier or address, it is understood that such identifiers can change and particular information on the internet can come and go, but equivalent information is known and can be readily accessed, such as by searching the internet and/or appropriate databases. Reference thereto evidences the availability and public dissemination of such information.

As used herein, "insulin" refers to a hormone, precursor or a synthetic or recombinant analog thereof that acts to increase glucose uptake and storage and/or decrease endogenous glucose production. An exemplary human insulin is translated as a 110 amino acid precursor polypeptide, preproinsulin (SEQ ID NO:101), containing a 24 amino acid signal peptide that directs the protein to the endoplasmic reticulum (ER) wherein the signal sequence is cleaved, resulting in proinsulin (SEQ

ID NO:102). Proinsulin is processed further to release the 31 amino acid C- or connecting chain peptide (corresponding to amino acid residues 57 to 87 of the preproinsulin polypeptide set forth in SEQ ID NO:101, and to amino acid residues 33 to 63 of the proinsulin polypeptide set forth in SEQ ID NO:102). The resulting insulin contains a 21 amino acid A-chain (corresponding to amino acid residues 90 to 110 of the preproinsulin polypeptide set forth in SEQ ID NO:101, and to amino acid residues 66 to 86 of the proinsulin polypeptide set forth in SEQ ID NO:102) and a 30 amino acid B-chain (corresponding to amino acid residues 25 to 54 of the preproinsulin polypeptide set forth in SEQ ID NO:101, and to amino acid residues 1 to 30 of the proinsulin polypeptide set forth in SEQ ID NO:102) which are cross-linked by disulfide bonds. A properly cross-linked human insulin contains three disulfide bridges: one between position 7 of the A-chain and position 7 of the B-chain, a second between position 20 of the A-chain and position 19 of the B-chain, and a third between positions 6 and 11 of the A-chain. Reference to insulin includes preproinsulin, proinsulin and insulin polypeptides in single-chain or two-chain forms, truncated forms thereof that have activity, and includes allelic variants and species variants, variants encoded by splice variants, and other variants, such as insulin analogs, including polypeptides that have at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the precursor polypeptide set forth in SEQ ID NO:101 or the mature form thereof. Exemplary insulin analogs include those set forth in SEQ ID NOS:147-149, 152, and those containing an A-chain set forth in SEQ ID NOS:150, 156, 158, 160, 162 and 164 and/or a B chain set forth in SEQ ID NOS:151, 153-155, 157, 159, 161, 163 and 165.

Exemplary insulin polypeptides are those of mammalian, including human, origin. Exemplary amino acid sequences of insulin of human origin are set forth in SEQ ID NOS: 101-104. Exemplary insulin analogs include those set forth in SEQ ID NOS:147-149, 152, and those containing an A-chain set forth in SEQ ID NOS:150, 156, 158, 160, 162 and 164 and/or a B chain set forth in SEQ ID NOS:151, 153-155, 157, 159, 161, 163 and 165. Insulin polypeptides also include any of non-human origin including, but not limited to, any of the precursor insulin polypeptides set forth

in SEQ ID NOS:105-146. Reference to an insulin includes monomeric and multimeric insulins, including hexameric insulins, as well as humanized insulins.

As used herein, "fast-acting insulin" refers to any insulin or fast-acting insulin composition for acute administration to a diabetic subject in response to an actual, perceived, or anticipated hyperglycemic condition in the subject arising at the time of, or within about four hours following, administration of the fast-acting insulin (such as a prandial hyperglycemic condition resulting or anticipated to result from, consumption of a meal), whereby the fast-acting insulin is able to prevent, control or ameliorate the acute hyperglycemic condition. Typically a fast-acting insulin is an insulin that exhibits peak insulin levels at or about not more than four hours following subcutaneous administration to a subject. Fast-acting insulins include recombinant insulins and isolated insulins (also referred to as "regular" insulins) such as the insulin sold as Humulin® R, porcine insulins and bovine insulins, as well as rapid acting insulin analogs (also termed fast-acting insulin analogs herein) designed to be rapid acting by virtue of amino acid changes. Exemplary regular insulin preparations include, but are not limited to, human regular insulins, such as those sold under the trademarks Humulin® R, Novolin® R and Velosulin®, Insulin Human, USP and Insulin Human Injection, USP, as well as acid formulations of insulin, such as, for example, Toronto Insulin, Old Insulin, and Clear Insulin, and regular pig insulins, such as Iletin II® (porcine insulin). Regular insulins typically have an onset of action of between 30 minutes to an hour, and a peak insulin level of 2-5 hours post administration.

As used herein, rapid acting insulin analogs (also called fast-acting insulin analogs) are insulins that have a rapid onset of action. Rapid insulins typically are insulin analogs that have been engineered, such as by the introduction of one or more amino acid substitutions, to be more rapid acting than regular insulins. Rapid acting insulin analogs typically have an onset of action of 10-30 minutes post injection, with peak insulin levels observed 30-90 minutes post injection. Exemplary rapid acting insulin analogs include, but are not limited to, for example, insulin lispro (*e.g.* Humalog® insulin), insulin aspart (*e.g.* NovoLog® insulin), and insulin glulisine (*e.g.* Apidra® insulin) the fast-acting insulin composition sold as VIAject® and VIAtab® (see, *e.g.*, U.S. Pat. No. 7,279,457). Also included are any other insulins that have an

onset of action of 30 minutes or less and a peak level before 90 minutes, typically 30-90 minutes, post injection.

As used herein, a human insulin refers to an insulin that is synthetic or recombinantly produced based upon the human polypeptide, including allelic variants and analogs thereof.

As used herein, fast-acting human insulins or human fast-acting insulin compositions include any human insulin or composition of a human insulin that is fast-acting, but excludes non-human insulins, such as regular pig insulin.

As used herein, the terms "basal-acting insulins," or "basal insulins" refer to insulins administered to maintain a basal insulin level as part of an overall treatment regimen for treating a chronic condition such diabetes. Typically, a basal-acting insulin is formulated to maintain an approximately steady state insulin level by the controlled release of insulin when administered periodically (*e.g.* once or twice daily). Basal-acting insulins include crystalline insulins (*e.g.* NPH and Lente®, protamine insulin, surfen insulin), basal insulin analogs (insulin glargine, HOE 901, NovoSol Basal) and other chemical formulations of insulin (*e.g.* gum arabic, lecithin or oil suspensions) that retard the absorption rate of regular insulin. As used herein, the basal-acting insulins can include insulins that are typically understood as long-acting (typically reaching a relatively low peak concentration, while having a maximum duration of action over about 20-30 hours) or intermediate-acting (typically causing peak insulin concentrations at about 4-12 hours after administration).

As used herein, the terms "hyperglycemic condition" or "hyperglycemia" refer to an undesired elevation in blood glucose.

As used herein, the term "hypoglycemic condition" or "hypoglycemia" refers to an undesired drop in blood glucose.

As used herein, glycemic control or "controlling blood glucose levels" refers to the maintenance of blood glucose concentrations at a desired level, typically between 70-130 mg/dL or 90-110 mg/dL.

As used herein, a closed loop system is an integrated system for providing continuous glycemic control. Closed loop systems contain a mechanism for measuring blood glucose, a mechanism for delivering one or more compositions, including an insulin composition, and a mechanism for determining the amount of

insulin needed to be delivered to achieve glycemic control. Typically, therefore, closed loop systems contain a glucose sensor, an insulin delivery device, such as an insulin pump, and a controller that receives information from the glucose sensor and provides commands to the insulin delivery device. The commands can be generated by software in the controller. The software typically includes an algorithm to determine the amount of insulin required to be delivered to achieve glycemic control, based upon the blood glucose levels detected by the glucose sensor or anticipated by the user. An open loop system refers to similar devices, except that the devices do not automatically measure and respond to glucose levels.

As used herein, dosing regime refers to the amount of insulin administered and the frequency of administration. The dosing regime is a function of the disease or condition to be treated, and thus can vary.

As used herein, a hyaluronan degrading enzyme refers to an enzyme that catalyzes the cleavage of a hyaluronan polymer (also referred to as hyaluronic acid or HA) into smaller molecular weight fragments. Exemplary of hyaluronan degrading enzymes are hyaluronidases, and particular chondroitinases and lyases that have the ability to depolymerize hyaluronan. Exemplary chondroitinases that are hyaluronan degrading enzymes include, but are not limited to, chondroitin ABC lyase (also known as chondroitinase ABC), chondroitin AC lyase (also known as chondroitin sulfate lyase or chondroitin sulfate eliminase) and chondroitin C lyase. Chondroitin ABC lyase contains two enzymes, chondroitin-sulfate-ABC endolyase (EC 4.2.2.20) and chondroitin-sulfate-ABC exolyase (EC 4.2.2.21). An exemplary chondroitin-sulfate-ABC endolyases and chondroitin-sulfate-ABC exolyases include, but are not limited to, those from *Proteus vulgaris* and *Flavobacterium heparinum* (the *Proteus vulgaris* chondroitin-sulfate-ABC endolyase is set forth in SEQ ID NO:98; Sato *et al.* (1994) *Appl. Microbiol. Biotechnol.* 41(1):39-46). Exemplary chondroitinase AC enzymes from the bacteria include, but are not limited to, those from *Flavobacterium heparinum*, set forth in SEQ ID NO:99, *Victivallis vadensis*, set forth in SEQ ID NO:100 and *Arthrobacter aurescens* (Tkalec *et al.* (2000) *Applied and Environmental Microbiology* 66(1):29-35; Ernst *et al.* (1995) *Critical Reviews in Biochemistry and Molecular Biology* 30(5):387-444). Exemplary chondroitinase C enzymes from the bacteria include, but are not limited to, those from *Streptococcus* and *Flavobacterium*

(Hibi *et al.* (1989) *FEMS-Microbiol-Lett.* 48(2):121-4; Michelacci *et al.* (1976) *J. Biol. Chem.* 251:1154-8; Tsuda *et al.* (1999) *Eur. J. Biochem.* 262:127-133).

As used herein, hyaluronidase refers to a class of hyaluronan degrading enzymes. Hyaluronidases include bacterial hyaluronidases (EC 4.2.2.1 or EC 4.2.99.1), hyaluronidases from leeches, other parasites, and crustaceans (EC 3.2.1.36), and mammalian-type hyaluronidases (EC 3.2.1.35). Hyaluronidases include any of non-human origin including, but not limited to, murine, canine, feline, leporine, avian, bovine, ovine, porcine, equine, piscine, ranine, bacterial, and any from leeches, other parasites, and crustaceans. Exemplary non-human hyaluronidases include, hyaluronidases from cows (SEQ ID NOS:10, 11, 64 and BH55 (U.S. Pat. Nos. 5,747,027 and 5,827,721), yellow jacket wasp (SEQ ID NOS:12 and 13), honey bee (SEQ ID NO:14), white-face hornet (SEQ ID NO:15), paper wasp (SEQ ID NO:16), mouse (SEQ ID NOS:17-19, 32), pig (SEQ ID NOS:20-21), rat (SEQ ID NOS:22-24, 31), rabbit (SEQ ID NO:25), sheep (SEQ ID NOS:26, 27, 63 and 65), orangutan (SEQ ID NO:28), cynomolgus monkey (SEQ ID NO:29), guinea pig (SEQ ID NO:30), chimpanzee (SEQ ID NO:185), rhesus monkey (SEQ ID NO:186), *Arthrobacter sp.* (strain FB24) (SEQ ID NO:67), *Bdellovibrio bacteriovorus* (SEQ ID NO:68), *Propionibacterium acnes* (SEQ ID NO:69), *Streptococcus agalactiae* (SEQ ID NO:70); 18RS21 (SEQ ID NO:71); serotype Ia (SEQ ID NO:72); serotype III (SEQ ID NO:73), *Staphylococcus aureus* (strain COL) (SEQ ID NO:74); strain MRSA252 (SEQ ID NOS:75 and 76); strain MSSA476 (SEQ ID NO:77); strain NCTC 8325 (SEQ ID NO:78); strain bovine RF122 (SEQ ID NOS:79 and 80); strain USA300 (SEQ ID NO:81), *Streptococcus pneumoniae* (SEQ ID NO:82); strain ATCC BAA-255 / R6 (SEQ ID NO:83); serotype 2, strain D39 / NCTC 7466 (SEQ ID NO:84), *Streptococcus pyogenes* (serotype M1) (SEQ ID NO:85); serotype M2, strain MGAS10270 (SEQ ID NO:86); serotype M4, strain MGAS10750 (SEQ ID NO:87); serotype M6 (SEQ ID NO:88); serotype M12, strain MGAS2096 (SEQ ID NOS:89 and 90); serotype M12, strain MGAS9429 (SEQ ID NO:91); serotype M28 (SEQ ID NO:92); *Streptococcus suis* (SEQ ID NOS:93-95); *Vibrio fischeri* (strain ATCC 700601/ ES114 (SEQ ID NO:96)), and the *Streptomyces hyaluronolyticus* hyaluronidase enzyme, which is specific for hyaluronic acid and does not cleave chondroitin or chondroitin sulfate (Ohya, T. and Kaneko, Y. (1970) *Biochim. Biophys.*

Acta 198:607). Hyaluronidases also include those of human origin. Exemplary human hyaluronidases include HYAL1 (SEQ ID NO:36), HYAL2 (SEQ ID NO:37), HYAL3 (SEQ ID NO:38), HYAL4 (SEQ ID NO:39), and PH20 (SEQ ID NO:1). Also included amongst hyaluronidases are soluble hyaluronidases, including, ovine and bovine PH20, soluble human PH20 and soluble rHuPH20. Examples of commercially available bovine or ovine soluble hyaluronidases Vitrase® (ovine hyaluronidase) and Amphadase® (bovine hyaluronidase).

Reference to hyaluronan degrading enzyme, hyaluronidase or PH20 includes precursor hyaluronan degrading enzyme polypeptides and mature hyaluronan degrading enzyme polypeptides (such as those in which a signal sequence has been removed), truncated forms thereof that have activity (e.g. C-terminally truncated forms), and includes allelic variants and species variants, variants encoded by splice variants, and other variants, including polypeptides that have at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the precursor polypeptides set forth in SEQ ID NOS: 1 and 10-48, 63-65, 67-100, or the mature form thereof . For example, reference to a hyaluronan-degrading enzyme (e.g. PH20) includes the mature human PH20 set forth in SEQ ID NO:2 and truncated forms thereof that have activity, and includes allelic variants and species variants, variants encoded by splice variants and other variants including polypeptides that have at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:2. For example, reference to hyaluronan degrading enzyme also includes the human PH20 precursor polypeptide variants set forth in SEQ ID NOS:50-51. Hyaluronan degrading enzymes also include those that contain chemical or posttranslational modifications and those that do not contain chemical or posttranslational modifications. Such modifications include, but are not limited to, pegylation, albumination, glycosylation, farnesylation, carboxylation, hydroxylation, phosphorylation, and other polypeptide modifications known in the art.

As used herein, PH20 refers to a type of hyaluronidase that occurs in sperm and is neutral-active. PH-20 occurs on the sperm surface, and in the lysosome-derived acrosome, where it is bound to the inner acrosomal membrane. PH20 includes those of any origin including, but not limited to, human, chimpanzee, Cynomolgus

monkey, Rhesus monkey, murine, bovine, ovine, guinea pig, rabbit and rat origin. Exemplary PH20 proteins include, but are not limited to, human (precursor polypeptide set forth in SEQ ID NO:1, mature polypeptide set forth in SEQ ID NO: 2), bovine (SEQ ID NOS: 11 and 64), rabbit (SEQ ID NO: 25), ovine PH20 (SEQ ID NOS: 27, 63 and 65), cynomolgus monkey (SEQ ID NO: 29), guinea pig (SEQ ID NO: 30), rat (SEQ ID NO: 31), mouse (SEQ ID NO: 32), chimpanzee (SEQ ID NO: 185) and rhesus monkey (SEQ ID NO:186) PH20 polypeptides. Reference to PH20 includes precursor PH20 polypeptides and mature PH20 polypeptides (such as those in which a signal sequence has been removed), truncated forms thereof that have activity, and includes allelic variants and species variants, variants encoded by splice variants, and other variants, including polypeptides that have at least 40%, 45%, 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the precursor polypeptides set forth in SEQ ID NO:1, 10, 25, 27, 30-31, 63-65, 185-186, or the mature forms thereof. PH20 polypeptides also include those that contain chemical or posttranslational modifications and those that do not contain chemical or posttranslational modifications. Such modifications include, but are not limited to, PEGylation, albumination, glycosylation, farnesylation, carboxylation, hydroxylation, phosphorylation, and other polypeptide modifications known in the art. Examples of commercially available bovine or ovine soluble hyaluronidases are Vitrase® hyaluronidase (ovine hyaluronidase) and Amphadase® hyaluronidase (bovine hyaluronidase).

As used herein, a soluble hyaluronidase refers to a polypeptide that is secreted from cells and is not membrane-anchored or associated, and hence can be characterized by its solubility under physiologic conditions. Soluble hyaluronidases can be distinguished, for example, by its partitioning into the aqueous phase of a Triton X-114 solution warmed to 37 °C (Bordier *et al.*, (1981) *J. Biol. Chem.*, 256:1604-7). Membrane-anchored, such as lipid anchored hyaluronidases, will partition into the detergent rich phase, but will partition into the detergent-poor or aqueous phase following treatment with Phospholipase-C. Included among soluble hyaluronidases are membrane anchored hyaluronidases in which one or more regions associated with anchoring of the hyaluronidase to the membrane has been removed or modified, where the soluble form retains hyaluronidase activity. Hence, soluble

hyaluronidase, such as soluble PH20 polypeptides, include truncated forms thereof, for example, C-terminally truncated forms in which one or more amino acids of the glycosylphosphatidylinositol (GPI) anchor is lacking. Soluble hyaluronidases include recombinant soluble hyaluronidases and those contained in or purified from natural sources, such as, for example, testes extracts from sheep or cows. Exemplary of such soluble hyaluronidases are soluble human PH20. Other soluble hyaluronidases include ovine (SEQ ID NOS:27, 63, 65) and bovine (SEQ ID NOS:11, 64) PH20.

As used herein, soluble human PH20 or sHuPH20 include mature PH20 polypeptides lacking all or a portion of the glycosylphosphatidylinositol (GPI) attachment site at the C-terminus such that upon expression, the polypeptides are not associated with the membrane of a host cell in which they are produced so that they are secreted and, thus, soluble in the cell culture medium. Hence, soluble human PH20 includes C-terminal truncated human PH20 polypeptides. Exemplary soluble or C-terminal truncated PH20 polypeptides include mature polypeptides having an amino acids sequence set forth in any one of SEQ ID NOS: 4-9, 47-48, 234-254, and 267-273, or a polypeptide that exhibits at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 4-9, 47-48, 234-254, and 267-273. Exemplary sHuPH20 polypeptides include mature polypeptides having an amino acid sequence set forth in any one of SEQ ID NOS:4-9 and 47-48. The precursor polypeptides for such exemplary sHuPH20 polypeptides include a signal sequence. Exemplary of the precursors are those set forth in SEQ ID NOS:3 and 40-46, each of which contains a 35 amino acid signal sequence at amino acid positions 1-35. Soluble HuPH20 polypeptides also include those degraded during or after the production and purification methods described herein.

As used herein, a recombinant human PH20 referred to as rHuPH20 refers to a secreted soluble form of human PH20 that is recombinantly expressed in Chinese Hamster Ovary (CHO) cells. Soluble rHuPH20 is the product produced by nucleic acid that encodes a signal sequence, such as the native signal sequence, and includes nucleic acid that encodes amino acids 36-482 and for which an exemplary sequence, including the nucleic acid encoding the native signal sequence is set forth in SEQ ID NO:49. Also included are DNA molecules that are allelic variants thereof and other

soluble variants. The nucleic acid encoding soluble rHuPH20 is expressed in CHO cells, which secrete the mature polypeptide. As produced in the culture medium, there is heterogeneity at the C-terminus so that the product includes a mixture of species that can include any one or more of SEQ ID NOS: 4-9 in various abundance. Corresponding allelic variants and other variants also are included, including those corresponding to the precursor human PH20 polypeptides set forth in SEQ ID NOS:50-51. Other variants can have 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity with any of SEQ ID NOS:4-9 and 47-48 as long they retain a hyaluronidase activity and are soluble.

As used herein, "super fast-acting insulin composition" refers to an insulin composition containing a fast-acting insulin, particularly a rapid acting insulin analog, such as an insulin analog with a more rapid onset insulin, and a hyaluronan degrading enzyme (such as a, but not limited to, rHuPH20 preparations), such that the insulin composition, over the first forty minutes following parenteral administration to a subject, provides a cumulative systemic insulin exposure in the subject that is greater than the cumulative systemic insulin exposure provided to the subject over the same period after administering the same dosage of a fast-acting insulin, by the same route, in the absence of the hyaluronan degrading enzyme. The super fast-acting insulin composition optionally can include a basal-acting insulin.

As used herein, a formulation refers to a composition containing at least one active or pharmaceutical agent and one or more excipients.

As used herein, a co-formulation refers to a composition containing two or more active or pharmaceutical agents and one or more excipients. For example, a co-formulation of a fast-acting insulin and a hyaluronan degrading enzyme contains a fast-acting insulin, a hyaluronan degrading enzyme, and one or more excipients.

As used herein, a composition is said to be stable under defined conditions if the active ingredients therein retains at least a requisite level of activity and/or purity and/or potency or recovery compared to the initial activity and/or purity and/or potency or recovery. For purposes herein, a composition is stable if it retains at least 50% of the hyaluronan-degrading enzyme activity and/or if it retains at least 90% of insulin potency or recovery and/or at least 90% of the insulin purity.

As used herein, a stable co-formulation, which contains at least two active ingredients, is stable if each active ingredient retains at least the requisite level of activity and/or purity and/or potency or recovery compared to the initial activity and/or purity and/or potency or recovery. For purposes herein, a coformulation is stable if it retains at least 50% of the hyaluronan-degrading enzyme activity and if it retains at least 90% of insulin potency or recovery and/or at least 90% of the insulin purity.

As used herein, defined conditions refer to conditions of storage and/or use.

As used herein, "storage" means that a formulation is not immediately administered to a subject once prepared, but is kept for a period of time under particular conditions (*e.g.* particular temperature; time, liquid or lyophilized form) prior to use. For example, a liquid formulation can be kept for days, weeks, months or years, prior to administration to a subject under varied temperatures such as refrigerated (0° to 10° C, such as 2° C to 8° C), room temperature (*e.g.* temperature up to 32° C, such as 18° C to about or at 32° C), or elevated temperature (*e.g.*, 30° C to 42° C, such as 32° C to 37° C or 35° C to 37° C).

As used herein, "use" with reference to a condition associated with stability refers to the act of employing the formulation for a specific purpose. Particular applications can influence the activity or properties of a protein or agent. For example, certain applications can require that the formulation is subjected to certain temperatures for certain time periods, is subjected to fluctuations in temperature and or is subjected to agitation, shaking, stirring or other similar motion that can affect the stability (e.g. activity and/or solubility) of the active agents. Exemplary of a condition is continuous infusion methods, whereby active agents are continuously infused to a subject from a user-associated pump or infuser over a course of several days. Such a condition can be associated with agitation and fluctuations in temperature.

As used herein, defined conditions for storage or use under which stability is measured includes temperature conditions, time of storage conditions and/or use conditions. For example, defined temperature conditions include low or refrigerated temperatures of 2° C to 8° C, ambient temperatures of 20° C to 30° C or elevated temperatures of 32° C to 40° C. In another example, defined time conditions refers to

the length of storage under varied temperature conditions, such as storage for days (at least 3 days, 4 days, 5 days, 6 days or 7 days), weeks (at least one week, at least two weeks, at least three weeks or at least for weeks) or months (at least 1 months, 2 months, 3 months, 4 months, 5 months, 6 months, 12 months, 18 months, 24 months or more). In a further example, defined use conditions refers to conditions that disturb or alter the composition mixture, such as conditions of agitation.

As used herein, a single dosage formulation refers to a formulation or co-formulation for direct administration. Generally, a single dosage formulation is a formulation that contains a single dose of therapeutic agent for direct administration. Single dosage formulations generally do not contain any preservatives.

As used herein, a multi-dose formulation refers to a formulation that contains multiple doses of a therapeutic agent and that can be directly administered to provide several single doses of the therapeutic agent. The doses can be administered over the course of minutes, hours, weeks, days or months. Multidose formulations can allow dose adjustment, dose-pooling and/or dose-splitting. Because multi-dose formulations are used over time, they generally contain one or more preservatives to prevent microbial growth. Multi-dose formulations can be formulated for injection or infusion (*e.g.* continuous infusion).

As used herein, a "stable multiple dose injection (MDI) co-formulation" refers to a stable co-formulation that is stable for at least 6 months at a temperature from or from about 2°C to 8°C and/or for at least 14 days at a temperature from or from about 20°C to 30°C, such that the requisite level of activity and/or purity and/or potency or recovery is retained over the defined time and temperature compared to the initial activity and/or purity and/or potency or recovery. For example, a stable multiple dose injection formulation retains at least 50% of the hyaluronan-degrading enzyme activity and at least 90% of insulin potency or recovery and/or at least 90% of the insulin purity for at least 6 months at a temperature from or from about 2°C to 8°C and/or for at least 14 days at a temperature from or from about 20°C to 30°C.

As used herein, a "stable continuous insulin infusion formulation" refers to a stable co-formulation that is stable for at least 3 days at a temperature from or from about 32°C to 40°C, such that the requisite level of activity and/or purity and/or potency or recovery is retained over the defined time and temperature compared to the

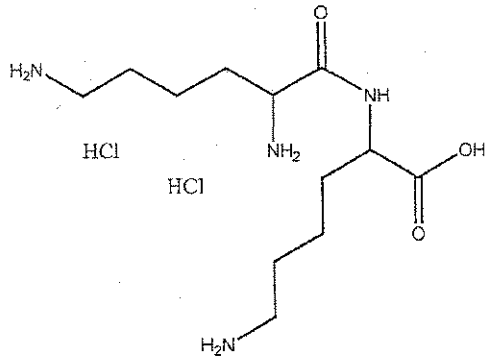
initial activity and/or purity and/or potency or recovery. For example, a stable continuous insulin infusion formulation retains at least 50% of the hyaluronan-degrading enzyme activity and at least 90% of insulin potency or recovery and/or at least 90% of the insulin purity for at least 3 days at a temperature from or from about 32°C to 40°C.

As used herein, continuous subcutaneous insulin infusion therapy (CSII) refers to an insulin dosage regimen whereby insulin is administered at programmed rates over a course of several days from a small infuser or pump subcutaneously via an infusion set connected to the pump. Typically, CSII therapy continues for 2-4 days before the infusion set and pump reservoir must be replaced. The treatment combines continuous baseline insulin release (basal rate) and additional insulin bolus doses before meals and in response to high glycaemia values (*i.e.* correction bolus). CSII therapy generally uses a battery powered syringe driver, insulin pump or other similar device to deliver a fast-acting insulin, in particular an insulin analog, according to the dosage regimen. Generally, scheduling of continuous baseline insulin release is set by a physician for each patient. Bolus doses are determined based on prandial needs and glycemic responses. Hence, CSII therapy is patient specific. It is well within the level of a skilled physician and patient to determine the particular insulin dosage regimen for each patient depending on the needs of the patients and other patient-specific parameters such as weight, age, exercise, diet and clinical symptoms of the patient.

As used herein, a stabilizing agent refers to compound added to the formulation to protect either the hyaluronan degrading enzyme or insulin or both from degradation, such as under the conditions of salt, pH and temperature at which the co-formulations herein are stored or used. Thus, included are agents that prevent proteins from degradation from other components in the compositions. protein stabilizing agents Exemplary of such agents are amino acids, amino acid derivatives, amines, sugars, polyols, salts and buffers, surfactants, inhibitors or substrates and other agents as described herein.

As used herein, Lysyl lysine (Lys-Lys or dilysine) refers to a Lys-Lys dipeptide, salt, derivative, analogue or mimetic thereof. For example, reference to Lys-Lys includes salts thereof, such as the dihydrochloride salt of Lys-Lys, *i.e.* Lys-

Lys dihydrochloride (or dilysine dihydrochloride; H-Lys-Lys-OH HCl;). Lys-Lys dihydrochloride is represented by the following formula:



Reference to Lys-Lys also includes derivatives thereof, such as derivatives containing a protected groups, such as an Fmoc-, Noc- or Cbz. Examples of commercially available Lys-Lys dihydrochlorides include, but are not limited to, those available from Sigma Aldrich, RnD Chem., MP Bio, Tetrahedron Scientific Inc., and Crescent Chemical Company. An exemplary commercially available Lys-Lys dihydrochloride is the Lys-Lys dihydrochloride available from Sigma Aldrich (Product No. L5502) or RnD Chem (Product No. G-2675).

As used herein, an antimicrobial effectiveness test demonstrates the effectiveness of the preservative system in a product. A product is inoculated with a controlled quantity of specific organisms. The test then compares the level of microorganisms found on a control sample versus the test sample over a period of 28 days. Parameters for performing an antimicrobial effectiveness test are known to one of skill in the art as described herein.

As used herein, an anti-microbially or anti-microbial effective amount of a preservative refers to an amount of the preservative that kills or inhibits the propagation of microbial organisms in a sample that may be introduced from storage or use. For example, for multiple-dose containers, an anti-microbially effective amount of a preservative inhibits the growth of microorganisms that may be introduced from repeatedly withdrawing individual doses. USP and EP (EPA and EPB) have anti-microbial requirements that determine preservative effectiveness, and that vary in stringency. For example, an anti-microbial effective amount of a preservative is an amount such that at least a 1.0 $\log_{10}$ unit reduction in bacterial organisms occurs at 7 days following inoculation in an antimicrobial preservative

effectiveness test (APET). In a particular example, an anti-microbial effective amount of a preservative is an amount such that at least a 1.0 $\log_{10}$ unit reduction in bacterial organisms occurs at 7 days following inoculation, at least a 3.0 $\log_{10}$ unit reduction of bacterial organisms occurs at 14 days following inoculation at least no further increase in bacterial organisms occurs after 28 days following inoculation; and at least no increase in fungal organisms occurs after 7 days following inoculation. In a further example, an anti-microbial effective amount of a preservative is an amount such that at least a 1.0 $\log_{10}$ unit reduction of bacterial organisms occurs at 24 hours following inoculation, at least a 3.0 $\log_{10}$ unit reduction of bacterial organisms occurs at 7 days following inoculation, no further increase in bacterial organisms occurs after 28 days following inoculation, at least a 1.0 $\log_{10}$ unit reduction of fungal organisms occurs at 14 days following inoculation, and at least no further increase in fungal organisms occurs after 28 days following inoculation. In an additional example, an anti-microbial effective amount of a preservative is an amount such that at least a 2.0 $\log_{10}$ unit reduction of bacterial organisms at 6 hours following inoculation, at least a 3.0 $\log_{10}$ unit reduction of bacterial organisms occurs at 24 hours following inoculation, no recovery of bacterial organisms occurs after 28 days following inoculation of the composition with the microbial inoculum, at least a 2.0 $\log_{10}$ unit reduction of fungal organisms occurs at 7 days following inoculation, and at least no further increase in fungal organisms occurs after 28 days following inoculation.

As used herein, the "excipient" refers to a compound in a formulation of an active agent that does not provide the biological effect of the active agent when administered in the absence of the active agent. Exemplary excipients include, but are not limited to, salts, buffers, stabilizers, tonicity modifiers, metals, polymers, surfactants, preservatives, amino acids and sugars.

As used herein, a "buffer" refers to a substance, generally a solution, that can keep its pH constant, despite the addition of strong acids or strong bases and external influences of temperature, pressure, volume or redox potential. Buffer prevents change in the concentration of another chemical substance, e.g. proton donor and acceptor systems that prevent marked changes in hydrogen ion concentration (pH). The pH values of all buffers are temperature and concentration dependent. The

choice of buffer to maintain a pH value or range can be empirically determined by one of skill in the art based on the known buffering capacity of known buffers. Exemplary buffers include but are not limited to, bicarbonate buffer, cacodylate buffer, phosphate buffer or Tris buffer. For example, Tris buffer (tromethamine) is an amine based buffer that has a pKa of 8.06 and has an effective pH range between 7.9 and 9.2. For Tris buffers, pH increases about 0.03 unit per °C temperature decrease, and decreases 0.03 to 0.05 unit per ten-fold dilution.

As used herein, activity refers to a functional activity or activities of a polypeptide or portion thereof associated with a full-length (complete) protein. Functional activities include, but are not limited to, catalytic or enzymatic activity, antigenicity (ability to bind or compete with a polypeptide for binding to an anti-polypeptide antibody), immunogenicity, ability to form multimers, and the ability to specifically bind to a receptor or ligand for the polypeptide.

As used herein, hyaluronidase activity refers to the ability to enzymatically catalyze the cleavage of hyaluronic acid. The United States Pharmacopeia (USP) XXII assay for hyaluronidase determines hyaluronidase activity indirectly by measuring the amount of higher molecular weight hyaluronic acid, or hyaluronan, (HA) substrate remaining after the enzyme is allowed to react with the HA for 30 min at 37 °C (USP XXII-NF XVII (1990) 644-645 United States Pharmacopeia Convention, Inc, Rockville, MD). A Reference Standard solution can be used in an assay to ascertain the relative activity, in units, of any hyaluronidase. *In vitro* assays to determine the hyaluronidase activity of hyaluronidases, such as soluble rHuPH20, are known in the art and described herein. Exemplary assays include the microturbidity assay described below (*see e.g.* Example 3) that measures cleavage of hyaluronic acid by hyaluronidase indirectly by detecting the insoluble precipitate formed when the uncleaved hyaluronic acid binds with serum albumin. Reference Standards can be used, for example, to generate a standard curve to determine the activity in Units of the hyaluronidase being tested.

As used herein, "functionally equivalent amount" or grammatical variations thereof, with reference to a hyaluronan degrading enzyme, refers to the amount of hyaluronan degrading enzyme that achieves the same effect as an amount (such as a known number of Units of hyaluronidase activity) of a reference enzyme, such as a

hyaluronidase. For example, the activity of any hyaluronan degrading enzyme can be compared to the activity of rHuPH20 to determine the functionally equivalent amount of a hyaluronan degrading enzyme that would achieve the same effect as a known amount of rHuPH20. For example, the ability of a hyaluronan degrading enzyme to act as a spreading or diffusing agent can be assessed by injecting it into the lateral skin of mice with trypan blue (*see e.g.* U.S. Pat. Publication No. 20050260186), and the amount of hyaluronan degrading enzyme required to achieve the same amount of diffusion as, for example, 100 units of a Hyaluronidase Reference Standard, can be determined. The amount of hyaluronan degrading enzyme required is, therefore, functionally equivalent to 100 units. In another example, the ability of a hyaluronan degrading enzyme to increase the level and rate of absorption of a co-administered insulin can be assessed in human subjects, such as described below in Example 1, and the amount of hyaluronan degrading enzyme required to achieve the same increase in the level and rate of absorption of insulin as, for example, the administered quantity of rHuPH20, can be determined (such as by assessing the maximum insulin concentration in the blood ($C_{max,}$) the time required to achieve maximum insulin concentration in the blood (t_{max}) and the cumulative systemic insulin exposure over e given period of time (AUC).

As used herein, the residues of naturally occurring α-amino acids are the residues of those 20 α-amino acids found in nature which are incorporated into protein by the specific recognition of the charged tRNA molecule with its cognate mRNA codon in humans.

As used herein, nucleic acids include DNA, RNA and analogs thereof, including peptide nucleic acids (PNA) and mixtures thereof. Nucleic acids can be single or double-stranded. When referring to probes or primers, which are optionally labeled, such as with a detectable label, such as a fluorescent or radiolabel, single-stranded molecules are contemplated. Such molecules are typically of a length such that their target is statistically unique or of low copy number (typically less than 5, generally less than 3) for probing or priming a library. Generally a probe or primer contains at least 14, 16 or 30 contiguous nucleotides of sequence complementary to or identical to a gene of interest. Probes and primers can be 10, 20, 30, 50, 100 or more nucleic acids long.

As used herein, a peptide refers to a polypeptide that is greater than or equal to two amino acids in length, and less than or equal to 40 amino acids in length.

As used herein, the amino acids that occur in the various sequences of amino acids provided herein are identified according to their known, three-letter or one-letter abbreviations (Table 1). The nucleotides that occur in the various nucleic acid fragments are designated with the standard single-letter designations used routinely in the art.

As used herein, an "amino acid" is an organic compound containing an amino group and a carboxylic acid group. A polypeptide contains two or more amino acids. For purposes herein, amino acids include the twenty naturally-occurring amino acids, non-natural amino acids and amino acid analogs (*i.e.*, amino acids wherein the α-carbon has a side chain).

As used herein, "amino acid residue" refers to an amino acid formed upon chemical digestion (hydrolysis) of a polypeptide at its peptide linkages. The amino acid residues described herein are presumed to be in the "L" isomeric form. Residues in the "D" isomeric form, which are so designated, can be substituted for any L-amino acid residue as long as the desired functional property is retained by the polypeptide. NH_2 refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxyl terminus of a polypeptide. In keeping with standard polypeptide nomenclature described in *J. Biol. Chem.*, 243: 3557-3559 (1968), and adopted 37 C.F.R. §§ 1.821-1.822, abbreviations for amino acid residues are shown in Table 1:

Table 1 – Table of Correspondence

SYMBOL		
1-Letter	**3-Letter**	**AMINO ACID**
Y	Tyr	Tyrosine
G	Gly	Glycine
F	Phe	Phenylalanine
M	Met	Methionine
A	Ala	Alanine
S	Ser	Serine
I	Ile	Isoleucine
L	Leu	Leucine
T	Thr	Threonine
V	Val	Valine
P	Pro	proline
K	Lys	Lysine

SYMBOL		
1-Letter	3-Letter	AMINO ACID
H	His	Histidine
Q	Gln	Glutamine
E	Glu	glutamic acid
Z	Glx	Glu and/or Gln
W	Trp	Tryptophan
R	Arg	Arginine
D	Asp	aspartic acid
N	Asn	asparagine
B	Asx	Asn and/or Asp
C	Cys	Cysteine
X	Xaa	Unknown or other

All amino acid residue sequences represented herein by formulae have a left to right orientation in the conventional direction of amino-terminus to carboxyl-terminus. In addition, the phrase "amino acid residue" is broadly defined to include the amino acids listed in the Table of Correspondence (Table 1) and modified and unusual amino acids, such as those referred to in 37 C.F.R. §§ 1.821-1.822, and incorporated herein by reference. Furthermore, a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino acid residues, to an amino-terminal group such as NH_2 or to a carboxyl-terminal group such as COOH.

As used herein, "naturally occurring amino acids" refer to the 20 L-amino acids that occur in polypeptides.

As used herein, "non-natural amino acid" refers to an organic compound that has a structure similar to a natural amino acid but has been modified structurally to mimic the structure and reactivity of a natural amino acid. Non-naturally occurring amino acids thus include, for example, amino acids or analogs of amino acids other than the 20 naturally-occurring amino acids and include, but are not limited to, the D-isostereomers of amino acids. Exemplary non-natural amino acids are described herein and are known to those of skill in the art.

As used herein, a DNA construct is a single- or double-stranded, linear or circular DNA molecule that contains segments of DNA combined and juxtaposed in a manner not found in nature. DNA constructs exist as a result of human manipulation, and include clones and other copies of manipulated molecules.

As used herein, a DNA segment is a portion of a larger DNA molecule having specified attributes. For example, a DNA segment encoding a specified polypeptide is a portion of a longer DNA molecule, such as a plasmid or plasmid fragment, which, when read from the 5' to 3' direction, encodes the sequence of amino acids of the specified polypeptide.

As used herein, the term polynucleotide means a single- or double-stranded polymer of deoxyribonucleotides or ribonucleotide bases read from the 5' to the 3' end. Polynucleotides include RNA and DNA, and can be isolated from natural sources, synthesized *in vitro*, or prepared from a combination of natural and synthetic molecules. The length of a polynucleotide molecule is given herein in terms of nucleotides (abbreviated "nt") or base pairs (abbreviated "bp"). The term nucleotides is used for single- and double-stranded molecules where the context permits. When the term is applied to double-stranded molecules it is used to denote overall length and will be understood to be equivalent to the term base pairs. It will be recognized by those skilled in the art that the two strands of a double-stranded polynucleotide can differ slightly in length and that the ends thereof can be staggered; thus all nucleotides within a double-stranded polynucleotide molecule may not be paired. Such unpaired ends will, in general, not exceed 20 nucleotides in length.

As used herein, "similarity" between two proteins or nucleic acids refers to the relatedness between the sequence of amino acids of the proteins or the nucleotide sequences of the nucleic acids. Similarity can be based on the degree of identity and/or homology of sequences of residues and the residues contained therein. Methods for assessing the degree of similarity between proteins or nucleic acids are known to those of skill in the art. For example, in one method of assessing sequence similarity, two amino acid or nucleotide sequences are aligned in a manner that yields a maximal level of identity between the sequences.

As used herein, "identity" refers to the extent to which the amino acid or nucleotide sequences are invariant. Alignment of amino acid sequences, and to some extent nucleotide sequences, also can take into account conservative differences and/or frequent substitutions in amino acids (or nucleotides). Conservative differences are those that preserve the physico-chemical properties of the residues involved. Alignments can be global (alignment of the compared sequences over the

entire length of the sequences and including all residues) or local (the alignment of a portion of the sequences that includes only the most similar region or regions). "Identity" per se has an art-recognized meaning and can be calculated using published techniques. (See, *e.g.*: *Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exists a number of methods to measure identity between two polynucleotide or polypeptides, the term "identity" is well known to skilled artisans (Carillo, H. & Lipton, D., *SIAM J Applied Math 48*:1073 (1988)).

As used herein, homologous (with respect to nucleic acid and/or amino acid sequences) means about greater than or equal to 25% sequence homology, typically greater than or equal to 25%, 40%, 50%, 60%, 70%, 80%, 85%, 90% or 95% sequence homology; the precise percentage can be specified if necessary. For purposes herein the terms "homology" and "identity" are often used interchangeably, unless otherwise indicated. In general, for determination of the percentage homology or identity, sequences are aligned so that the highest order match is obtained (see, *e.g.*: *Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data, Part I*, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; Carillo *et al.* (1988) *SIAM J Applied Math 48*:1073). By sequence homology, the number of conserved amino acids is determined by standard alignment algorithms programs, and can be used with default gap penalties established by each supplier. Substantially homologous nucleic acid molecules would hybridize typically at moderate stringency or at high stringency all along the length of the nucleic acid of interest. Also contemplated are nucleic acid molecules that contain degenerate codons in place of codons in the hybridizing nucleic acid molecule.

Whether any two molecules have nucleotide sequences or amino acid sequences that are at least 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% "identical" or "homologous" can be determined using known computer algorithms such as the "FASTA" program, using for example, the default parameters as in Pearson *et al.* (1988) *Proc. Natl. Acad. Sci. USA 85*:2444 (other programs include the GCG program package (Devereux, J., *et al., Nucleic Acids Research 12(I)*:387 (1984)), BLASTP, BLASTN, FASTA (Atschul, S.F., *et al., J Molec Biol 215*:403 (1990)); Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo *et al.* (1988) *SIAM J Applied Math 48*:1073). For example, the BLAST function of the National Center for Biotechnology Information database can be used to determine identity. Other commercially or publicly available programs include, DNAStar "MegAlign" program (Madison, WI) and the University of Wisconsin Genetics Computer Group (UWG) "Gap" program (Madison WI). Percent homology or identity of proteins and/or nucleic acid molecules can be determined, for example, by comparing sequence information using a GAP computer program (*e.g.*, Needleman *et al.* (1970) *J. Mol. Biol.* 48:443, as revised by Smith and Waterman (1981) *Adv. Appl. Math.* 2:482). Briefly, the GAP program defines similarity as the number of aligned symbols (*i.e.*, nucleotides or amino acids), which are similar, divided by the total number of symbols in the shorter of the two sequences. Default parameters for the GAP program can include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) and the weighted comparison matrix of Gribskov *et al.* (1986) *Nucl. Acids Res.* 14:6745, as described by Schwartz and Dayhoff, eds., *ATLAS OF PROTEIN SEQUENCE AND STRUCTURE*, National Biomedical Research Foundation, pp. 353-358 (1979); (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps.

Therefore, as used herein, the term "identity" or "homology" represents a comparison between a test and a reference polypeptide or polynucleotide. As used herein, the term at least "90% identical to" refers to percent identities from 90 to 100% relative to the reference nucleic acid or amino acid sequence of the polypeptide. Identity at a level of 90% or more is indicative of the fact that, assuming for exemplification purposes a test and reference polypeptide length of 100 amino

acids are compared. No more than 10% (*i.e.*, 10 out of 100) of the amino acids in the test polypeptide differs from that of the reference polypeptide. Similar comparisons can be made between test and reference polynucleotides. Such differences can be represented as point mutations randomly distributed over the entire length of a polypeptide or they can be clustered in one or more locations of varying length up to the maximum allowable, *e.g.* 10/100 amino acid difference (approximately 90% identity). Differences are defined as nucleic acid or amino acid substitutions, insertions or deletions. At the level of homologies or identities above about 85-90%, the result should be independent of the program and gap parameters set; such high levels of identity can be assessed readily, often by manual alignment without relying on software.

As used herein, an aligned sequence refers to the use of homology (similarity and/or identity) to align corresponding positions in a sequence of nucleotides or amino acids. Typically, two or more sequences that are related by 50% or more identity are aligned. An aligned set of sequences refers to 2 or more sequences that are aligned at corresponding positions and can include aligning sequences derived from RNAs, such as ESTs and other cDNAs, aligned with genomic DNA sequence.

As used herein, substantially identical to a product means sufficiently similar so that the property of interest is sufficiently unchanged so that the substantially identical product can be used in place of the product.

As used herein, it also is understood that the terms "substantially identical" or "similar" varies with the context as understood by those skilled in the relevant art.

As used herein, an allelic variant or allelic variation references any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and can result in phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or can encode polypeptides having altered amino acid sequence. The term "allelic variant" also is used herein to denote a protein encoded by an allelic variant of a gene. Typically the reference form of the gene encodes a wildtype form and/or predominant form of a polypeptide from a population or single reference member of a species. Typically, allelic variants, which include variants between and among species typically have at least 80%, 90% or greater amino acid identity with a

wildtype and/or predominant form from the same species; the degree of identity depends upon the gene and whether comparison is interspecies or intraspecies. Generally, intraspecies allelic variants have at least about 80%, 85%, 90% or 95% identity or greater with a wildtype and/or predominant form, including 96%, 97%, 98%, 99% or greater identity with a wildtype and/or predominant form of a polypeptide. Reference to an allelic variant herein generally refers to variations in proteins among members of the same species.

As used herein, "allele," which is used interchangeably herein with "allelic variant" refers to alternative forms of a gene or portions thereof. Alleles occupy the same locus or position on homologous chromosomes. When a subject has two identical alleles of a gene, the subject is said to be homozygous for that gene or allele. When a subject has two different alleles of a gene, the subject is said to be heterozygous for the gene. Alleles of a specific gene can differ from each other in a single nucleotide or several nucleotides, and can include modifications such as substitutions, deletions and insertions of nucleotides. An allele of a gene also can be a form of a gene containing a mutation.

As used herein, species variants refer to variants in polypeptides among different species, including different mammalian species, such as mouse and human.

As used herein, modification is in reference to modification of a sequence of amino acids of a polypeptide or a sequence of nucleotides in a nucleic acid molecule and includes deletions, insertions, and replacements of amino acids and nucleotides, respectively. Methods of modifying a polypeptide are routine to those of skill in the art, such as by using recombinant DNA methodologies.

As used herein, isolated or purified polypeptide or protein or biologically-active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue from which the protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. Preparations can be determined to be substantially free if they appear free of readily detectable impurities as determined by standard methods of analysis, such as thin layer chromatography (TLC), gel electrophoresis and high performance liquid chromatography (HPLC), used by those of skill in the art to assess such purity, or sufficiently pure such that further purification would not detectably alter the physical

and chemical properties, such as enzymatic and biological activities, of the substance. Methods for purification of the compounds to produce substantially chemically pure compounds are known to those of skill in the art. A substantially chemically pure compound, however, can be a mixture of stereoisomers. In such instances, further purification might increase the specific activity of the compound.

The term substantially free of cellular material includes preparations of proteins in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly-produced. In one embodiment, the term substantially free of cellular material includes preparations of enzyme proteins having less that about 30% (by dry weight) of non-enzyme proteins (also referred to herein as a contaminating protein), generally less than about 20% of non-enzyme proteins or 10% of non-enzyme proteins or less that about 5% of non-enzyme proteins. When the enzyme protein is recombinantly produced, it also is substantially free of culture medium, *i.e.*, culture medium represents less than about or at 20%, 10% or 5% of the volume of the enzyme protein preparation.

As used herein, the term substantially free of chemical precursors or other chemicals includes preparations of enzyme proteins in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. The term includes preparations of enzyme proteins having less than about 30% (by dry weight), 20%, 10%, 5% or less of chemical precursors or non-enzyme chemicals or components.

As used herein, synthetic, with reference to, for example, a synthetic nucleic acid molecule or a synthetic gene or a synthetic peptide refers to a nucleic acid molecule or polypeptide molecule that is produced by recombinant methods and/or by chemical synthesis methods.

As used herein, production by recombinant means by using recombinant DNA methods means the use of the well known methods of molecular biology for expressing proteins encoded by cloned DNA.

As used herein, vector (or plasmid) refers to discrete elements that are used to introduce a heterologous nucleic acid into cells for either expression or replication thereof. The vectors typically remain episomal, but can be designed to effect integration of a gene or portion thereof into a chromosome of the genome. Also

contemplated are vectors that are artificial chromosomes, such as yeast artificial chromosomes and mammalian artificial chromosomes. Selection and use of such vehicles are well known to those of skill in the art.

As used herein, an expression vector includes vectors capable of expressing DNA that is operatively linked with regulatory sequences, such as promoter regions, that are capable of effecting expression of such DNA fragments. Such additional segments can include promoter and terminator sequences, and optionally can include one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal, and the like. Expression vectors are generally derived from plasmid or viral DNA, or can contain elements of both. Thus, an expression vector refers to a recombinant DNA or RNA construct, such as a plasmid, a phage, recombinant virus or other vector that, upon introduction into an appropriate host cell, results in expression of the cloned DNA. Appropriate expression vectors are well known to those of skill in the art and include those that are replicable in eukaryotic cells and/or prokaryotic cells and those that remain episomal or those which integrate into the host cell genome.

As used herein, vector also includes "virus vectors" or "viral vectors." Viral vectors are engineered viruses that are operatively linked to exogenous genes to transfer (as vehicles or shuttles) the exogenous genes into cells.

As used herein, operably or operatively linked when referring to DNA segments means that the segments are arranged so that they function in concert for their intended purposes, *e.g.*, transcription initiates downstream of the promoter and upstream of any transcribed sequences. The promoter is usually the domain to which the transcriptional machinery binds to initiate transcription and proceeds through the coding segment to the terminator.

As used herein, the term assessing is intended to include quantitative and qualitative determination in the sense of obtaining an absolute value for the activity of a protease, or a domain thereof, present in the sample, and also of obtaining an index, ratio, percentage, visual or other value indicative of the level of the activity. Assessment can be direct or indirect and the chemical species actually detected need not of course be the proteolysis product itself but can for example be a derivative thereof or some further substance. For example, detection of a cleavage product of a

complement protein, such as by SDS-PAGE and protein staining with Coomassie blue.

As used herein, biological activity refers to the *in vivo* activities of a compound or physiological responses that result upon *in vivo* administration of a compound, composition or other mixture. Biological activity, thus, encompasses therapeutic effects and pharmaceutical activity of such compounds, compositions and mixtures. Biological activities can be observed in *in vitro* systems designed to test or use such activities. Thus, for purposes herein a biological activity of a protease is its catalytic activity in which a polypeptide is hydrolyzed.

As used herein equivalent, when referring to two sequences of nucleic acids, means that the two sequences in question encode the same sequence of amino acids or equivalent proteins. When equivalent is used in referring to two proteins or peptides, it means that the two proteins or peptides have substantially the same amino acid sequence with only amino acid substitutions that do not substantially alter the activity or function of the protein or peptide. When equivalent refers to a property, the property does not need to be present to the same extent (*e.g.*, two peptides can exhibit different rates of the same type of enzymatic activity), but the activities are usually substantially the same.

As used herein, a composition refers to any mixture. It can be a solution, suspension, liquid, powder, paste, aqueous, non-aqueous or any combination thereof.

As used herein, a combination refers to any association between or among two or more items. The combination can be two or more separate items, such as two compositions or two collections, can be a mixture thereof, such as a single mixture of the two or more items, or any variation thereof. The elements of a combination are generally functionally associated or related.

As used herein, "disease or disorder" refers to a pathological condition in an organism resulting from cause or condition including, but not limited to, infections, acquired conditions, genetic conditions, and characterized by identifiable symptoms. Diseases and disorders of interest herein include diabetes mellitus.

As used herein, "treating" a subject with a disease or condition means that the subject's symptoms are partially or totally alleviated, or remain static following treatment. Hence treatment encompasses prophylaxis, therapy and/or cure.

Prophylaxis refers to prevention of a potential disease and/or a prevention of worsening of symptoms or progression of a disease. Treatment also encompasses any pharmaceutical use of a co-formulation of insulin and hyaluronan degrading enzyme provided herein.

As used herein, a pharmaceutically effective agent, includes any therapeutic agent or bioactive agents, including, but not limited to, for example, anesthetics, vasoconstrictors, dispersing agents, conventional therapeutic drugs, including small molecule drugs and therapeutic proteins.

As used herein, treatment means any manner in which the symptoms of a condition, disorder or disease or other indication, are ameliorated or otherwise beneficially altered.

As used herein, a therapeutic effect means an effect resulting from treatment of a subject that alters, typically improves or ameliorates the symptoms of a disease or condition or that cures a disease or condition. A therapeutically effective amount refers to the amount of a composition, molecule or compound which results in a therapeutic effect following administration to a subject.

As used herein, the term "subject" refers to an animal, including a mammal, such as a human being.

As used herein, a patient refers to a human subject exhibiting symptoms of a disease or disorder.

As used herein, amelioration of the symptoms of a particular disease or disorder by a treatment, such as by administration of a pharmaceutical composition or other therapeutic, refers to any lessening, whether permanent or temporary, lasting or transient, of the symptoms that can be attributed to or associated with administration of the composition or therapeutic.

As used herein, prevention or prophylaxis refers to methods in which the risk of developing disease or condition is reduced.

As used herein, a "therapeutically effective amount" or a "therapeutically effective dose" refers to the quantity of an agent, compound, material, or composition containing a compound that is at least sufficient to produce a therapeutic effect. Hence, it is the quantity necessary for preventing, curing, ameliorating, arresting or partially arresting a symptom of a disease or disorder.

As used herein, a therapeutically effective insulin dosage is the amount of insulin required or sufficient to achieve glycemic control. This amount can be determined empirically, such as by glucose or meal challenge. The compositions provided herein contain a therapeutically effective amount or concentration of insulin so that therapeutically effective dosages are administered.

As used herein, unit dose form refers to physically discrete units suitable for human and animal subjects and packaged individually as is known in the art.

As used herein, an "article of manufacture" is a product that is made and sold. As used throughout this application, the term is intended to encompass a fast-acting insulin composition and hyaluronan degrading enzyme composition contained in the same or separate articles of packaging.

As used herein, fluid refers to any composition that can flow. Fluids thus encompass compositions that are in the form of semi-solids, pastes, solutions, aqueous mixtures, gels, lotions, creams and other such compositions.

As used herein, a "kit" refers to a combination of compositions provided herein and another item for a purpose including, but not limited to, reconstitution, activation, instruments/devices for delivery, administration, diagnosis, and assessment of a biological activity or property. Kits optionally include instructions for use.

As used herein, animal includes any animal, such as, but are not limited to primates including humans, gorillas and monkeys; rodents, such as mice and rats; fowl, such as chickens; ruminants, such as goats, cows, deer, sheep; pigs and other animals. Non-human animals exclude humans as the contemplated animal. The enzymes provided herein are from any source, animal, plant, prokaryotic and fungal. Most enzymes are of animal origin, including mammalian origin.

As used herein, a control refers to a sample that is substantially identical to the test sample, except that it is not treated with a test parameter, or, if it is a plasma sample, it can be from a normal volunteer not affected with the condition of interest. A control also can be an internal control.

As used herein, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to a compound, comprising "an extracellular domain"" includes compounds with one or a plurality of extracellular domains.

As used herein, ranges and amounts can be expressed as "about" a particular value or range. About also includes the exact amount. Hence "about 5 bases" means "about 5 bases" and also "5 bases."

As used herein, "optional" or "optionally" means that the subsequently described event or circumstance does or does not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not. For example, an optionally substituted group means that the group is unsubstituted or is substituted.

As used herein, the abbreviations for any protective groups, amino acids and other compounds, are, unless indicated otherwise, in accord with their common usage, recognized abbreviations, or the IUPAC-IUB Commission on Biochemical Nomenclature (see, (1972) *Biochemistry* 11:1726).

B. HYALURONAN-DEGRADING ENZYME FORMULATIONS AND GENERATING INSULIN CO-FORMULATIONS

Provided herein are stable formulations of a hyaluronan-degrading enzyme, such as a soluble hyaluronidase, for example a PH20. Generally, hyaluronan-degrading enzymes requires a relative high salt in order to retain enzymatic activity (*see e.g.* U.S. Patent Publication No. US20110066111). Also, existing formulations also contain human serum albumin (HSA) for stability. It is found herein that Lys-Lys and magnesium chloride ($MgCl_2$) stabilize hyaluronan-degrading enzymes (*e.g.* a soluble hyaluronidase, for example PH20) more than NaCl. Further, in the presence of Lys-Lys or $MgCl_2$, HSA is not necessary nor required. The formulations provided herein offer advantages over existing formulations, including increased stability, in particular at higher temperatures and for longer times. Provided herein are stable formulations containing Lys-Lys and/or $MgCl_2$. In particular, provided herein are stable formulations of a hyaluronan-degrading enzyme (*e.g.* a soluble hyaluronidase, for example PH20) containing Lys-Lys as a stabilizer.

Co-formulations of a hyaluronan-degrading enzyme with another therapeutic agent also should exhibit stability under various conditions. This can be a problem when the formulation requirements of the other therapeutic agent differs, and sometimes opposes, the formulation requirements required for a hyaluronan-degrading enzyme. It is found herein that this is the case for co-formulations of a

hyaluronan-degrading enzyme and insulin when admixed. Provided herein are stable co-formulations of a hyaluronan-degrading enzyme (e.g. a soluble hyaluronidase such as a PH20) and an insulin, for example, a fast-acting insulin or insulin analog.

Stable formulations, including the marketed formulations, of fast-acting insulins, typically contain different excipients and components and/or different concentrations of excipients and components, which are required for the stability, solubility and activity of the insulin, than those found in stable formulations of hyaluronan degrading enzymes, such as soluble hyaluronidases. These optimized formulations of insulins and soluble hyaluronidases are incompatible when mixed together in a co-formulation, such that the stability, solubility and/or activity of the co-formulated insulin and soluble hyaluronidase are greatly reduced. Such incompatibility is a major barrier to developing stable co-formulations of these agents.

1. Hyaluronan-Degrading Enzyme Formulations

Existing formulations of a hyaluronan degrading enzyme generally contain NaCl, typically 130 mM to 150 mM NaCl. For example, Hylenex® recombinant (hyaluronidase human injection) contains, per mL, 8.5 mg NaCl (145 mM), 1.4 mg dibasic sodium phosphate (9.9 mM), 1.0 mg human albumin, 0.9 mg edetate disodium (2.4 mM), 0.3 mg $CaCl_2$ (2.7 mM) and NaOH to adjust the pH to 7.4. Other formulations of human soluble hyaluronidase, such as the rHuPH20 formulations described in U.S. Pat. Pub. No. US2011/0053247, include 130 mM NaCl, 10 mM Hepes, pH 7.0; or 10 mM histidine, 130 mM NaCl, pH 6.0.

Formulations of hyaluronan degrading enzymes, such as hyaluronidases, including a PH20 such as rHuPH20, contain different components than formulations of insulins. For example, PH20 is most stable at lower pH values between pH 5.5 to 6.5. In addition to lower pH, human hyaluronidase formulations contain more NaCl than insulin formulations, both of which promote stability of the protein and maintain enzymatic activity. Also, human soluble hyaluronidase formulations, such as Hylenex® recombinant (hyaluronidase human injection) have to date been single dose formulations. As such, they do not contain any preservatives.

2. Fast-Acting Insulin Formulations

Fast-acting insulins, including regular insulin and rapid-acting insulin analogs, typically are formulated to optimize insulin solubility, stability and purity at

refrigerated temperatures (*e.g.* 4 °C, such as for long term storage) as well as elevated temperatures (*e.g.* 25 °C and 30 °C). The formulations are made to confer stability over time, in particular, for multidose use and packaging. For example, the labels for marketed insulin products, including Humulin®, Humalog®, Novolog® and Apidra®, indicate stability of at least 24 months at storage temperature of 2-8 °C and 28 days at 25 °C or 30 °C storage. Also, the formulations are believed to be stable for at least 6 days at storage temperatures of or of about 37 °C.

Although the optimal formulations for each insulin can differ, there typically are some commonalities in the formulations. For example, insulin formulations typically contain a buffer, tonicity modifier(s) and one or more preservatives. Many fast-acting insulins also contain zinc, while some also contain a stabilizer. Further, insulin formulations typically are prepared at high neutral pH (*e.g.* 7.0-7.8). Table 2 below sets forth the select marketed formulations of four fast-acting insulins, including a regular insulin and three rapid-acting insulin analogs.

Table 2. Select fast-acting insulin formulations

Insulin	API (U/mL)	pH	Zn	Buffer	Tonicity Modifier		Stabilizer	Preservative	
					NaCl	Glycerin		Phenol	m-cresol
Humulin® recombinant insulin	100 or 500	7.0-7.8	0.017 or 0.085 mg/mL	Dibasic sodium phosphate	-	16 mg/mL (170 mM)	-		
Humalog® insulin lispro	100	7.0-7.8	0.0197 mg/mL	1.88 mg/mL (13.2 mM) dibasic sodium phosphate	-	16 mg/mL (170 mM)	-	trace	3.15 mg/mL (0.315 %)
NovoLog® insulin aspart	100	7.2-7.6	0.0196 mg/mL	1.25 mg/mL (7 mM) disodium hydrogen phosphate dihydrate	0.58 mg/mL (10 mM)	16 mg/mL (170 mM)	-	1.5 mg/mL (0.15 %)	1.72 mg/mL (0.172 %)
Apidra® insulin glulisine	100	7.3	-	6 mg/mL (50 mM) Tris	5 mg/mL (85 mM)	-	0.01 % poly-sorbate 20	-	3.15 mg/mL (0.315 %)

The fast-acting insulin formulations contain a preservative, which prevents microbial contamination being introduced into the formulation by repeated access, such as repeated insertion of a needle, for multiple dosing. Although many

preservatives currently are used in approved parenteral drug products, phenolic compounds such as phenol, metacresol (m-cresol) and parabens are most commonly used in insulin formulations. These phenolic compounds serve not only as effective anti-microbial agents, but also can bind to allosteric sites on the insulin hexamer and change the overall conformation of the higher order structure of insulin. This stabilizes the hexamers by inhibiting the formation of filamentous aggregates (fibrils), which form more readily with insulin monomers than hexamers (Rahuel-Clermont *et al.* (1997) *Biochemistry* 36:5837-5845). Although these preservatives can act as stabilizers, they also can reduce solubility of the insulin if present at too high a concentration. Thus, the concentration of preservatives in insulin is critical to the stability and solubility of the agent.

One or more tonicity modifiers typically are included in the insulin formulations to adjust the isotonicity of the preparation. Exemplary tonicity modifiers that often are present in the insulin formulations include glycerin and/or NaCl. In addition to affecting isotonicity, NaCl affects the solubility of the insulin, such that an increase in NaCl concentration results in reduced solubility. Various insulins, including insulin analogs, have different apparent solubility. Thus, the amount of NaCl that can be present in the formulation without adversely affecting solubility will differ between insulins. For example, insulin glulisine (*e.g.* Apidra® insulin glulisine) is more soluble than insulin aspart (*e.g.* NovoLog® insulin aspart), and thus tolerates more NaCl in the formulation. By comparison, insulin lispro and regular insulin are the least soluble of the fast acting insulins and typically contain no NaCl in their formulations.

Other components also can be included in the insulin formulations. Many insulin formulations, including regular insulin, insulin aspart and insulin lispro formulations, contain Zn^{2+} ions, which promote and stabilize hexamer formation. Although insulin glulisine formulations do not contain zinc, they do contain polysorbate 20 (P20; Tween 20) as a protein stabilizer. Buffers used in the fast acting insulin formulations can include, for example, dibasic sodium phosphate buffer and Trometamol (also known as Tris or THAM).

3. Hyaluronan-Degrading Enzyme and Insulin Co-Formulations

Compositions containing a fast-acting insulin and a hyaluronan degrading enzyme (such as a soluble hyaluronidase, *e.g.* rHuPH20) produce a super fast-acting insulin composition that more closely mimics the endogenous (*i.e.*, natural) post-prandial insulin release of a nondiabetic subject compared to the fast-acting insulin alone (*see e.g.* U.S. Pub No. US20090304665). Thus, such super fast-acting insulin compositions can be used by diabetic subjects to more accurately control blood glucose levels and reduce hyperglycemic excursions, compared to fast-acting insulins alone, thus providing a substantial benefit to the patient.

Multi-dose formulations of fast-acting insulins and formulations of hyaluronan degrading enzymes, however, are incompatible and the mixing of the two typically results in rapid loss of stability and activity of the hyaluronan degrading enzyme in addition to rapid loss of insulin solubility and stability. Until now, therefore, administration of a super fast-acting composition must be performed immediately after combining the insulin and the hyaluronan degrading enzyme to prevent loss of activity. This is impractical and an unacceptable burden for the diabetic patient.

Thus, provided herein are stable co-formulations of a fast-acting insulin and a hyaluronan degrading enzyme (such as a soluble hyaluronidase, *e.g.* rHuPH20). The co-formulations provided herein can be used as therapeutics for the treatment of diabetes mellitus, in particular for the control of post prandial blood glucose levels. The stable co-formulations include those that are multidose formulations that can be provided in a vial, syringe, pen, reservoir for a pump or in a closed loop system, or any other suitable container.

a. Opposing Requirements for Stability

Major barriers preventing the development of stable co-formulations of insulin and hyaluronan degrading enzymes, such as soluble hyaluronidases (*e.g.* rHuPH20), include the crystallization and precipitation of fast acting insulins at refrigerated temperatures, and the stability of the hyaluronan-degrading enzyme at elevated temperatures. Typically, the excipients and conditions that normally prevent such outcomes are different for the two active agents. Some excipients and conditions that are optimal for maintaining solubility and stability of insulin formulations can have a negative effect on the stability and activity of hyaluronan degrading enzymes, such as soluble hyaluronidases (*e.g.* rHuPH20). Conversely, the excipients and conditions

that are optimal for stability of a hyaluronan degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20) have a negative effect of the stability and solubility of insulins.

Merely mixing the existing formulations of insulin, including rapid-acting insulin analogs, and the existing formulations of soluble hyaluronidases, such as rHuPH20, results in a formulation that is not stable under long-term refrigerated storage, or storage at ambient temperature or elevated temperature. This is due to rapid aggregation of rHuPH20 and loss of enzymatic activity, as well as loss of insulin activity. These detrimental effects are the result of multiple incompatible excipients and conditions, including, but not limited to, the type and concentration of preservatives, NaCl concentration, zinc concentration, pH and storage temperature. Thus, identifying formulations in which both agents remain soluble, stable and active is extremely challenging.

i. Preservatives

Preservatives are included in multidose insulin formulations to prevent microbial contamination, which can be introduced into the formulation through repeated access to the vial, pen cartridge, or other multi-dose container containing the formulation. The preservatives typically must be present at sufficient concentrations to satisfy regulatory rules. For example, regulatory requirements assert that the antimicrobial efficacy of the formulation must satisfy the preservative efficacy test (PET) requirements of the target markets. The PET requirements of the United States Pharmacopoeia (USP) and the European Pharmacopoeia (EP) differ considerably, imposing additional constraints in developing multidose formulations.

Marketed insulin formulations typically contain phenol, meta-cresol (m-cresol) and/or methylparaben. These compounds not only serve as effective anti-microbial agents, but also can act to stabilize the hexameric forms of the insulin molecules. However, the concentration and type of preservative used in the insulin formulations is important. For example, although phenolic compounds can stabilize hexameric insulin molecules at optimal concentrations, the solubility of the insulin decreases as the concentration of the preservative increases. Thus, the concentration of preservative in the insulin formulations is critical for both stability and solubility, as well as providing essential anti-microbial activity.

Although a necessary component, preservatives pose a significant problem in the development of stable, multidose formulations of proteins because they typically induce aggregation of the protein in aqueous solution. For example, preservatives such as phenol, m-cresol, and benzyl alcohol have been shown to induce aggregation of human growth hormone (Maa and Hsu (1996) *Int. J. Pharm.*140:155–168), recombinant interleukin-1 receptor (Remmele (1998) *Pharm. Res.*15:200–208), human insulin-like growth factor I (Fransson (1997) *Pharm. Res.* 14:606–612), rhIFN-γ (Lam (1997) *Pharm. Res.* 14:725–729) and cytochrome c (Singh *et al.* (2011) *J. Pharm Sci.*, 100:1679-89). The destabilizing effect that preservatives have on proteins in solution has been a limiting factor in the development of multidose formulations, and to date, most protein therapeutics have been formulated for single use only.

Like most other protein therapeutics, PH20 hyaluronidase, such as rHuPH20, rapidly loses activity in the presence of preservatives, likely due to unfolding of the protein and subsequent aggregate formation. For example, as shown in the Examples herein, preservatives reduce PH20 enzymatic activity, particularly at elevated temperatures. The results herein show by dynamic light scattering (DLS), differential scanning calorimetry (DSC) and other physical chemical characterization techniques that the melting temperature of the exemplary hyaluronan-degrading enzyme rHuPH20 is reduced significantly when phenolic preservatives, such as m-cresol, are added to the formulation. For example, the unfolding temperature of rHuPH20 is reduced from 44 °C to 24 °C. The lower PH20 unfolding temperature leads to increased PH20 aggregation, especially at elevated temperatures, and reduced enzyme activity.

As noted above, these phenolic compounds, such as phenol, m-cresol, and parabens, are the very preservatives used in insulin formulations. The destabilizing effect is likely due to the hydrophobic nature of the phenolic preservatives. The hydrophobicity of the phenolic compounds can lead to interaction with rHuPH20 through nonspecific binding to the protein, ultimately perturbing the structural integrity of rHuPH20. This translates to a significant loss of rHuPH20 enzymatic activity in the presence of preservatives.

As demonstrated in the Examples herein, as the level of phenolic preservative (*e.g.* phenol, m-cresol and methylparaben) increases, and/or the temperature increases, the negative impact on rHuPH20 enzymatic activity also increases. For example, the enzymatic activity of rHuPH20 was significantly reduced after one week of incubation at 35° C when the overall preservative level is relatively high (>0.2%). At room temperature and lower preservative concentrations, the enzyme maintains its activity relatively well for at least one month. Further, the type of phenolic compound also impacts the activity of rHuPH20, such that m-cresol is the most detrimental to rHuPH20 activity, followed by phenol and then methylparaben. However, methylparaben, although the least detrimental to rHuPH20 activity of the phenolic compounds, is also the least effective as an anti-microbial, and thus not an optimal preservative. Other preservatives, such as thimerosal and chlorhexidine salts, appear more compatible with rHuPH20 but are not widely accepted. Thus, formulations that contain these non-traditional preservatives face additional regulatory hurdles.

The detrimental effect of preservatives on the exemplary hyaluronan-degrading enzyme rHuPH20 enzymatic activity is greatly enhanced at elevated temperatures. As shown in the Examples, phenolic preservatives have a negative effect on the melting temperature (T_m) of the enzyme. For example, the T_m for rHuPH20 dropped from above 40° C in the absence of preservative, down to about 26° C in the presence of, for example, 0.25 % m-cresol. Thus, the T_m of rHuPH20 significantly decreases when preservative is added to rHuPH20 in solution. As a result, at elevated temperatures, the soluble hyaluronidase unfolds. As shown in the Examples, this denaturation and subsequent aggregation is reflected in the increased size of the rHuPH20 molecules in the presence of preservatives at elevated temperatures.

Hence, preservatives, although required for their anti-microbial activities and useful for their stabilizing effect on hexameric insulin, can have a deleterious effect on the stability and activity of hyaluronan degrading enzymes, such as rHuPH20, and on the solubility of insulin.

ii. NaCl and pH

Another particular challenge in developing stable co-formulations of insulin and hyaluronan degrading enzymes (*e.g.* rHuPH20) is the fact that the optimal pH and

NaCl concentration ranges for insulin solubility are different than the optimal pH and NaCl concentration ranges for rHuPH20. For example, the solubility of insulin and insulin analogs tends to increase with a higher pH (*e.g.* >7.2) and lower NaCl concentration (*e.g.* <140 mM), conditions which typically have a deleterious effect on the stability of the exemplary hyaluronan-degrading enzyme rHuPH20, particularly at elevated temperatures and over long term storage. This difference is exacerbated even further in the presence of preservatives, which tend to reduce insulin solubility and rHuPH20 stability.

The apparent solubility of regular insulin and the rapid acting insulin analogs varies, with solubility increasing from regular insulin, which is the least soluble, to insulin lispro, then insulin aspart and finally insulin glulisine, which is the most soluble. The solubility is directly related to the tolerance for NaCl in the formulation, such that no NaCl is present in marketed solutions containing regular insulin and insulin lispro, a small amount of NaCl (10 mM) is present in marketed formulations of insulin aspart, and a larger amount of NaCl (85 mM) is present in marketed formulations of insulin glulisine.

Increasing the NaCl concentration of the insulin formulations can result in crystallization/aggregation of the insulin, particularly at lower temperatures. The solubility also is greatly affected by NaCl. As demonstrated in the Examples, when the NaCl concentration of refrigerated insulin solutions increased from 50 mM to 140 mM, the solubility of regular insulin, insulin aspart and insulin lispro decreased significantly. As demonstrated in the Examples, however, the opposite is true for the exemplary hyaluronan-degrading enzyme rHuPH20 stability. The stability of rHuPH20 in solution at elevated temperatures (*e.g.* 25° C and 30° C) is greatly reduced over time as the NaCl concentration is decreased from 140 mM to 50 mM.

The solubility of insulin also is greatly affected by pH. Similar to effects of higher concentrations of NaCl on insulin solubility, a similar negative effect on insulin solubility was observed by decreasing the pH from 7.6 to 6.6. Thus, at low pH and high NaCl, insulin solubility is greatly reduced. Conversely, insulin solubility is maximal at low NaCl and high pH. Similar to the opposite requirements of NaCl concentration between insulin and PH20, pH requirements also are opposite. The stability of rHuPH20 in solution at elevated temperatures (*e.g.* 25° C and 30° C) is

greatly reduced over time as the pH is increased from 7.0 to 7.6. At refrigerated temperatures, rHuPH20 is relatively stable regardless of pH and NaCl concentration.

Thus, the optimal NaCl concentration and pH for insulin solubility and hyaluronan degrading enzyme (*e.g.* rHuPH20) stability appear incompatible. Insulin solubility is maximal at higher pH and lower NaCl concentration. These conditions, however, are detrimental to the exemplary hyaluronan-degrading enzyme rHuPH20, which loses stability at higher pH and lower NaCl concentration. The stability of rHuPH20 can be increased by increasing NaCl concentrations and lowering pH. However, such conditions have a negative effect of the solubility of insulin and insulin analogs, which precipitate at low pH and high NaCl concentration. Hence, one of the major challenges to the development of stable co-formulations of insulin and a hyaluronan degrading enzymes (e.g. rHuPH20 or other hyaluronan-degrading enzyme) is identifying an NaCl concentration and pH in which insulin remains soluble and active and the hyaluronan-degrading enzyme (e.g. rHuPH20) remains stable and active. This has been achieved herein.

b. Compatible Co-Formulation

The opposing requirements of insulin and hyaluronidase, such as PH20 hyaluronidase, for stability mean that several parameters must be balanced to optimize compatibility in a co-formulation. The stable co-formulations provided herein contain the required balance of preservatives, salt (*e.g.* NaCl), pH, stabilizer(s), and/or buffer to retain acceptable levels of hyaluronan degrading enzyme activity and insulin solubility and activity. As discussed above, the challenges in identifying this balance were several-fold. In the first instance, preservatives, such as phenolic preservatives, which are required as anti-bacterials in multidose formulations, have significant destabilizing effects on hyaluronan degrading enzymes, such as rHuPH20, resulting in rapid loss of activity. Secondly, the optimal NaCl concentrations and pH for insulin solubility and stability are very different to those for stability of hyaluronan degrading enzymes. Insulin solubility is maximal at higher pH and lower NaCl concentration. These conditions, however, are detrimental to the exemplary hyaluronan-degrading enzyme rHuPH20, which loses stability at higher pH and lower salt concentration. This instability of rHuPH20 is exacerbated even further in the presence of preservatives. The stability of rHuPH20 can be increased by increasing NaCl

concentrations and lowering pH. However, such conditions have a negative effect on the solubility of insulin and insulin analogs, which precipitate at low pH and high salt concentration.

Thus, identifying conditions under which both the hyaluronan degrading enzyme and the fast-acting insulin remain soluble, stable and active is extremely challenging. The co-formulations provided herein nonetheless provide these conditions. Not only are optimal salt (*e.g.* NaCl), pH and preservative combinations identified, but additional stabilizers and buffers also are identified that, when combined with each other and, in some instances, the described salt, pH and preservatives, further stabilize the hyaluronan degrading enzyme and insulin as well as maintain solubility of the insulin. For example, it is found herein that Lys-Lys is a stabilizer that in some cases, and with some insulin analogs, can be used as a substitute for NaCl such that no or lower concentrations of NaCl can be used in the formulation while retaining enzyme activity and insulin solubility.

The following sections describe exemplary hyaluronan-degrading enzymes and insulins for inclusion in the formulations or co-formulations, exemplary stable formulations and co-formulations, methods of assessing the stability and activity of formulations and co-formulations, and methods of using the formulations or co-formulations in various diseases and conditions.

C. HYALURONAN DEGRADING ENZYMES

Provided herein are stable formulations of a hyaluronan-degrading enzyme. Also provided herein are stable co-formulations containing an insulin and a hyaluronan-degrading enzyme. For example, the description and examples herein show that stable co-formulations of an insulin and a hyaluronan-degrading enzyme, such as a hyaluronidase, can be made even though each individually has opposing requirements for stability and activity. This is exemplified herein with PH20 (*e.g.* rHuPH20), but can be generalized to other hyaluronan-degrading enzymes, such as soluble hyaluronidases or other PH20 polypeptides.

In particular, provided herein are formulations or co-formulations containing a hyaluronan-degrading enzyme that is a hyaluronidase such as a truncated hyaluronidase (*e.g.* C-terminally truncated) lacking all or a portion of a GPI anchor motif. Such hyaluronidase polypeptides can be recombinantly expressed and secreted

from cells into the media upon expression therefrom. By virtue of secretion into the media, hyaluronidases that are normally associated with the cell membrane, when truncated, can exist as soluble protein products. It is within the level of one of skill int the art to generate and/or express hyaluronan-degrading enzymes as provided herein or known in the art, and make stable formulations or co-formulations based on the description and teachings herein.

Hyaluronan-degrading enzymes act to degrade hyaluronan by cleaving hyaluronan polymers, which are composed of repeating disaccharides units, D-glucuronic acid (GlcA) and N-acetyl-D-glucosamine (GlcNAc), linked together via alternating β-1→4 and β-1→3 glycosidic bonds. Hyaluronan chains can reach about 25,000 disaccharide repeats or more in length and polymers of hyaluronan can range in size from about 5,000 to 20,000,000 Da *in vivo*. Hyaluronan, also called hyaluronic acid or hyaluronate, is a non-sulfated glycosaminoglycan that is widely distributed throughout connective, epithelial, and neural tissues. Hyaluronan is an essential component of the extracellular matrix and a major constituent of the interstitial barrier. By catalyzing the hydrolysis of hyaluronan, hyaluronan-degrading enzymes lower the viscosity of hyaluronan, thereby increasing tissue permeability and increasing the absorption rate of fluids administered parenterally. As such, hyaluronan-degrading enzymes, such as hyaluronidases, have been used, for example, as spreading or dispersing agents in conjunction with other agents, drugs and proteins to enhance their dispersion and delivery.

Accordingly, hyaluronan-degrading enzymes include any enzyme having the ability to catalyze the cleavage of a hyaluronan disaccharide chain or polymer. In some examples the degrading enzyme cleaves the β-1→4 glycosidic bond in the hyaluronan chain or polymer. In other examples, the degrading enzyme catalyze the cleavage of the β-1→3 glycosidic bond in the hyaluronan chain or polymer. Exemplary of hyaluronan degrading enzymes in the co-formulations provided herein are hyaluronidases that are secreted into the media when expressed from a cell expression system, including natural hyalurondiases that do not contain a a glycosylphosphatidylinositol (GPI) anchor or truncated hyaluronidases that lack one or more amino acids of the GPI anchor or hyaluronidases that are otherwise not associated with the cell membrane when expressed therefrom. Such hyaluronidases

can be produced recombinantly or synthetically. Other exemplary hyaluronan degrading enzymes include, but are not limited to particular chondroitinases and lyases that have the ability to cleave hyaluronan.

Hyaluronan-degrading enzymes provided in the co-formulations herein also include allelic or species variants or other variants, of a hyaluronan-degrading enzyme as described herein. For example, hyaluronan-degrading enzymes can contain one or more variations in its primary sequence, such as amino acid substitutions, additions and/or deletions. A variant of a hyaluronan-degrading enzyme generally exhibits at least or about 60 %, 70 %, 80 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or more sequence identity compared to the hyaluronan-degrading enzyme not containing the variation. Any variation can be included in the hyaluronan degrading enzyme for the purposes herein provided the enzyme retains hyaluronidase activity, such as at least or about 5 %, 10 %, 15 %, 20 %, 25 %, 30 %, 35 %, 40 %, 45 %, 50 %, 55 %, 60 %, 65 %, 70 %, 75 %, 80 %, 85 %, 90 %, 95 % or more of the activity of a hyaluronan degrading enzyme not containing the variation (as measured by *in vitro* and/or *in vivo* assays well known in the art and described herein).

Various forms of hyaluronan degrading enzymes, including hyaluronidases have been prepared and approved for therapeutic use in subjects, including humans. For example, animal-derived hyaluronidase preparations include Vitrase® (ISTA Pharmaceuticals), a purified ovine testicular hyaluronidase, and Amphadase® (Amphastar Pharmaceuticals), a bovine testicular hyaluronidase. Hylenex® (Baxter) is a human recombinant hyaluronidase produced by genetically engineered Chinese Hamster Ovary (CHO) cells containing nucleic acid encoding a truncated human PH20 polypeptide (designated rHuPH20). It is understand that any hyaluronan-degrading enzyme, such as any hyaluronidase can be included in the stable co-formulations provided herein (see, *e.g.*, U.S. Pat. No. 7,767,429, and U.S. Publication Nos. 20040268425 and 20100143457, which are incorporated by reference in their entirety).

Typically, for use in the formulations and co-formulations herein, a human hyaluronan degrading enzyme, such as a human PH20 and in particular a C-terminal truncated human PH20 as described herein, is used. Although hyaluronan degrading enzymes, such as PH20, from other animals can be utilized, such preparations are

potentially immunogenic, since they are animal proteins. For example, a significant proportion of patients demonstrate prior sensitization secondary to ingested foods, and since these are animal proteins, all patients have a risk of subsequent sensitization. Thus, non-human preparations may not be suitable for chronic use. If non-human preparations are desired, they can be prepared to have reduced immunogenicity. Such modifications are within the level of one of skill in the art and can include, for example, removal and/or replacement of one or more antigenic epitopes on the molecule.

Hyaluronan degrading enzymes, including hyaluronidases (*e.g.*, PH20), used in the formulations and co-formulations provided herein can be recombinantly produced or can be purified or partially-purified from natural sources, such as, for example, from testes extracts. Methods for production of recombinant proteins, including recombinant hyaluronan degrading enzymes, are provided elsewhere herein and are well known in the art.

1. Hyaluronidases

Hyaluronidases are members of a large family of hyaluronan degrading enzymes. There are three general classes of hyaluronidases: mammalian-type hyaluronidases, bacterial hyaluronidases and hyaluronidases from leeches, other parasites and crustaceans. Such enzymes can be used in the co-formulations provided herein.

a. Mammalian-type hyaluronidases

Mammalian-type hyaluronidases (EC 3.2.1.35) are *endo-β-N*-acetyl-hexosaminidases that hydrolyze the β-1→4 glycosidic bond of hyaluronan into various oligosaccharide lengths such as tetrasaccharides and hexasaccharides. These enzymes have both hydrolytic and transglycosidase activities, and can degrade hyaluronan and chondroitin sulfates (CS), generally C4-S and C6-S. Hyaluronidases of this type include, but are not limited to, hyaluronidases from cows (bovine) (SEQ ID NOS:10, 11 and 64 and BH55 (U.S. Pat. Nos. 5,747,027 and 5,827,721)), sheep (*Ovis aries*) (SEQ ID NO: 26, 27, 63 and 65), yellow jacket wasp (SEQ ID NOS:12 and 13), honey bee (SEQ ID NO:14), white-face hornet (SEQ ID NO:15), paper wasp (SEQ ID NO:16), mouse (SEQ ID NOS:17-19, 32), pig (SEQ ID NOS:20-21), rat (SEQ ID NOS:22-24, 31), rabbit (SEQ ID NO:25), orangutan (SEQ ID NO:28),

cynomolgus monkey (SEQ ID NO:29), guinea pig (SEQ ID NO:30), chimpanzee (SEQ ID NO:185), rhesus monkey (SEQ ID NO:186) and human hyaluronidases.

Mammalian hyaluronidases can be further subdivided into those that are neutral active, predominantly found in testes extracts, and acid active, predominantly found in organs such as the liver. Exemplary neutral active hyaluronidases include PH20, including but not limited to, PH20 derived from different species such as ovine (SEQ ID NO:27), bovine (SEQ ID NO:11) and human (SEQ ID NO:1). Human PH20 (also known as SPAM1 or sperm surface protein PH20), is generally attached to the plasma membrane via a glycosylphosphatidyl inositol (GPI) anchor. It is naturally involved in sperm-egg adhesion and aids penetration by sperm of the layer of cumulus cells by digesting hyaluronic acid. Exemplary of hyaluronidases used in the co-formulations here are neutral active hyaluronidases.

Besides human PH20 (also termed SPAM1), five hyaluronidase-like genes have been identified in the human genome, HYAL1, HYAL2, HYAL3, HYAL4 and HYALP1. HYALP1 is a pseudogene, and HYAL3 (SEQ ID NO:38) has not been shown to possess enzyme activity toward any known substrates. HYAL4 (precursor polypeptide set forth in SEQ ID NO:39) is a chondroitinase and exhibits little activity towards hyaluronan. HYAL1 (precursor polypeptide set forth in SEQ ID NO:36) is the prototypical acid-active enzyme and PH20 (precursor polypeptide set forth in SEQ ID NO:1) is the prototypical neutral-active enzyme. Acid-active hyaluronidases, such as HYAL1 and HYAL2 (precursor polypeptide set forth in SEQ ID NO:37) generally lack catalytic activity at neutral pH (*i.e.* pH 7). For example, HYAL1 has little catalytic activity *in vitro* over pH 4.5 (Frost *et al.* (1997) *Anal. Biochem.* 251:263-269). HYAL2 is an acid-active enzyme with a very low specific activity *in vitro*. The hyaluronidase-like enzymes also can be characterized by those which are generally attached to the plasma membrane via a glycosylphosphatidyl inositol (GPI) anchor such as human HYAL2 and human PH20 (Danilkovitch-Miagkova, *et al.* (2003) *Proc Natl Acad Sci USA* 100(8):4580-5), and those which are generally soluble such as human HYAL1 (Frost *et al.* (1997) *Biochem Biophys Res Commun.* 236(1):10-5).

PH20

PH20, like other mammalian hyaluronidases, is an endo-β-N-acetyl-hexosaminidase that hydrolyzes the β1→4 glycosidic bond of hyaluronic acid into

various oligosaccharide lengths such as tetrasaccharides and hexasaccharides. They have both hydrolytic and transglycosidase activities and can degrade hyaluronic acid and chondroitin sulfates, such as C4-S and C6-S. PH20 is naturally involved in sperm-egg adhesion and aids penetration by sperm of the layer of cumulus cells by digesting hyaluronic acid. PH20 is located on the sperm surface, and in the lysosome-derived acrosome, where it is bound to the inner acrosomal membrane. Plasma membrane PH20 has hyaluronidase activity only at neutral pH, while inner acrosomal membrane PH20 has activity at both neutral and acid pH. In addition to being a hyaluronidase, PH20 also appears to be a receptor for HA-induced cell signaling, and a receptor for the zona pellucida surrounding the oocyte.

Exemplary PH20 proteins include, but are not limited to, human (precursor polypeptide set forth in SEQ ID NO:1, mature polypeptide set forth in SEQ ID NO: 2), bovine (SEQ ID NOS: 11 and 64), rabbit (SEQ ID NO: 25), ovine PH20 (SEQ ID NOS: 27, 63 and 65), cynomolgus monkey (SEQ ID NO: 29), guinea pig (SEQ ID NO: 30), rat (SEQ ID NO: 31), mouse (SEQ ID NO: 32), chimpanzee (SEQ ID NO: 185) and rhesus monkey (SEQ ID NO:186) PH20 polypeptides.

Bovine PH20 is a 553 amino acid precursor polypeptide (SEQ ID NO:11). Alignment of bovine PH20 with the human PH20 shows only weak homology, with multiple gaps existing from amino acid 470 through to the respective carboxy termini due to the absence of a GPI anchor in the bovine polypeptide (*see e.g.*, Frost GI (2007) *Expert Opin. Drug. Deliv.* 4: 427-440). In fact, clear GPI anchors are not predicted in many other PH20 species besides humans. Thus, PH20 polypeptides produced from ovine and bovine naturally exist as soluble forms. Though bovine PH20 exists very loosely attached to the plasma membrane, it is not anchored via a phospholipase sensitive anchor (Lalancette *et al.* (2001) *Biol Reprod.* 65(2):628-36). This unique feature of bovine hyaluronidase has permitted the use of the soluble bovine testes hyaluronidase enzyme as an extract for clinical use (Wydase®, Hyalase®).

The human PH20 mRNA transcript is normally translated to generate a 509 amino acid precursor polypeptide (SEQ ID NO:1) containing a 35 amino acid signal sequence at the N-terminus (amino acid residue positions 1-35) and a 19 amino acid glycosylphosphatidylinositol (GPI) anchor attachment signal sequence at the C-

terminus (amino acid residue positions 491-509). The mature PH20 is, therefore, a 474 amino acid polypeptide set forth in SEQ ID NO:2. Following transport of the precursor polypeptide to the ER and removal of the signal peptide, the C-terminal GPI-attachment signal peptide is cleaved to facilitate covalent attachment of a GPI anchor to the newly-formed C-terminal amino acid at the amino acid position corresponding to position 490 of the precursor polypeptide set forth in SEQ ID NO:1. Thus, a 474 amino acid GPI-anchored mature polypeptide with an amino acid sequence set forth in SEQ ID NO:2 is produced.

Although human PH20 is a neutral active hyaluronidase when it exists at the plasma membrane via a GPI anchor, it exhibits activity at both neutral and acidic pH when it is expressed on the inner acrosomal membrane. It appears that PH20 contains two catalytic sites at distinct regions of the polypeptide: the Peptide 1 and Peptide 3 regions (Cherr *et al.*, (2001) *Matrix Biology* 20:515-525). The Peptide 1 region of PH20, which corresponds to amino acid positions 107-137 of the mature polypeptide set forth in SEQ ID NO:2 and positions 142-172 of the precursor polypeptide set forth in SEQ ID NO:1, is required for enzyme activity at neutral pH. Amino acids at positions 111 and 113 (corresponding to the mature PH20 polypeptide set forth in SEQ ID NO:2) within this region appear to be important for activity, as mutagenesis by amino acid replacement results in PH20 polypeptides with 3% hyaluronidase activity or undetectable hyaluronidase activity, respectively, compared to the wild-type PH20 (Arming *et al.*, (1997) *Eur. J. Biochem.* 247:810-814).

The Peptide 3 region, which corresponds to amino acid positions 242-262 of the mature polypeptide set forth in SEQ ID NO:2, and positions 277-297 of the precursor polypeptide set forth in SEQ ID NO:1, appears to be important for enzyme activity at acidic pH. Within this region, amino acids at positions 249 and 252 of the mature PH20 polypeptide appear to be essential for activity, and mutagenesis of either one results in a polypeptide essentially devoid of activity (Arming *et al.*, (1997) *Eur. J. Biochem.* 247:810-814).

In addition to the catalytic sites, PH20 also contains a hyaluronan-binding site. Experimental evidence shows that this site is located in the Peptide 2 region, which corresponds to amino acid positions 205-235 of the precursor polypeptide set forth in SEQ ID NO:1 and positions 170-200 of the mature polypeptide set forth in SEQ ID

NO:2. This region is highly conserved among hyaluronidases and is similar to the heparin binding motif. Mutation of the arginine residue at position 176 (corresponding to the mature PH20 polypeptide set forth in SEQ ID NO:2) to a glycine results in a polypeptide with only about 1% of the hyaluronidase activity of the wild type polypeptide (Arming *et al.*, (1997) *Eur. J. Biochem.* 247:810-814).

There are seven potential N-linked glycosylation sites in human PH20 at N82, N166, N235, N254, N368, N393, N490 of the polypeptide exemplified in SEQ ID NO:1. Because amino acids 36 to 464 of SEQ ID NO:1 appears to contain the minimally active human PH20 hyaluronidase domain, the N-linked glycosylation site N-490 is not required for proper hyaluronidase activity. There are six disulfide bonds in human PH20. Two disulfide bonds between the cysteine residues C60 and C351 and between C224 and C238 of the polypeptide exemplified in SEQ ID NO:1 (corresponding to residues C25 and C316, and C189 and C203 of the mature polypeptide set forth in SEQ ID NO:2, respectively). A further four disulfide bonds are formed between between the cysteine residues C376 and C387; between C381 and C435; between C437 and C443; and between C458 and C464 of the polypeptide exemplified in SEQ ID NO:1 (corresponding to residues C341 and C352; between C346 and C400; between C402 and C408; and between C423 and C429 of the mature polypeptide set forth in SEQ ID NO:2, respectively).

b. Bacterial hyaluronidases

Bacterial hyaluronidases (EC 4.2.2.1 or EC 4.2.99.1) degrade hyaluronan and, to various extents, chondroitin sulfates and dermatan sulfates. Hyaluronan lyases isolated from bacteria differ from hyaluronidases (from other sources, *e.g.*, hyaluronoglucosaminidases, EC 3.2.1.35) by their mode of action. They are endo-β-N-acetylhexosaminidases that catalyze an elimination reaction, rather than hydrolysis, of the β1→4-glycosidic linkage between N-acetyl-beta-D-glucosamine and D-glucuronic acid residues in hyaluronan, yielding 3-(4-deoxy-β-D-gluc-4-enuronosyl)-N-acetyl-D-glucosamine tetra- and hexasaccharides, and disaccharide end products. The reaction results in the formation of oligosaccharides with unsaturated hexuronic acid residues at their nonreducing ends.

Exemplary hyaluronidases from bacteria for co-formulations provided herein include, but are not limited to, hyaluronan degrading enzymes in microorganisms,

including strains of *Arthrobacter*, *Bdellovibrio*, *Clostridium*, *Micrococcus*, *Streptococcus*, *Peptococcus*, *Propionibacterium*, *Bacteroides*, and *Streptomyces*. Particular examples of such enzymes include, but are not limited to *Arthrobacter sp.* (strain FB24) (SEQ ID NO:67), *Bdellovibrio bacteriovorus* (SEQ ID NO:68), *Propionibacterium acnes* (SEQ ID NO:69), *Streptococcus agalactiae* ((SEQ ID NO:70); 18RS21 (SEQ ID NO:71); serotype Ia (SEQ ID NO:72); serotype III (SEQ ID NO:73)), *Staphylococcus aureus* (strain COL) (SEQ ID NO:74); strain MRSA252 (SEQ ID NOS:75 and 76); strain MSSA476 (SEQ ID NO:77); strain NCTC 8325 (SEQ ID NO:78); strain bovine RF122 (SEQ ID NOS:79 and 80); strain USA300 (SEQ ID NO:81), *Streptococcus pneumoniae* ((SEQ ID NO:82); strain ATCC BAA-255 / R6 (SEQ ID NO:83); serotype 2, strain D39 / NCTC 7466 (SEQ ID NO:84), *Streptococcus pyogenes* (serotype M1) (SEQ ID NO:85); serotype M2, strain MGAS10270 (SEQ ID NO:86); serotype M4, strain MGAS10750 (SEQ ID NO:87); serotype M6 (SEQ ID NO:88); serotype M12, strain MGAS2096 (SEQ ID NOS:89 and 90); serotype M12, strain MGAS9429 (SEQ ID NO:91); serotype M28 (SEQ ID NO:92); *Streptococcus suis* (SEQ ID NOS:93-95); *Vibrio fischeri* (strain ATCC 700601/ ES114 (SEQ ID NO:96)), and the *Streptomyces hyaluronolyticus* hyaluronidase enzyme, which is specific for hyaluronic acid and does not cleave chondroitin or chondroitin sulfate (Ohya, T. and Kaneko, Y. (1970) *Biochim. Biophys. Acta* 198:607).

c. Hyaluronidases from leeches, other parasites and crustaceans

Hyaluronidases from leeches, other parasites, and crustaceans (EC 3.2.1.36) are endo-β-glucuronidases that generate tetra- and hexasaccharide end-products. These enzymes catalyze hydrolysis of 1→3-linkages between β-D-glucuronate and N-acetyl-D-glucosamine residues in hyaluronate. Exemplary hyaluronidases from leeches include, but are not limited to, hyaluronidase from Hirudinidae (*e.g.*, *Hirudo medicinalis*), Erpobdellidae (*e.g.*, *Nephelopsis obscura* and *Erpobdella punctata,*), Glossiphoniidae (*e.g.*, *Desserobdella picta, Helobdella stagnalis, Glossiphonia complanata, Placobdella ornata* and *Theromyzon sp.*) and Haemopidae (*Haemopis marmorata*) (Hovingh *et al.* (1999) *Comp Biochem Physiol B Biochem Mol Biol.* 124(3):319-26). An exemplary hyaluronidase from bacteria that has the same

mechanism of action as the leech hyaluronidase is that from the cyanobacteria, *Synechococcus sp.* (strain RCC307, SEQ ID NO:97).

2. Other hyaluronan degrading enzymes

In addition to the hyaluronidase family, other hyaluronan degrading enzymes can be used in the stable formulations provided herein or the co-formulations with insulin provided herein. For example, enzymes, including particular chondroitinases and lyases, that have the ability to cleave hyaluronan can be employed. Exemplary chondroitinases that can degrade hyaluronan include, but are not limited to, chondroitin ABC lyase (also known as chondroitinase ABC), chondroitin AC lyase (also known as chondroitin sulfate lyase or chondroitin sulfate eliminase) and chondroitin C lyase. Methods for production and purification of such enzymes for use in the compositions, combinations, and methods provided are known in the art (*e.g.*, U.S. Pat. No. 6,054,569; Yamagata, *et al.* (1968) *J. Biol. Chem.* 243(7):1523-1535; Yang *et al.* (1985) *J. Biol. Chem.* 160(30):1849-1857).

Chondroitin ABC lyase contains two enzymes, chondroitin-sulfate-ABC endolyase (EC 4.2.2.20) and chondroitin-sulfate-ABC exolyase (EC 4.2.2.21) (Hamai *et al.* (1997) *J Biol Chem.* 272(14):9123-30), which degrade a variety of glycosaminoglycans of the chondroitin-sulfate- and dermatan-sulfate type. Chondroitin sulfate, chondroitin-sulfate proteoglycan and dermatan sulfate are the preferred substrates for chondroitin-sulfate-ABC endolyase, but the enzyme also can act on hyaluronan at a lower rate. Chondroitin-sulfate-ABC endolyase degrades a variety of glycosaminoglycans of the chondroitin-sulfate- and dermatan-sulfate type, producing a mixture of Δ4-unsaturated oligosaccharides of different sizes that are ultimately degraded to Δ4-unsaturated tetra- and disaccharides. Chondroitin-sulfate-ABC exolyase has the same substrate specificity but removes disaccharide residues from the non-reducing ends of both polymeric chondroitin sulfates and their oligosaccharide fragments produced by chondroitin-sulfate-ABC endolyase (Hamai, A. *et al.* (1997) *J. Biol. Chem.* 272:9123-9130). A exemplary chondroitin-sulfate-ABC endolyases and chondroitin-sulfate-ABC exolyases include, but are not limited to, those from *Proteus vulgaris* and *Flavobacterium heparinum* (the *Proteus vulgaris* chondroitin-sulfate-ABC endolyase is set forth in SEQ ID NO: 98 (Sato *et al.* (1994) *Appl. Microbiol. Biotechnol.* 41(1):39-46).

Chondroitin AC lyase (EC 4.2.2.5) is active on chondroitin sulfates A and C, chondroitin and hyaluronic acid, but is not active on dermatan sulfate (chondroitin sulfate B). Exemplary chondroitinase AC enzymes from the bacteria include, but are not limited to, those from *Flavobacterium heparinum* and *Victivallis vadensis*, set forth in SEQ ID NOS:99 and 100, respectively, and *Arthrobacter aurescens* (Tkalec *et al.* (2000) *Applied and Environmental Microbiology* 66(1):29-35; Ernst *et al.* (1995) *Critical Reviews in Biochemistry and Molecular Biology* 30(5):387-444).

Chondroitinase C cleaves chondroitin sulfate C producing tetrasaccharide plus an unsaturated 6-sulfated disaccharide (delta Di-6S). It also cleaves hyaluronic acid producing unsaturated non-sulfated disaccharide (delta Di-OS). Exemplary chondroitinase C enzymes from the bacteria include, but are not limited to, those from *Streptococcus* and *Flavobacterium* (Hibi *et al.* (1989) *FEMS-Microbiol-Lett.* 48(2):121-4; Michelacci *et al.* (1976) *J. Biol. Chem.* 251:1154-8; Tsuda *et al.* (1999) *Eur. J. Biochem.* 262:127-133)

3. Truncated hyaluronan degrading enzymes or other soluble forms

Hyaluronan-degrading enzymes can exist in membrane-bound or membrane-associated form, or can be secreted into the media when expressed from cells and thereby exist in soluble form. For purposes herein, hyaluronan degrading enzymes include any hyaluronan degrading enzymes that when expressed and secreted from cells are not associated with the cell membrane, and thereby exist in soluble form. Soluble hyaluronan-degrading enzymes include, but are not limited to hyaluronidases, including non-human hyaluronidases (*e.g.* animal or bacterial hyaluronidases), such as bovine PH20 or ovine PH20, and human hyaluronidases such as Hyal1, or truncated forms of non-human or human membrane-associated hyaluronidases, in particular truncated forms of human PH20, allelic variants thereof and other variants thereof. Exemplary of hyaluronan-degrading enzymes in the co-formulations herein are truncated forms of a hyaluronan-degrading enzyme that lack one or more amino acid residues of a glycosylphosphatidylinositol (GPI) anchor and that retain hyaluornidase activity. In one example, the human hyaluronidase PH20, which is normally membrane anchored via a GPI anchor, can be made soluble by truncation of and removal of all or a portion of the GPI anchor at the C-terminus.

Thus, in some instances, a hyaluronan degrading enzyme that is normally GPI-anchored (such as, for example, human PH20) is rendered soluble by truncation at the C-terminus. Such truncation can remove all of the GPI anchor attachment signal sequence, or can remove only some of the GPI anchor attachment signal sequence. The resulting polypeptide, however, is soluble. In instances where the soluble hyaluronan degrading enzyme retains a portion of the GPI anchor attachment signal sequence, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid residues in the GPI-anchor attachment signal sequence can be retained, provided the polypeptide is soluble (*i.e.* secreted when expressed from cells) and active. One of skill in the art can determine whether a polypeptide is GPI-anchored using methods well known in the art. Such methods include, but are not limited to, using known algorithms to predict the presence and location of the GPI-anchor attachment signal sequence and ω-site, and performing solubility analyses before and after digestion with phosphatidylinositol-specific phospholipase C (PI-PLC) or D (PI-PLD).

Exemplary of a soluble hyaluronidase is PH20 from any species, such as any set forth in any of SEQ ID NOS: 1, 2, 11, 25, 27, 30-32, 63-65 and 185-186, or truncated forms thereof lacking all or a portion of the C-terminal GPI anchor, so long as the hyaluronidase is soluble and retains hyaluronidase activity. Exemplary soluble hyaluronidases that are C-terminally truncated and lack all or a portion of the GPI anchor attachment signal sequence include, but are not limited to, PH20 polypeptides of primate origin, such as, for example, human and chimpanzee PH20 polypeptides. For example, soluble PH20 polypeptides can be made by C-terminal truncation of any of the mature or precursor polypeptides set forth in SEQ ID NOS:1, 2 or 185, or allelic or other variation thereof, including active fragment thereof, wherein the resulting polypeptide is soluble and lacks all or a portion of amino acid residues from the GPI-anchor attachment signal sequence. Also included among soluble hyaluronidases are allelic variants or other variants of any of SEQ ID NOS: 1, 2, 11, 25, 27, 30-32, 63-65 and 185-186, or truncated forms thereof. Allelic variants and other variants are known to one of skill in the art, and include polypeptides having 60 %, 70 %, 80 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 % or more sequence identity to any of SEQ ID NOS: 1, 2, 11, 25, 27, 30-32, 63-65 and 185-186, or truncated forms thereof. Amino acid variants include conservative and non-

conservative mutations. It is understood that residues that are important or otherwise required for the activity of a hyaluronidase, such as any described above or known to skill in the art, are generally invariant and cannot be changed. These include, for example, active site residues. Thus, for example, amino acid residues 111, 113 and 176 (corresponding to residues in the mature PH20 polypeptide set forth in SEQ ID NO:2) of a human PH20 polypeptide, or soluble form thereof, are generally invariant and are not altered. Other residues that confer glycosylation and formation of disulfide bonds required for proper folding also can be invariant.

a. C-terminal Truncated Human PH20

Exemplary of a soluble hyaluronidase is a C-terminal truncated human PH20. C-terminal truncated forms of recombinant human PH20 have been produced and can be used in the co-formulations described herein. The production of such soluble forms of PH20 is described in U.S. Pat. No. 7,767,429 and U.S. Pat. Application Nos. US20040268425, US20050260186, US20060104968 and US20100143457.

For example, C-terminal truncated PH20 polypeptides include polypeptides that at least contain amino acids 36-464 (the minimal portion required for hyaluronidase activity), or include a sequence of amino acids that has at least 85 %, for example at least 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 % sequence identity to a sequence of amino acids that includes at least amino acids 36-464 of SEQ ID NO:1 and retain hyaluronidase activity. Included among these polypeptides are human PH20 polypeptides that completely lack all the GPI-anchor attachment signal sequence. Also include among these polypeptides are human PH20 polypeptides that lack a portion of contiguous amino acid residues of the GPI-anchor attachment signal sequence (termed extended soluble PH20 (esPH20); see e.g. US20100143457). C-terminally truncated PH20 polypeptides can be C-terminally truncated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60 or more amino acids compared to the full length wild type polypeptide, such as a full length wild type polypeptide with a sequence set forth in SEQ ID NOS:1 or 2, or allelic or species variants or other variants thereof. Thus, instead of having a GPI-anchor covalently attached to the C-terminus of the protein in the ER and being anchored to the extracellular leaflet of the plasma membrane, these polypeptides are secreted when expressed from cells and are soluble.

Exemplary C-terminally truncated human PH20 polypeptides provided herein include any that include at least amino acids 36-464 of SEQ ID NO:1 and are C-terminally truncated after amino acid position 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499 or 500 of the sequence of amino acids set forth in SEQ ID NO:1, or a variant thereof that exhibits at least 85 % sequence identity, such as at least 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 % sequence identity thereto and retains hyaluronidase activity. Table 3 provides non-limiting examples of exemplary C-terminally truncated PH20 polypeptides. In Table 3 below, the length (in amino acids) of the precursor and mature polypeptides, and the sequence identifier (SEQ ID NO) in which exemplary amino acid sequences of the precursor and mature polypeptides of the C-terminally truncated PH20 proteins are set forth, are provided. The wild-type PH20 polypeptide also is included in Table 3 for comparison. For example, exemplary C-terminally truncated PH20 polypeptides include, but are not limited to, polypeptides set forth in any one of SEQ ID NOS: 4-9, 47-48, 234-254, and 267-273, or a polypeptide that exhibits at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any one of SEQ ID NOS: 4-9, 47-48, 234-254, and 267-273.

Table 3. Exemplary C-terminally truncated PH20 polypeptides

Polypeptide	Precursor (amino acids)	Precursor SEQ ID NO	Mature (amino acids)	Mature SEQ ID NO
wildtype	509	1	474	2
SPAM1-SILF	500	223	465	267
SPAM-VSIL	499	190	464	234
SPAM1-IVSI	498	224	463	268
SPAM1-FIVS	497	191	462	235
SPAM1-MFIV	496	225	461	269
SPAM1-TMFI	495	192	460	236
SPAM1-ATMF	494	226	459	270
SPAM1-SATM	493	193	458	237
SPAM1-LSAT	492	227	457	271
SPAM1-TLSA	491	194	456	238
SPAM1-STLS	490	196	455	240
SPAM1-PSTL	489	195	454	239
SPAM1-SPST	488	228	453	272
SPAM1-ASPS	487	197	452	241

SPAM1-NASP	486	229	451	273
SPAM1-YNAS	485	198	450	242
SPAM1-FYNA	484	199	449	243
SPAM1-IFYN	483	46	448	48
SPAM1-QIFY	482	3	447	4
SPAM1-PQIF	481	45	446	5
SPAM1-EPQI	480	44	445	6
SPAM1-EEPQ	479	43	444	7
SPAM1-TEEP	478	42	443	8
SPAM1-ETEE	477	41	442	9
SPAM1-METE	476	200	441	244
SPAM1-PMET	475	201	440	245
SPAM1-PPME	474	202	439	246
SPAM1-KPPM	473	203	438	247
SPAM1-LKPP	472	204	437	248
SPAM1-FLKP	471	205	436	249
SPAM1-AFLK	470	206	435	250
SPAM1-DAFL	469	207	434	251
SPAM1-IDAF	468	208	433	252
SPAM1-CIDA	467	40	432	47
SPAM1-VCID	466	209	431	253
SPAM1-GVCI	465	200	430	254

b. rHuPH20

Exemplary of a C-terminal truncated form of SEQ ID NO:1 is a polypeptide thereof that is truncated after amino acid 482 of the sequence set forth in SEQ ID NO:1. Such a polypeptide can be generated from a nucleic acid molecule encoding amino acids 1-482 (set forth in SEQ ID NO:3). Such an exemplary nucleic acid molecule is set forth in SEQ ID NO:49. Post translational processing removes the 35 amino acid signal sequence, leaving a 447 amino acid soluble recombinant human PH20 (SEQ ID NO:4). As produced in the culture medium there is heterogeneity at the C-terminus such that the product, designated rHuPH20, includes a mixture of species that can include any one or more of SEQ ID NOS:4-9 in various abundance. Typically, rHuPH20 is produced in cells that facilitate correct N-glycosylation to retain activity, such as CHO cells (*e.g.* DG44 CHO cells).

4. Glycosylation of hyaluronan degrading enzymes

Glycosylation, including N- and O-linked glycosylation, of some hyaluronan degrading enzymes, including hyaluronidases, can be important for their catalytic activity and stability. While altering the type of glycan modifying a glycoprotein can

have dramatic affects on a protein's antigenicity, structural folding, solubility, and stability, most enzymes are not thought to require glycosylation for optimal enzyme activity. For some hyaluronidases, removal of N-linked glycosylation can result in near complete inactivation of the hyaluronidase activity. Thus, for such hyaluronidases, the presence of N-linked glycans is critical for generating an active enzyme.

N-linked oligosaccharides fall into several major types (oligomannose, complex, hybrid, sulfated), all of which have $(Man)_3$-GlcNAc-GlcNAc-cores attached via the amide nitrogen of Asn residues that fall within -Asn-Xaa-Thr/Ser-sequences (where Xaa is not Pro). Glycosylation at an -Asn-Xaa-Cys- site has been reported for coagulation protein C. In some instances, a hyaluronan degrading enzyme, such as a hyaluronidase, can contain both N-glycosidic and O-glycosidic linkages. For example, PH20 has O-linked oligosaccharides as well as N-linked oligosaccharides. There are seven potential N-linked glycosylation sites at N82, N166, N235, N254, N368, N393, N490 of human PH20 exemplified in SEQ ID NO: 1. Amino acid residues N82, N166 and N254 are occupied by complex type glycans whereas amino acid residues N368 and N393 are occupied by high mannose type glycans. Amino acid residue N235 is occupied by approximately 80 % high mannose type glycans and 20 % complex type glycans. As noted above, N-linked glycosylation at N490 is not required for hyaluronidase activity.

In some examples, the hyaluronan degrading enzymes for use in the co-formulations provided are glycosylated at one or all of the glycosylation sites. For example, for human PH20, or a soluble form thereof, 2, 3, 4, 5, or 6 of the N-glycosylation sites corresponding to amino acids N82, N166, N235, N254, N368, and N393 of SEQ ID NO:1 are glycosylated. In some examples the hyaluronan degrading enzymes are glycosylated at one or more native glycosylation sites. Generally soluble forms of PH20 are produced using protein expression systems that facilitate correct N-glycosylation to ensure the polypeptide retains activity, since glycosylation is important for the catalytic activity and stability of hyaluronidases. Such cells include, for example Chinese Hamster Ovary (CHO) cells (*e.g.* DG44 CHO cells).

In other examples, the hyaluronan degrading enzymes are modified at one or more non-native glycosylation sites to confer glycosylation of the polypeptide at one

or more additional site. In such examples, attachment of additional sugar moieties can enhance the pharmacokinetic properties of the molecule, such as improved half-life and/or improved activity.

In other examples, the hyaluronan degrading enzymes, such as a PH20 or human PH20, included in the co-formulations provided herein are partially deglycosylated (or N-partially glycosylated polypeptides) (*see e.g.* U.S. Pat. Publication No. US20100143457). Glycosidases, or glycoside hydrolases, are enzymes that catalyze the hydrolysis of the glycosidic linkage to generate two smaller sugars. The major types of N-glycans in vertebrates include high mannose glycans, hybrid glycans and complex glycans. There are several glycosidases that result in only partial protein deglycosylation, including: EndoF1, which cleaves high mannose and hybrid type glycans; EndoF2, which cleaves biantennary complex type glycans; EndoF3, which cleaves biantennary and more branched complex glycans; and EndoH, which cleaves high mannose and hybrid type glycans. For example, treatment of PH20 (*e.g.* a recombinant PH20 designated rHuPH20) with one or all of the above glycosidases (*e.g.* EndoF1, EndoF2 EndoF3 and/or EndoH) results in partial deglycosylation. These partially deglycosylated PH20 polypeptides can exhibit hyaluronidase enzymatic activity that is comparable to the fully glycosylated polypeptides. In contrast, treatment of PH20 with PNGaseF, a glycosidase that cleaves all N-glycans, or with the GlcNAc phosphotransferase (GPT) inhibitor tunicamycin, results in complete deglycosylation of all N-glycans and thereby renders PH20 enzymatically inactive. Thus, although all N-linked glycosylation sites (such as, for example, those at amino acids N82, N166, N235, N254, N368, and N393 of human PH20, exemplified in SEQ ID NO:1) can be glycosylated, treatment with one or more glycosidases can render the extent of glycosylation reduced compared to a hyaluronidase that is not digested with one or more glycosidases.

Hence, partially deglycosylated hyaluronan degrading enzymes, such as partially deglycosylated soluble hyaluronidases, can be produced by digestion with one or more glycosidases, generally a glycosidase that does not remove all N-glycans but only partially deglycosylates the protein. The partially deglycosylated hyaluronan degrading enzymes, including partially deglycosylated soluble PH20 polypeptides, can have 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 % or 80 % of the level of

glycosylation of a fully glycosylated polypeptide. In one example, 1, 2, 3, 4, 5 or 6 of the N-glycosylation sites corresponding to amino acids N82, N166, N235, N254, N368, and N393 of SEQ ID NO:1 are partially deglycosylated, such that they no longer contain high mannose or complex type glycans, but rather contain at least an N-acetylglucosamine moiety. In some examples, 1, 2 or 3 of the N-glycosylation sites corresponding to amino acids N82, N166 and N254 of SEQ ID NO:1 are deglycosylated, that is, they do not contain a sugar moiety. In other examples, 3, 4, 5, or 6 of the N-glycosylation sites corresponding to amino acids N82, N166, N235, N254, N368, and N393 of SEQ ID NO:1 are glycosylated. Glycosylated amino acid residues minimally contain an N-acetylglucosamine moiety. Typically, the partially deglycosylated hyaluronan degrading enzymes, including partially deglycosylated soluble PH20 polypeptides, exhibit hyaluronidase activity that is 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 %, 100 %, 110 %, 120 %, 130 %, 140 %, 150 %, 200 %, 300 %, 400 %, 500 %, 1000 % or more of the hyaluronidase activity exhibited by the fully glycosylated polypeptide.

5. Modifications of hyaluronan degrading enzymes to improve their pharmacokinetic properties

Hyaluronan degrading enzymes can be modified to improve their pharmacokinetic properties, such as increasing their half-life *in vivo* and/or activities. The modification of hyaluronan degrading enzymes for use in the stable formulations or co-formulations provided herein or in any compositions, combinations and/or methods provided can include attaching, directly or indirectly via a linker, such as covalently or by other stable linkage, a polymer, such as dextran, a polyethylene glycol (pegylation(PEG)) or sialyl moiety, or other such polymers, such as natural or sugar polymers.

Pegylation of therapeutics is known to increase resistance to proteolysis, increase plasma half-life, and decrease antigenicity and immunogenicity. Covalent or other stable attachment (conjugation) of polymeric molecules, such as polyethylene glycol moiety (PEG), to the hyaluronan degrading enzyme thus can impart beneficial properties to the resulting enzyme-polymer composition. Such properties include improved biocompatibility, extension of protein (and enzymatic activity) half-life in the blood, cells and/or in other tissues within a subject, effective shielding of the

protein from proteases and hydrolysis, improved biodistribution, enhanced pharmacokinetics and/or pharmacodynamics, and increased water solubility.

Exemplary polymers that can be conjugated to the hyaluronan degrading enzyme, include natural and synthetic homopolymers, such as polyols (*i.e.* poly-OH), polyamines (*i.e.* poly-NH_2) and polycarboxyl acids (*i.e.* poly-COOH), and further heteropolymers *i.e.* polymers comprising one or more different coupling groups *e.g.* a hydroxyl group and amine groups. Examples of suitable polymeric molecules include polymeric molecules selected from among polyalkylene oxides (PAO), such as polyalkylene glycols (PAG), including polypropylene glycols (PEG), methoxypolyethylene glycols (mPEG) and polypropylene glycols, PEG-glycidyl ethers (Epox-PEG), PEG-oxycarbonylimidazole (CDI-PEG) branched polyethylene glycols (PEGs), polyvinyl alcohol (PVA), polycarboxylates, polyvinylpyrrolidone, poly-D,L-amino acids, polyethylene-co-maleic acid anhydride, polystyrene-co-maleic acid anhydride, dextrans including carboxymethyl-dextrans, heparin, homologous albumin, celluloses, including methylcellulose, carboxymethylcellulose, ethylcellulose, hydroxyethylcellulose carboxyethylcellulose and hydroxypropylcellulose, hydrolysates of chitosan, starches such as hydroxyethyl-starches and hydroxypropyl-starches, glycogen, agaroses and derivatives thereof, guar gum, pullulan, inulin, xanthan gum, carrageenan, pectin, alginic acid hydrolysates and bio-polymers.

Typically, the polymers are polyalkylene oxides (PAO), such as polyethylene oxides, such as PEG, typically mPEG, which, in comparison to polysaccharides such as dextran, pullulan and the like, have few reactive groups capable of cross-linking. Typically, the polymers are non-toxic polymeric molecules such as (m)polyethylene glycol (mPEG) which can be covalently conjugated to the hyaluronan degrading enzyme (*e.g.*, to attachment groups on the protein surface) using relatively simple chemistry.

Suitable polymeric molecules for attachment to the hyaluronan degrading enzyme include, but are not limited to, polyethylene glycol (PEG) and PEG derivatives such as methoxy-polyethylene glycols (mPEG), PEG-glycidyl ethers (Epox-PEG), PEG-oxycarbonylimidazole (CDI-PEG), branched PEGs, and polyethylene oxide (PEO) (*see e.g.* Roberts *et al.*, Advanced Drug Delivery Review

2002, 54: 459-476; Harris and Zalipsky, S (eds.) "Poly(ethylene glycol), Chemistry and Biological Applications" ACS Symposium Series 680, 1997; Mehvar *et al.*, *J. Pharm. Pharmaceut. Sci.*, 3(1):125-136, 2000; Harris, *Nature Reviews* 2:215 (2003); and Tsubery, *J Biol. Chem* 279(37):38118-24, 2004). The polymeric molecule can be of a molecular weight typically ranging from about 3 kDa to about 60 kDa. In some embodiments the polymeric molecule that is conjugated to a protein, such as rHuPH20, has a molecular weight of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 or more than 60 kDa.

Various methods of modifying polypeptides by covalently attaching (conjugating) a PEG or PEG derivative (*i.e.* "PEGylation") are known in the art (*see e.g.*, U.S. Pat. Pub. Nos. 20060104968 and U.S. 20040235734; U.S. Pat. Nos. 5,672,662 and U.S. 6,737,505). Techniques for PEGylation include, but are not limited to, specialized linkers and coupling chemistries (*see e.g.*, Harris, *Adv. Drug Deliv. Rev.* 54:459-476, 2002), attachment of multiple PEG moieties to a single conjugation site (such as via use of branched PEGs; *see e.g.*, Veronese *et al.*, *Bioorg. Med. Chem. Lett.* 12:177-180, 2002), site-specific PEGylation and/or mono-PEGylation (*see e.g.*, Chapman *et al., Nature Biotech.* 17:780-783, 1999), and site-directed enzymatic PEGylation (*see e.g.*, Sato, *Adv. Drug Deliv. Rev.,* 54:487-504, 2002) (see, also, for example, Lu and Felix (1994) *Int. J. Peptide Protein Res.* 43:127-138; Lu and Felix (1993) *Peptide Res.* 6:142-6, 1993; Felix *et al.* (1995) *Int. J. Peptide Res.* 46:253-64; Benhar *et al.* (1994) *J. Biol. Chem.* 269:13398-404; Brumeanu *et al.* (1995) *J Immunol.* 154:3088-95; see also, Caliceti *et al.* (2003) *Adv. Drug Deliv. Rev.* 55(10):1261-77 and Molineux (2003) *Pharmacotherapy* 23 (8 Pt 2):3S-8S). Methods and techniques described in the art can produce proteins having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more than 10 PEG or PEG derivatives attached to a single protein molecule (*see e.g.*, U.S. Pat. Pub. No. 20060104968).

Numerous reagents for PEGylation have been described in the art. Such reagents include, but are not limited to, N-hydroxysuccinimidyl (NHS) activated PEG, succinimidyl mPEG, mPEG2-N-hydroxysuccinimide, mPEG succinimidyl alpha-methylbutanoate, mPEG succinimidyl propionate, mPEG succinimidyl butanoate, mPEG carboxymethyl 3-hydroxybutanoic acid succinimidyl ester, homobifunctional PEG-succinimidyl propionate, homobifunctional PEG

propionaldehyde, homobifunctional PEG butyraldehyde, PEG maleimide, PEG hydrazide, p-nitrophenyl-carbonate PEG, mPEG-benzotriazole carbonate, propionaldehyde PEG, mPEG butyraldehyde, branched mPEG2 butyraldehyde, mPEG acetyl, mPEG piperidone, mPEG methylketone, mPEG "linkerless" maleimide, mPEG vinyl sulfone, mPEG thiol, mPEG orthopyridylthioester, mPEG orthopyridyl disulfide, Fmoc-PEG-NHS, Boc-PEG-NHS, vinylsulfone PEG-NHS, acrylate PEG-NHS, fluorescein PEG-NHS, and biotin PEG-NHS (*see e.g.*, Monfardini *et al.*, *Bioconjugate Chem.* 6:62-69, 1995; Veronese *et al.*, *J. Bioactive Compatible Polymers* 12:197-207, 1997; U.S. 5,672,662; U.S. 5,932,462; U.S. 6,495,659; U.S. 6,737,505; U.S. 4,002,531; U.S. 4,179,337; U.S. 5,122,614; U.S. 5,324, 844; U.S. 5,446,090; U.S. 5,612,460; U.S. 5,643,575; U.S. 5,766,581; U.S. 5,795, 569; U.S. 5,808,096; U.S. 5,900,461; U.S. 5,919,455; U.S. 5,985,263; U.S. 5,990, 237; U.S. 6,113,906; U.S. 6,214,966; U.S. 6,258,351; U.S. 6,340,742; U.S. 6,413,507; U.S. 6,420,339; U.S. 6,437,025; U.S. 6,448,369; U.S. 6,461,802; U.S. 6,828,401; U.S. 6,858,736; U.S. 2001/0021763; U.S. 2001/0044526; U.S. 2001/0046481; U.S. 2002/0052430; U.S. 2002/0072573; U.S. 2002/0156047; U.S. 2003/0114647; U.S. 2003/0143596; U.S. 2003/0158333; U.S. 2003/0220447; U.S. 2004/0013637; US 2004/0235734; U.S. 2005/0114037; U.S. 2005/0171328; U.S. 2005/0209416; EP 01064951; EP 0822199; WO 00176640; WO 0002017; WO 0249673; WO 0500360; WO 9428024; and WO 0187925).

D. STABLE HYALURONAN-DEGRADING ENZYME FORMULATIONS

Provided herein are stable formulations of a hyaluronan-degrading enzyme containing a stabilizing excipient that is a divalent cation. Examples of divalent cations include, but are not limited to, lysyl-lysine (dilysine; Lys-Lys) or magnesium (*e.g.* $MgCl_2$), or salts, derivatives, analogues or mimetics thereof. In particular examples, the stable formulations of a hyaluronan-degrading enzyme contains Lys-Lys, or salts, derivatives, analogues or mimetics thereof, as a stabilizing excipient. In other examples, the stable formulations of a hyaluronan-degrading enzyme contain $MgCl_2$, or derivatives, analogues or mimetics thereof, as a stabilizing agent. Hyaluronan-degrading enzymes containing a divalent cation, for example Lys-Lys or $MgCl_2$, are stable at temperatures of greater than or equal to 37°C for at least three (3) days, and generally at least six days, 7 days (one week), two weeks, three weeks or

four weeks (one month). For example, such formulations are stable at temperatures of greater than or equal to 37°C to 42°C, such as at least or approximately or about 40°C, for at least one months, two months, three months, four months, five months, six months, seven months, eight months, nine months, ten months, eleven months, twelve months or more.

Existing formulations of hyaluronan-degrading enzymes contain human serum albumin (HSA) as a stabilizer. For example, Hylenex® recombinant contains 1.0 mg human albumin. Stable HSA-free hyaluronan-degrading enzyme formulations are desired for several reasons. First, HSA is a blood derived product, and thus it often is not pure. Degradants and contaminants of HSA can interfere with activity of the enzyme. In addition, HSA itself also is subject to stability challenges, since it can form aggregates under certain conditions. It is found herein that stable HSA-free formulations of a hyaluronan-degrading enzyme, for example a hyaluronidase such as a PH20, can be made by inclusion of a divalent cation, for example, Lys-Lys.

Also, as discussed elsewhere herein, most existing formulations of a hyaluronan-degrading enzyme, for example a hyaluronidase such as PH20, also contain NaCl as a stabilizing agent. The presence of NaCl at high amounts of between or about between 130 mM to 150 mM NaCl or higher is generally required for optimal activity and stability of the enzyme. For example, the commercial PH20 formulation Hylenex® contains 145 mM NaCl. As demonstrated in the examples herein, the divalent cation Lys-Lys and $MgCl_2$ exhibit stability effects on the exemplary hyaluronan-degrading enzyme PH20 that is improved over NaCl. This is advantageous, since it is found herein that NaCl does not efficiently stabilize PH20 upon incubation at elevated or accelerated temperatures of greater than 37°C (*see e.g.* Examples 23 and 24). In contrast, the activity of PH20 in formulations containing divalent cations, such as Lys-Lys, is retained at elevated temperatures, such that formulations can exhibit up to 70% or more of activity, and generally at least or about at least 80%, 85%, 90%, or more activity, after incubation for 4 weeks at temperatures of greater than or equal to 37°C, such as greater than or equal to 37°C to 42°C, such as at least or approximately or about 40°C, for at least one month. In examples of formulations herein containing a divalent cation as a stabilizer, for example Lys-Lys, the activity of the hyaluronan-degrading enzyme at elevated temperatures of at least

or about at least 38°C to 42°C, and in particular at 40°C, is increased by greater than or at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more compared to the activity of a hyaluronan-degrading enzyme that does not contain the divalent cation (*e.g.* contains NaCl as a stabilizing agent).

Provided herein are stable hyaluronan-degrading enzyme formulations that contain a therapeutically effective amount of a hyaluronan-degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20) and an amount of a divalent cation, such as Lys-Lys or $MgCl_2$, to render the formulation stable at temperatures of greater than or equal to 37°C for at least one month. In particular examples, provided herein are stable hyaluronan-degrading enzyme formulations that contain a therapeutically effective amount of a hyaluronan-degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20) and an amount of Lys-Lys to render the formulation stable at temperatures of greater than or equal to 37°C for at least one month. For example, such formulations are stable at temperatures of greater than or equal to 37°C to 42°C, such as at least or approximately or about 40°C, for at least one month. The formulations generally also contain a surfactant, an anti-oxidation agent (*e.g.* methionine), a pH of between or about between 6.5 to 7.8 and a buffering agent that maintains the pH range. Optionally, the formulations can contain one or more other stabilizing agents, tonicity modifiers, preservative(s) or excipients.

Typically, the compounds are formulated into pharmaceutical compositions using techniques and procedures well known in the art (*see e.g.*, Ansel *Introduction to Pharmaceutical Dosage Forms*, Fourth Edition, 1985, 126). Pharmaceutically acceptable compositions are prepared in view of approvals for a regulatory agency or other agency prepared in accordance with generally recognized pharmacopeia for use in animals and in humans. The formulation should suit the mode of administration.

The stable formulations can be provided as a pharmaceutical preparation in liquid form as solutions, syrups or suspensions. In liquid form, the pharmaceutical preparations can be provided as a concentrated preparation to be diluted to a therapeutically effective concentration before use. Generally, the preparations are provided in a dosage form that does not require dilution for use, *i.e.* formulations for direct administration. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*,

sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). In another example, pharmaceutical preparations can be presented in lyophilized form for reconstitution with water or other suitable vehicle before use. The formulations can be prepared as single dose or multiple dose formulations.

The volume of the formulations provided herein can be any volume suitable for the container in which it is provided. In some examples, the formulations are provided in a vial, syringe, or any other suitable container. For example, the stable formulations provided herein are between or about between 0.1 mL to 500 mL, such as 0.1 mL to 100 mL, 1 mL to 100 mL, 0.1 mL to 50 mL, such as at least or about at least or about or 0.1 mL, 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, 10 mL, 15 mL, 20 mL, 30 mL, 40 mL, 50 mL or more.

Provided below is a description of the components that are provided in the stable hyaluronan-degrading enzyme formulations herein. The following stable formulations are exemplary only and provide a platform from which minor adjustments can be made. It is understood that very small changes in the concentrations of the various excipients and other components (*e.g.* ± 15 % of the stated concentrations), or small changes in pH, can be made while retaining some if not all of the hyaluronan degrading enzyme stability. Further changes also can be made by adding or removing excipients. For example, the type of stabilizing surfactant can be changed.

1. Hyaluronan-Degrading Enzyme

The amount of hyaluronan degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20), in the stable formulations provided herein is an amount for direct administration sufficient to achieve a therapeutic effect. In one example, the amount is an amount for direct administration sufficient to degrade hyaluronic acid (HA) in the subcutaneous space beneath the outer surface of the human skin. For example, the amount of hyaluronan-degrading enzyme in the formulation is an amount for direct administration to increase the dispersion and absorption of a co-injected or co-administered therapeutic agent. In another example,

the amount is an amount for direct administration sufficient to degrade hyaluronic aid (HA) that is associated with a diseased tissue or cell. For example, the amount is an amount for direct administration sufficient to degrade HA associated with tumor cells. In such examples, the amount is an amount to decrease or lower the interstitial fluid pressure (IFP) or increase tumor vascular volume.

For example, the amount is functionally equivalent to at least or about at least 30 Units/mL. For example, the formulations provided herein contain a hyaluronan-degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20) in an amount between or about between 30 Units/mL to 20,000 U/mL, 300 U/mL to 15,000 U/mL, 300 U/mL to 10,000 U/mL, 300 U/mL to to 5,000 U/mL, 300 U/mL to 3000 U/mL, 300 U/mL to 2000 U/mL, 600 U/mL to 20,000 U/mL, 600 U/mL to 15,000 U/mL, 600 U/mL to 10,000 U/mL, 600 U/mL to 6000 U/mL, 600 U/mL to 4000 U/mL, 600 U/mL to 2000 U/mL, 600 U/mL to 1000 U/mL, 60 U/mL to 600 U/mL, or 100 U/mL to 300 U/mL, such as at least or about at least 30 U/mL, 35 U/mL, 40 U/mL, 50 U/mL, 100 U/mL, 200 U/mL, 300 U/mL, 400 U/mL, 500 U/mL, 600 U/mL, 700 U/mL, 800 U/mL, 900 U/mL, 1000 U/ml, 2000 U/mL, 3000 U/mL, 4000 U/mL, 5000 U/mL, 6000 U/mL, 7000 U/mL, 8000 U/mL, 9000 U/mL, 10,000 U/mL, 12,000 U/mL, 15,000 U/mL or 20,000 U/mL. For example, the formulations provided herein contain a PH20 (*e.g.* rHuPH20) that is in an amount that is at least 100 U/mL to 300 U/mL, for example at least or about at least or about or 100 U/mL, 115 U/mL, 120 U/mL, 125 U/mL, 130 U/mL, 135 U/mL, 140 U/mL, 145 U/mL, 150 U/mL, 155 U/mL, 160 U/mL, 165 U/mL, 170 U/mL, 175 U/mL, 180 U/mL, 185 U/mL, 190 U/mL, 200 U/mL, 220 U/mL, 240 U/mL, 260 U/mL, 280 U/mL or 300 U/mL.

In the stable formulations provided herein the stability of a hyaluronan-degrading enzyme, including a hyaluronidase such as a PH20 (*e.g.* rHuPH20), in the formulations is a function of the recovery and/or activity of the enzyme at elevated temperatures of greater than or equal to 37°C to 42°C, such as at least or approximately or about 37°C or 40°C, for at least three (3) days, and generally at least one month as described above. Assays to assess these parameters are described herein. The formulations provided herein retain hyaluronidase recovery and/or activity such that the formulations are suitable for therapeutic use as described herein. In the stable formulations provided herein, the activity of the hyaluronan degrading

enzyme, such as a hyaluronidase, for example a PH20, typically is greater than or about 50%, such as greater than or at least 55%, 60 %, 65 %, 70 %, 80 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or more of the initial activity of enzyme in the formulation prior to exposure to temperatures of greater than or equal to 37°C to 42°C for at least three (3) days, and generally at least one month as described herein. For example, the activity of the hyaluronan-degrading enzyme, such as a hyaluronidase, for example a PH20, typically is greater than or about 50%, such as greater than or at least 55%, 60 %, 65 %, 70 %, 80 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % of the activity of the same enzyme formulation when stored at 4°C for at least one month. Typically, the stable hyaluronan-degrading enzyme formulations provided herein exhibit at least 70 % of the initial activity of the enzyme for at least one month under storage or use at temperatures of greater than or equal to 37°C to 42°C, such as at least or approximately or about 37°C or 40°C. Thus, for example, in a solution formulated with 600 U/mL of a hyaluronan-degrading enzyme, for example rHuPH20, at least or about at least 360 Units/mL, 365 U/mL, 370 U/mL, 375 U/mL, 380 U/mL, 390 U/mL, 420 U/mL, 480 U/mL, 540 U/mL, 546 U/mL, 552 U/mL, 558 U/mL, 564 U/mL, 570 U/mL, 576 U/mL, 582 U/mL, 588 U/mL, 594 U/mL or more activity is retained at temperatures of greater than or equal to 37°C to 42°C, such as at least or approximately or about 37°C or 40°C, for at least one month. In other examples, stability can be assessed as function of recovery of the enzyme, for example, using RP-HPLC. In such examples, in the formulations provided herein the hyaluronidase enzyme recovery is from between or about between 60 % to 140 %. For example, in the formulations provided herein the hyaluronidase enzyme recovery is from between or about between 3-7 μg/mL.

2. Divalent Cation

The stable hyaluronan-degrading enzyme formulations provided herein contain an amount of a divalent cation to achieve at least 50%, and generally at least 70%, of the initial enzymatic activity of the hyaluronan-degrading enzyme at temperatures of between or approximately between 37°C to 42°C, such as at least or about or approximately 37°C or 40 °C, for at least three (3) days and generally at least one month (*e.g.* 4 weeks) as described herein. For example, the amount of divalent

cation is an amount to achieve at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of the initial enzymatic activity of the hyaluronan-degrading enzyme for at least three (3) days, and generally for at least 4 weeks at temperatures between or approximately between 37°C to 42°C, such as at least or about or approximately 40 °C.

For example, stable hyaluronan-degrading formulations provided herein can contain an amount of Lys-Lys, salt, derivative, analogue or mimetic thereof, to achieve at least 50%, and generally at least 70%, of the initial enzymatic activity of the hyaluronan-degrading enzyme at temperatures between or approximately between 37°C to 42°C, such as at least or about or approximately 40 °C, for at least three (3) days and generally for at least 4 weeks. Such stable hyaluronan-degrading enzyme (*e.g.* a hyaluronidase for example a PH20) formulation provided herein contain between or about between 5 mM to 300 mM Lys-Lys, such as 10 mM to 200 mM, 50 mM to 150 mM or 10 mM to 50 mM. For example, stable hyaluronan-degrading enzyme (*e.g.* a hyaluronidase for example a PH20) formulation provided herein contains at least or about at least or 5 mM, 10 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, 100 mM, 125 mM, 150 mM, 200 mM, 300 mM or more Lys-Lys.

In another example, stable hyaluronan-degrading formulations provided herein can contain an amount of $MgCl_2$, derivative, analogue or mimetic thereof, to achieve at least 50%, and generally at least 70%, of the initial enzymatic activity of the hyaluronan-degrading enzyme at temperatures between or approximately between 37°C to 42°C, such as at least or about or approximately 40 °C, for at least three (3) days and generally for at least 4 weeks. Such stable hyaluronan-degrading enzyme (*e.g.* a hyaluronidase for example a PH20) formulation provided herein contain between or about between 5 mM to 300 mM $MgCl_2$, such as 10 mM to 200 mM, 50 mM to 150 mM or 10 mM to 50 mM. For example, stable hyaluronan-degrading enzyme (*e.g.* a hyaluronidase for example a PH20) formulation provided herein contains at least or about at least or 5 mM, 10 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, 100 mM, 125 mM, 150 mM, 200 mM, 300 mM or more $MgCl_2$.

As discussed below, formulations containing a divalent cation (e.g. Lys-Lys), if necessary, also can contain a tonicity modifier (*e.g.* NaCl).

3. pH and Buffer

Provided herein are stable formulations of a hyaluronan-degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20) that have a pH of between or of about between 6.5 to 7.8 or 6.8 to 7.8 such as between or about between 6.5 to 7.5 or 7.0 to 7.6. Reference to pH herein is based on measurement of pH at room temperature. It is understood that the pH can change during storage over time, but typically will remain between or between about pH 6.5 to 7.8, for example between or about between 6.8 to or to about 7.8. For example, the pH can vary by ± 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2, 1.3, 1.4, 1.5 or more. Thus, it is understood that reference to a formulation that has a pH of about or at least pH 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4 or 7.6 includes co-formulations that have a pH of or of about or at least 6.5 ± 0.2, 6.6 ± 0.2, 6.7 ± 0.2, 6.8 ± 0.2, 6.9 ± 0.2, 7.0 ± 0.2, 7.1 ± 0.2, 7.2 ± 0.2, 7.3 ± 0.2, 7.4 ± 0.2, 7.5 ± 0.2 or 7.6 ± 0.2 when prepared.

If necessary, pH can be adjusted using acidifying agents to lower the pH or alkalizing agents to increase the pH. Exemplary acidifying agents include, but are not limited to, acetic acid, citric acid, sulfuric acid, hydrochloric acid, monobasic sodium phosphate solution, and phosphoric acid. Exemplary alkalizing agents include, but are not limited to, dibasic sodium phosphate solution, sodium carbonate, or sodium hydroxide.

Any buffer can be used in co-formulations provided herein so long as it does not adversely affect the stability of the formulation, and supports the requisite pH range required. Examples of particularly suitable buffers include Tris, succinate, acetate, phosphate buffers, histidine, citrate, aconitate, malate and carbonate. Those of skill in the art, however, will recognize that formulations provided herein are not limited to a particular buffer, so long as the buffer provides an acceptable degree of pH stability, or "buffer capacity" in the range indicated. Generally, a buffer has an adequate buffer capacity within about 1 pH unit of its pK (Lachman *et al.* 1986). Buffer suitability can be estimated based on published pK tabulations or can be determined empirically by methods well known in the art. The pH of the solution can

be adjusted to the desired endpoint within the range as described above, for example, using any acceptable acid or base.

Buffers that can be included in the co-formulations provided herein include, but are not limited to, Tris (Tromethamine), histidine, phosphate buffers, such as dibasic sodium phosphate, and citrate buffers. For example, the buffer can be a histidine hydrochloride (histidine/HCl) buffer. Generally, the buffering agent is present in an amount herein to maintain the pH range of the co-formulation between or about between 6.5 to 7.8, for example between or about between 6.8 to 7.8 such as between or about between 7.0 to 7.6. Such buffering agents can be present in the formulations at concentrations between or about between 1 mM to 100 mM, such as 10 mM to 50 mM or 20 mM to 40 mM, such as at or about 30 mM. For example, such buffering agents can be present in the co-formulations in a concentration of or about or at least 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, 15 mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, 55 mM, 60 mM, 65 mM, 70 mM, 75 mM, or more.

In some examples, a buffering agent is not required.

4. Surfactant

The stable formulations provided herein contain one or more surfactants. Such surfactants inhibit aggregation of the hyaluronan-degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20) and minimize absorptive loss. The surfactants generally are non-ionic surfactants. Surfactants that can be included in the formulations herein include, but are not limited to, partial and fatty acid esters and ethers of polyhydric alcohols such as of glycerol, or sorbitol, poloxamers and polysorbates. For example, exemplary surfactants in the formulations herein include any one or more of poloxamer 188 (PLURONICS® such as PLURONIC® F68), TETRONICS®, polysorbate 20, polysorbate 80, PEG 400, PEG 3000, Tween® (*e.g.* Tween® 20 or Tween® 80), Triton® X-100, SPAN®, MYRJ®, BRIJ®, CREMOPHOR®, polypropylene glycols or polyethylene glycols. In some examples, the formulations herein contain poloxamer 188, polysorbate 20, polysorbate 80, generally poloxamer 188 (pluronic F68). The formulations provided herein generally contain at least one surfactant, such as 1, 2 or 3 surfactants.

In the formulations provided herein, the total amount of the one or more surfactants as a percentage (%) of mass concentration (w/v) in the formulation can be, for example, between from or between about from 0.0005% to 1.0%, such as between or about between 0.0005% to 0.005%, 0.001% to .01%, 0.01% to 0.5%, 0.01% to 0.1% or 0.01% to 0.02%. Generally, the formulations contain at least 0.01% surfactant and contain less than 1.0%, such as less than 0.5% or less than 0.1% surfactant. For example, the formulations provided herein can contain at or about 0.001%, 0.005%, 0.01%, 0.015%, 0.02%, 0.025%, 0.03%, 0.035%, 0.04%, 0.045%, 0.05%, 0.055%, 0.06%, 0.065%, 0.07%, 0.08%, or 0.09% surfactant. In particular examples, the formulations provided herein contain or contain about 0.01% to or to about 0.05% surfactant.

It is found herein that oxidation of the enzyme is increased with increasing levels of surfactant. Also, the surfactant poloxamer 188 causes less oxidation than the polysorbates. Hence, the formulations herein generally contain poloxamer 188. Thus, although surfactants are able to stabilize a hyaluronan-degrading enzyme, the inclusion of surfactants in the formulations provided herein can result in oxidation of the hyaluronan-degrading enzyme at high concentrations. Thus, generally lower concentrations of surfactant are used in the co-formulations herein, for example, as a percentage (%) of mass concentration (w/v) of less than 1.0 % and generally between or about between 0.01 % or 0.05 %, such as 0.01%. Also, as provided herein below, optionally an anti-oxidation agent can be included in the formulation to reduce or prevent oxidation.

5. Anti-Oxidation Agent

The formulations provided herein also can contain antioxidants to reduce or prevent oxidation, in particular oxidation of the hyaluronan-degrading enzyme. For example, the examples herein show that oxidation can be effected by high concentrations of surfactant. Exemplary antioxidants include, but are not limited to, cysteine, tryptophan and methionine. In particular examples, the anti-oxidant is methionine. The formulations provided herein can include an antioxidant at a concentration from between or from about between 5 mM to or to about 50 mM, such as 5 mM to 40 mM, 5 mM to 20 mM or 10 mM to 20 mM. For example, methionine can be provided in the formulations herein at a concentration from between or from

about between 5 mM to or to about 50 mM, such as 5 mM to 40 mM, 5 mM to 20 mM or 10 mM to 20 mM. For example, an antioxidant, for example methionine, can be included at a concentration that is or is about or is at least 5 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, 15 mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 21 mM, 22 mM, 23 mM, 24 mM, 25 mM, 26 mM, 27 mM, 28 mM, 29 mM, 30 mM, 35 mM, 40 mM, 45 mM or 50 mM. In some examples, the co-formulations contain 10 mM to 20 mM methionine, such as or about or at least 10 mM or 20 mM methionine.

6. Tonicity Modifier

Optionally, the stable hyaluronan-degrading enzyme formulations provided herein can contain a tonicity modifier. In particular, a tonicity modifier is necessary in formulations containing lower concentrations of a divalent cation, such as Lys-Lys, since sufficient tonicity is not achieved.

For example, a tonicity modifier is included in the formulations herein to produce a solution with the desired osmolarity. The formulations provided herein have an osmolarity of between or about between 245 mOsm/kg to 500 mOsm/kg. For example, the osmolarity is or is about or at least 245 mOsm/kg, 250 mOsm/kg, 255 mOsm/kg, 260 mOsm/kg, 265 mOsm/kg, 270 mOsm/kg, 275 mOsm/kg, 280 mOsm/kg, 285 mOsm/kg, 290 mOsm/kg, 295 mOsm/kg, 300 mOsm/kg, 350 mOsm/kg, 400 mOsm/kg, 450 mOsm/kg or 500 mOsm/kg. Typically, a tonicity modified is included in the formulations herein that contain a divalent cation, such as Lys-Lys, in a concentration that is less than 100 mM, such as less than 80 mM, 70 mM, 60 mM, 50 mM, 40 mM, 30 mM, 20 mM, 10 mM or less. For example, a tonicity modified is included in the formulations herein that contain a divalent cation, such as Lys-Lys, at a concentration of between or about between 10 mM to 50 mM, such as about or approximately 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM or 50 mM.

Tonicity modifiers include, but are not limited to, glycerin, NaCl, amino acids, polyalcohols, trehalose, and other salts and/or sugars. For example, the formulations provided herein can optionally include NaCl as a tonicity modifier. The NaCl can be included at a concentration of between or about between 0 mM to 200 mM, such as generally 30 mM to 100 mM, 50 mM to 160 mM, for example 50 mM to 120 mM or 80 mM to 140 mM. Generally, the NaCl is less than 150 mM, and generally less than

140 mM, 130 mM, 120 mM, 110 mM, 100 mM, 90 mM, 80 mM, 70 mM, 60 mM, 50 mM, 40 mM, 30 mM, 20 mM, 10 mM or less. The particular amount is a function of the concentration of divalent cation, for example Lys-Lys. For example, the higher concentration of Lys-Lys, the lower the concentration of NaCl (or no NaCl). The particular amount can be empirically determined in order to retain enzyme activity and/or tonicity.

In another example, glycerin (glycerol) is optionally included in the stable formulations. For example, formulations provided herein typically contain less than 60 mM glycerin, such as less than 55 mM, less than 50 mM, less than 45 mM, less than 40 mM, less than 35 mM, less than 30 mM, less than 25 mM, less than 20 mM, less than 15 mM, 10 mM or less. The amount of glycerin typically depends on the amount of divalent cation (e.g. Lys-Lys) present: the more divalent cation (e.g. Lys-Lys) present in the formulation, the less glycerin is required to achieve the desired osmolarity. Thus, in some cases, little or no glycerin need be included in the formulation.

7. Other Agents or Excipients

The stable formulations provided herein can optionally contain one or more other agents, carriers, excipients or preservatives. For example, exemplary stabilizers that optionally can be included in the stable hyaluronan-degrading enzyme formulations provided herein include, but are not limited to, amino acids, amino acid derivatives, amines, sugars, polyols, salts and buffers, surfactants, and other agents. For example, included among the types of stabilizers that optionally can be contained in the formulations herein is an amino acid stabilizer or a hyaluronidase inhibitor (e.g. a hyaluronidase substrate, such as hyaluronan). Exemplary amino acid stabilizers, amino acid derivatives or amines include, but are not limited to, L-Arginine, Glutamine, glycine, Lysine, Methionine, Proline, Lys-Lys, Gly-Gly, Trimethylamine oxide (TMAO) or betaine. Exemplary of sugars and polyols include, but are not limited to, glycerol, sorbitol, mannitol, inositol, sucrose or trehalose. Exemplary of salts and buffers include, but are not limited to, magnesium chloride, sodium sulfate, Tris such as Tris (100 mM), or sodium Benzoate. Exemplary surfactants include, but are not limited to, poloxamer 188 (*e.g.* Pluronic® F68), polysorbate 80 (PS80), polysorbate 20 (PS20). Other stabilizers include, but are not limited to, hyaluronic

acid (HA), human serum albumin (HSA), phenyl butyric acid, taurocholic acid, polyvinylpyrolidone (PVP) or zinc. In particular examples herein, the stable hyaluronan-degrading enzymes do not contain HSA and are HSA-free formulations.

For stable formulations formulated for multiple dose administration, the formulations also can optionally contain an amount of preservative(s) that, when combined with the components set forth above, result in a stable formulation. When included, the preservatives are present in a sufficient concentration to provide the anti-microbial requirements of, for example, the United States Pharmacopoeia (USP) and the European Pharmacopoeia (EP). Table 22, in Example 7E below, sets forth these requirements, including the minimum EP anti-microbial requirements (EPA) and the preferred EP anti-microbial requirements (EPB). Typically, formulations that meet EP (EPA or EPB) anti-microbial requirements contain more preservative than those formulated only to meet USP anti-microbial requirements. Generally, when included, the formulations provided herein contain preservative(s) in an amount that exhibits anti-microbial activity by killing or inhibiting the propagation of microbial organisms in a sample of the composition as assessed in an antimicrobial preservative effectiveness test (APET) as discussed elsewhere herein. Non-limiting examples of preservatives that can be included in the formulations provided herein include, but are not limited to, phenol, meta-cresol (m-cresol), methylparaben, benzyl alcohol, thimerosal, benzalkonium chloride, 4-chloro-1-butanol, chlorhexidine dihydrochloride, chlorhexidine digluconate, L-phenylalanine, EDTA, bronopol (2-bromo-2-nitropropane-1,3-diol), phenylmercuric acetate, glycerol (glycerin), imidurea, chlorhexidine, sodium dehydroacetate, ortho-cresol (o-cresol), para-cresol (p-cresol), chlorocresol, cetrimide, benzethonium chloride, ethylparaben, propylparaben or butylparaben and any combination thereof. In one example, the preservative in the formulation contains at least one phenolic preservative. For example, the formulation contains phenol, m-cresol or phenol and m-cresol. When included in the formulations provided herein, the total amount of the one or more preservative agents as a percentage (%) of mass concentration (w/v) in the formulation can be, for example, between from or between about from 0.1% to 0.4%, such as 0.1% to 0.3%, 0.15% to 0.325%, 0.15% to 0.25%, 0.1% to 0.2%, 0.2% to 0.3%, or 0.3% to 0.4%, and generally less than 0.4% (w/v) preservative, for example,

at least or about at least 0.1% , 0.12%, 0.125%, 0.13%, 0.14%, 0.15%, 0.16%, 0.17%, 0.175%, 0.18%, 0.19%, 0.2%, 0.25%, 0.3%, 0.325%, 0.35% but less than 0.4% total preservative.

Optionally, the formulations can include carriers such as a diluent, adjuvant, excipient, or vehicle with which the formulation is administered. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin. Such compositions will contain a therapeutically effective amount of the compound, generally in purified form or partially purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, and sesame oil. Water is a typical carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions also can be employed as liquid carriers, particularly for injectable solutions.

For example, pharmaceutically acceptable carriers used in parenteral preparations include aqueous vehicles, nonaqueous vehicles, antimicrobial agents, isotonic agents, buffers, antioxidants, local anesthetics, suspending and dispersing agents, emulsifying agents, sequestering or chelating agents and other pharmaceutically acceptable substances. Examples of aqueous vehicles include Sodium Chloride Injection, Ringers Injection, Isotonic Dextrose Injection, Sterile Water Injection, Dextrose and Lactated Ringers Injection. Nonaqueous parenteral vehicles include fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil and peanut oil. Antimicrobial agents in bacteriostatic or fungistatic concentrations can be added to parenteral preparations packaged in multiple-dose containers, which include phenols or cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride. Isotonic agents include sodium chloride and dextrose. Buffers include phosphate and citrate. Antioxidants include sodium bisulfate. Local anesthetics include procaine hydrochloride. Suspending and dispersing agents include sodium carboxymethylcellulose, hydroxypropyl methylcellulose and polyvinylpyrrolidone. Emulsifying agents include Polysorbate 80 (TWEEN 80). A sequestering or chelating

agent of metal ions include EDTA. Pharmaceutical carriers also include ethyl alcohol, polyethylene glycol and propylene glycol for water miscible vehicles and sodium hydroxide, hydrochloric acid, citric acid or lactic acid for pH adjustment.

Compositions can contain along with an active ingredient: a diluent such as lactose, sucrose, dicalcium phosphate, or carboxymethylcellulose; a lubricant, such as magnesium stearate, calcium stearate and talc; and a binder such as starch, natural gums, such as gum acacia, gelatin, glucose, molasses, polyvinylpyrrolidone, celluloses and derivatives thereof, povidone, crospovidones and other such binders known to those of skill in the art.

For example, an excipient protein can be added to the formulation that can be any of a number of pharmaceutically acceptable proteins or peptides. Generally, the excipient protein is selected for its ability to be administered to a mammalian subject without provoking an immune response. For example, human serum albumin is generally well-suited for use in pharmaceutical formulations, although it typically is not included in the stable formulations herein.. Other known pharmaceutical protein excipients include, but are not limited to, starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, and ethanol. The excipient is included in the formulation at a sufficient concentration to prevent adsorption of the protein to the holding vessel or vial. The concentration of the excipient will vary according to the nature of the excipient and the concentration of the protein in the co-formulation.

A composition, if desired, also can contain minor amounts of wetting or emulsifying agents, or pH buffering agents, for example, acetate, sodium citrate, cyclodextrin derivatives, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, and other such agents.

8. Exemplary Stable Hyaluronan-Degrading Enzyme Formulations

Provided herein are stable hyaluronan-degrading enzyme formulations that are stable at temperatures of 37 °C to 42 °C , such as greater than or equal to 37°C or 40 °C, for at least three (3) days, and generally at least one month that contain.

In one example, an exemplary formulation contains: 100 U/mL to 1000 U/mL, such as 100 U/mL to 500 U/mL or 100 U/mL to 300 U/mL of a hyaluronan-degrading

enzyme such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20), and in particular at least or about at least or about 155 U/mL of a hyaluronan-degrading enzyme such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20); from or from about 5 mM to or to about 200 mM, such as 10 mM to 50 mM or 5 mM to 20 mM Lys-Lys (*e.g.* at least or about at least 10 mM, 20 mM, 30 mM, 40 mM or 50 mM); from or from about 0 mM to or to about 300 mM dibasic sodium phosphate (*e.g.* from or from about 0 mM to 150 mM or 5 mM to 50 mM dibasic sodium phosphate, such as at least or about at least 5 mM, 10 mM, 20 mM, 30 mM, 40 mM or 50 mM, 100 mM or 150 mM); 0 mM to or to about 50 mM methionine (*e.g.* between or about between 5 mM to 20 mM, such as at least or about at least 5 mM, 10 mM, 20 mM, 30 mM, 40 mM or 50 mM methionine); and from or from about 0.01 % to or to about 0.5 % poloxamer 188, such as 0.01% to 0.05% (*e.g.* at least or about at least 0.01%, 0.02%, 0.03%, 0.04% or 0.05% polysorbate 80). The formulations are prepared with a pH from or from about 6.5 to 7.6, such as from or from about 6.5 to 7.2 or 7.0 to or to about 7.6 (*e.g.* at least or about at least pH 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5 or 7.6). In further examples, NaCl is included at a concentration less than 140 mM. For example, NaCl is included in a concentration of or about 50 mM to 150 mM, such as at least or about at least 50 mM, 60 mM, 70 mM, 80 mM, 90 mM or 100 mM.

In another example, an exemplary formulation contains: 100 U/mL to 1000 U/mL, such as 100 U/mL to 500 U/mL or 100 U/mL to 300 U/mL of a hyaluronan-degrading enzyme such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20), and in particular at least or about at least or about 155 U/mL of a hyaluronan-degrading enzyme such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20); from or from about 5 mM to or to about 200 mM, such as between or about between 50 mM to 150 mM $MgCl_2$ (*e.g.* at least or about at least 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, 100 mM, 110 mM, 120 mM, 130 mM or 150 mM); from or from about 0 mM to or to about 300 mM histidine hydrochloride (*e.g.* from or from about 0 mM to 150 mM or 5 mM to 50 mM histidine hydrochloride, such as at least or about at least 5 mM, 10 mM, 20 mM, 30 mM, 40 mM or 50 mM, 100 mM or 150 mM); from or from about 0 mM to or to about 50 mM methionine (*e.g.* between or about between 5 mM to 20 mM, such as at least or about at least 5 mM, 10 mM, 20 mM, 30 mM, 40 mM or 50 mM methionine); and from or from about 0.01 % to or to about 0.5 % poloxamer 188,

such as 0.01% to 0.05% (*e.g.* at least or about at least 0.01%, 0.02%, 0.03%, 0.04% or 0.05% polysorbate 80). The formulations are prepared with a pH from or from about 6.5 to 7.6, such as from or from about 6.5 to 7.2 or 7.0 to or to about 7.6 (*e.g.* at least or about at least pH 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5 or 7.6).

E. INSULIN POLYPEPTIDES

Provided herein are co-formulations of a hyaluronan-degrading enzyme and an insulin. The co-formulations provided herein contain a fast-acting insulin, such as a regular insulin or an insulin analog (*e.g.* called a fast-acting analog or a rapid-acting analog, used interchangeably herein) that is modified (*e.g.* by amino acid replacement) to reduce self-association of insulin and result in more rapid dissociation of hexamers.

Insulin is a polypeptide composed of 51 amino acid residues that is 5808 daltons in molecular weight. It is produced in the beta-cell islets of Langerhans in the pancreas. An exemplary human insulin is translated as a 110 amino acid precursor polypeptide, preproinsulin (SEQ ID NO:101), containing a 24 amino acid signal peptide to ER, the signal sequence is cleaved, resulting in proinsulin (SEQ ID NO:102). The proinsulin molecule is subsequently converted into a mature insulin by actions of proteolytic enzymes, known as prohormone convertases (PC1 and PC2) and by actions of the exoprotease carboxypeptidase E. This results in removal of 4 basic amino acid residues and the remaining 31 amino acid C-peptide or connecting chain (corresponding to amino acid residues 57 to 87 of the preproinsulin polypeptide set forth in SEQ ID NO:101) The resulting insulin contains a 21 amino acid A-chain (corresponding to amino acid residues 66 to 86 of the proinsulin polypeptide set forth in SEQ ID NO:102) and a 30 amino acid B-chain (corresponding to amino acid residues 1 to 30 of the proinsulin polypeptide set forth in SEQ ID NO:102), which are cross-linked by disulfide bonds. Typically, mature insulin contains three disulfide bridges: one between position 7 of the A-chain and position 7 of the B-chain, a second between position 20 of the A-chain and position 19 of the B-chain, and a third between positions 6 and 11 of the A-chain. The sequence of the A chain of a mature insulin is set forth in SEQ ID NO:103 and the sequence of the B-chain is set forth in SEQ ID NO:104.

Reference to insulin includes preproinsulin, proinsulin and insulin polypeptides in single-chain or two-chain forms, truncated forms thereof that have activity, and includes allelic and species variants, variants encoded by splice variants and other variants, such as insulin analogs or other derivatized forms, including polypeptides that have at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the precursor polypeptide set forth in SEQ ID NO:101 or the mature form thereof, so long as the insulin binds to the human insulin receptor to initiate a signaling cascade that results in an increase of glucose uptake and storage and/or a decrease of endogenous glucose production. For example, insulins include species variants of insulin. These include, but are not limited to, insulins derived from bovine (set forth in SEQ ID NO:133) and porcine (SEQ ID NO:123). Bovine insulin differs from human insulin at amino acids 8 and 10 of the A chain, and amino acid 30 of the B chain. Porcine insulin only differs from human insulin at amino acid 30 in the B chain where, like the bovine sequence, there is an alanine substitution in place of threonine. Other exemplary species variants of insulin are set forth in any of SEQ ID NOS: 105-146.

Also included among variants of insulin are insulin analogs that contain one or more amino acid modifications compared to a human insulin set forth in SEQ ID NO: 103 and 104 (A and B chains). These variants include fast-acting or longer-acting insulin analogs (all designated herein as a fast-acting insulin analog, although it is understood that for purposes herein this includes rapid-acting and longer-acting insulin analog forms). Exemplary insulin analogs (A and B chains), including fast-acting and longer-acting analog forms, are set forth in SEQ ID NOS:147-165, 182-184). For example, insulin analogs include, but are not limited to, glulisine (LysB3, GluB29; set forth in SEQ ID NO:103 (A-chain) and SEQ ID NO:149 (B-chain)), HMR-1 153 (LysB3, IleB28; set forth in SEQ ID NO:103 (A-chain) and SEQ ID NO:182 (B-chain)), HMR-1423 (GlyA21, HisB31, HisB32; set forth in SEQ ID NO:183 (A-chain) and SEQ ID NO:184 (B-chain)), insulin aspart (AspB28; set forth in SEQ ID NO:103 (A-chain) and SEQ ID NO:147 (B-chain)), and insulin lispro (LysB28, ProB29; set forth in SEQ ID NO:103 (A-chain) and SEQ ID NO:148 (B-chain)). In every instance above, the nomenclature of the analogs is based on a description of the amino acid substitution at specific positions on the A or B chain of

insulin, numbered from the N-terminus of the chain, in which the remainder of the sequence is that of natural human insulin.

Hence, regular insulin as provided in co-formulations herein is a mature insulin that contains a sequence of amino acids set forth in SEQ ID NOS: 103 and 104. Exemplary of a regular human insulin is recombinant human insulin designated Humulin® R. Regular insulins also includes species variants of mature insulin having an A and B chain, for example, mature forms of any of SEQ ID NOS: 105-146. Other exemplary insulin analogs included in the co-formulations herein include, but are not limited to an insulin that has a sequence of amino acids set forth in SEQ ID NO:103 (A-chain) and SEQ ID NO:149 (B-chain); a sequence of amino acids set forth in SEQ ID NO:103 (A-chain) and SEQ ID NO:147 (B-chain); or a sequence of amino acids set forth in SEQ ID NO:103 (A-chain) and SEQ ID NO:148 (B-chain).

Any of the above insulin polypeptides include those that are produced by the pancreas from any species, such as a human, and also include insulins that are produced synthetically or using recombinant techniques. For example, as described elsewhere herein, insulin can be produced biosynthetically by expressing synthetic genes for A and B chains of insulin, by expressing the entire proinsulin and exposing it to the appropriate enzymatic and chemical methods to generate a mature insulin, or by expressing A and B chains connected by a linker peptide (*see e.g.*, DeFelippis *et al.* (2002) *Insulin Chemistry and Pharmacokinetics*. In Ellenberg and Rifkin's Diabetes Mellitus (pp. 481-500) McGraw-Hill Professional).

Insulins also include monomeric and oligomeric forms, such as hexameric forms. Insulin can exist as a monomer as it circulates in the plasma, and it also binds to its receptor while in a monomeric form. Insulin, however, has a propensity to self-associate into dimers, and in the presence of metal ions such as Zn^{2+} can readily associate into higher order structures such as hexamers. There are two symmetrical high affinity binding sites for Zn^{2+}, although other weaker zinc-binding sites also have been reported (*see e.g.*, DeFelippis *et al.* (2002) *Insulin Chemistry and Pharmacokinetics*. In Ellenberg and Rifkin's Diabetes Mellitus (pp. 481-500) McGraw-Hill Professional). Self-association is important for the stability of the molecule to prevent chemical degradation and physical denaturation. Thus, in storage vesicles in pancreatic beta-cells, insulin exists as a hexamer. Upon release into the

extracellular space, however, it is believed that the insulin hexamers can experience a change in pH to more neutral conditions and the zinc ion-containing hexamers are diluted, which destabilizes the hexamer. There may be other reasons contributing to the destabilization of the insulin hexamer in the extracellular space. Insulin is thus predominantly found in the blood as a monomer. To take advantage of the stabilizing effects, most commercial formulations of insulin contain zinc ions in sufficient amounts to promote self-association into hexamers. The hexameric structure, however, slows down the absorption rate of these formulations upon subcutaneous administration.

Insulin is used as a therapeutic for glycemic control, such as in diabetic patients. There are various types of insulin formulations that exist, depending on whether the insulin is being administered to control glucose for basal therapy, for prandial therapy, or for a combination thereof. Insulin formulations can be provided solely as fast-acting formulations, solely as basal-acting formulations (*i.e.*, intermediate-acting and/or long-acting forms), or as mixtures thereof (*see e.g.*, Table 2) . Typically, mixtures contain a fast-acting and an intermediate- or long-acting insulin. For example, fast-acting insulins can be combined with an NPH insulin (an exemplary intermediate-acting insulin as discussed below) in various mixture ratios including 10:90, 20:80, 30:70, 40:60, and 50:50. Such premixed preparations can reduce the number of daily insulin injections by conveniently providing both meal-related and basal insulin requirements in a single formulation.

Preparations of insulin include an insulin polypeptide or variant (*i.e.* analog) thereof formulated in a specific manner. In some instances, it is the components and substances in the formulation that impart different properties on the insulin, such as different duration of action. For example, most insulin preparations contain a metal ion, such as zinc, in the formulation, which stabilizes the insulin by promoting self-association of the molecule. Self-association into hexameric forms can affect the absorption of insulin upon administration. Further, some longer-acting basal insulin formulations are prepared by precipitating insulin from an acetate buffer (instead of phosphate) by the addition of zinc. Large crystals of insulin with high zinc content, when collected and resuspended in a solution of sodium acetate-sodium chloride (pH 7.2 to 7.5), are slowly absorbed after subcutaneous injection and exert an action of

long duration. This crystal preparation is named extended insulin zinc suspension (ultralente insulin). Other zinc-containing insulin preparations include, for example, semilente insulins (prompt insulin zinc suspensions) and lente insulins (insulin zinc suspensions), which differ predominantly in the zinc concentration used. Zinc-containing insulin preparations also include those that are modified by protamine, such as NPH insulin.

In another example, a precipitation agent, such as protamine, can be added to an insulin polypeptide to generate a microcrystalline suspension. Typically, crystalline insulins have a prolonged duration of action compared to insulins that do not exist in crystalline form. A protamine zinc insulin, when injected subcutaneously in an aqueous suspension, dissolves only slowly at the site of deposition, and the insulin is absorbed at a retarded rate. Protamine zinc suspension insulin has largely been replaced by isophane insulin suspension, also known as NPH insulin. It is a modified protamine zinc insulin suspension that is crystalline. The concentrations of insulin, protamine, and zinc are so arranged that the preparation has an onset and a duration of action intermediate between those of regular insulin and protamine zinc insulin suspension.

Further, pH differences in the preparations also influence the type and property of insulin. Most insulins are formulated at neutral pH. One exception is insulin glargine, which is provided as a commercial formulation at pH 4.0. By virtue of the addition of two arginines to the C-terminus of the B-chain, the isoelectric point of the glargine insulin is shifted making it more soluble at an acidic pH. An additional amino acid change exists in the A chain (N21G) to prevent deamidation and dimerization resulting from an acid-sensitive asparagine. The sequence of the A chain of glargine insulin is set forth in SEQ ID NO:150 and the B-chain is set forth in SEQ ID NO:151. Since exposure to physiologic pH occurs upon administration, microprecipitates are formed, which make glargine similar to a crystalline, long-acting insulin.

Table 4 below summarizes various types of insulin, their onset of action and their application.

TABLE 4: Types of Insulins

Type	Brand name	Onset	Peak	Duration	Application
Fast-acting:	Lispro (*e.g.*	5-15	45-90	3-4 hours	Post-prandial

Insulin analogs	Humalog®); Aspart (*e.g.*, NovoLog®); Glulisine	minutes	minutes		glucose control
Fast-acting: Regular insulin	Regular Insulin (*e.g.*, Humulin® R; Novolin® R; Velosulin® Human)	30 minutes – 1 hour	2-5 hours	5-8 hours	Post-prandial glucose control
Intermediate-Acting	Lente® (*e.g.*, Humulin® L, Novolin® L); NPH (*e.g.*, Humulin® N, Novolin® N);	1-3 hours	6-12 hours	20-24 hours	Basal insulin supplementation
Long-lasting	Ultralente (*e.g.* Humulin® U); glargine; detemir (an analog)	4-6 hours	18-28 hours	28 hours	Basal insulin supplementation
Mixtures	Humulin® 50/50; Humulin® 70/30; Novolin® 70/30; Humalog® Mix 75/25	Varies	Varies	Varies	

The most commonly used insulins are fast-acting insulins, which include regular insulin (*i.e.* native or wildtype insulin, including allelic and species variants thereof) and fast-acting insulin analogs. For purposes herein, reference to insulin is a fast-acting insulin, unless specifically noted otherwise.

Fast-Acting Insulins

Fact-acting insulins that can be used in the co-formulations provided herein of insulin and a hyaluronan degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20), include regular insulin, which is the wild-type or native insulin, and fast-acting insulin analogs. By virtue of their fast absorption rate compared to basal-acting insulins, fast-acting insulins are used predominantly for post-prandial control purposes. Exemplary fast-acting insulins are set forth in Table 5 below. Fast-acting insulins also include any known in the art, such as, but not limited to, any insulin preparations and devices disclosed in U.S. Pat. No. 7,279,457 and U.S. Pat. Pub. Nos. 20070235365, 20080039368, 20080039365, 20070086952, 20070244467, and 20070191757. Any fast-acting insulin can be combined in co-formulations with a hyaluronan degrading enzyme provided herein. Such a

formulation also can further include a mixture of a fast-acting insulin with an intermediate or long-acting insulin, in addition to a hyaluronan degrading enzyme.

TABLE 5. Fast Acting Insulins				
Name	**Species**	**A-chain (SEQ ID NO)**	**B-chain (SEQ ID NO)**	**Commercial Name**
Regular Insulin	Human	103	104	Humulin R®; Novolin® R; Velosulin®
Regular Insulin	Porcine	88-108 of SEQ ID NO:123	25-54 of SEQ ID NO:123	Iletin II®;
Insulin Aspart	Human analog	103	147	Novolog®
Insulin Lispro	Human analog	103	148	Humalog®
Insulin Glulisine	Human analog	103	149	Apidra®

a. Regular Insulin

Regular insulins include the native or wildtype insulin polypeptide. These include human insulin, as well as insulins from bovine, porcine and other species. Regular human insulins are marketed as Humulin® R, Novolin® R and Velosulin®. Porcine insulin was marketed as Iletin II®. Generally, regular insulin, when administered subcutaneously alone, has an onset of action of 30 minutes. Maximal plasma levels are seen in 1-3 hours and the duration of intensity increases with dosage. The plasma half-life following subcutaneous administration is about 1.5 hours.

b. Fast-Acting Analogs (also called rapid-acting insulins)

Fast-Acting insulin analogs, which are often called rapid-acting insulins in the art, are modified forms of insulin that typically contain one or more amino acid changes. The analogs are designed to reduce the self-association of the insulin molecule for the purpose of increasing the absorption rate and onset of action as compared to regular insulin. Generally, such analogs are formulated in the presence of zinc, and thus exist as stable zinc hexamers. Due to the modification, however, they have a quicker dissociation from the hexameric state after subcutaneous administration compared to regular insulin.

i. Insulin Lispro

Human insulin lispro is an insulin polypeptide formulation containing amino acid changes at position 28 and 29 of the B-chain such that the Pro-Lys at this position in wild-type insulin B-chain set forth in SEQ ID NO:104 is inverted to Lys-Pro. The sequence of insulin lispro is set forth in SEQ ID NO:103 (A-chain) and SEQ ID NO: 148 (B-chain). It is marketed under the name Humalog® (insulin lispro, rDNA origin). The result of the inversion of these two amino acids is a polypeptide with a decreased propensity to self-associate, which allows for a more rapid onset of action. Specifically, the sequence inversion in the B-chain results in the elimination of two hydrophobic interactions and weakening of two beta-pleated sheet hydrogen bonds that stabilize the dimer (*see e.g.*, DeFelippis *et al.* (2002) *Insulin Chemistry and Pharmacokinetics.* In Ellenberg and Rifkin's Diabetes Mellitus (pp. 481-500) McGraw-Hill Professional). The polypeptide self-associates and forms hexamers as a result of excipients provided in the formulation, such as antimicrobial agents (*e.g.* m-cresol) and zinc for stabilization. Nevertheless, due to the amino acid modification, insulin lispro is more rapidly acting then regular insulin.

ii. Insulin Aspart

Human insulin aspart is an insulin polypeptide formulation containing an amino acid substitution at position 28 of the B-chain of human insulin set forth in SEQ ID NO:104 from a proline to an aspartic acid. The sequence of insulin aspart is set forth in SEQ ID NO:103 (A-chain) and SEQ ID NO:147 (B-chain). It is marketed under the name Novolog® (insulin aspart [rDNA origin] injection). The modification in insulin aspart confers a negatively-charged side-chain carboxyl group to create charge repulsion and destabilize the monomer-monomer interaction. Further, the removal of the proline eliminates a key hydrophobic interaction between monomers (*see e.g.*, DeFelippis *et al.* (2002) *Insulin Chemistry and Pharmacokinetics*. In Ellenberg and Rifkin's Diabetes Mellitus (pp. 481-500) McGraw-Hill Professional). The analog exists largely as a monomer, and is less prone to aggregate compared to other fast-acting analogs such as lispro. Generally, insulin aspart and insulin lispro are similar in their respective pharmacokinetic and phamacodynamic properties.

iii. Insulin Glulisine

Human insulin glulisine is an insulin polypeptide formulation containing an amino acid substitution in the B-chain at position B3 from asparagine to lysine and at

amino acid B29 from lysine to glutamic acid compared to the sequence of the B-chain of human insulin set forth in SEQ ID NO:104. The sequence of insulin glulisine is set forth in SEQ ID NO:103 (A-chain) and SEQ ID NO:149 (B-chain). It is marketed under the name Apidra® (insulin glulisine [rDNA origin] injection). The modifications render the polypeptide molecule less prone to self-association compared to human insulin. Unlike other insulin analogs, the polypeptide is commercially formulated in the absence of the hexamer-promoting zinc (Becker *et al.* (2008) *Clinical Pharmacokinetics*, 47:7-20). Hence, insulin glulisine has a more rapid rate of onset than insulin lispro and insulin aspart.

F. STABLE CO-FORMULATIONS OF INSULIN AND HYALURONAN-DEGRADING ENZYME

Provided herein are stable co-formulations of insulin, in particular fast-acting insulins including regular insulin and rapid acting insulin analogs (also called fast-acting insulin analogs), and hyaluronan degrading enzymes, such as soluble hyaluronidases (*e.g.* rHuPH20). Exemplary of the formulations provided herein are stable co-formulations of a rapid acting insulin analog and a PH20 or C-terminally truncated fragment thereof that is soluble and active (*e.g.* rHuPH20). The provided compositions containing a hyaluronan degrading enzyme and a fast-acting insulin are formulated for stability at various temperatures or under various conditions. The co-formulations provided herein are stable at from or about from 0° C to 40° C or under various stress conditions (*e.g.* agitation) for several hours, days, weeks, months or years as described herein. Hence, the formulations are suitable for multi-dose use or are suitable for other use conditions that require elevated temperatures or agitation. For example, the co-formulations are suitable for multi-dose injectable (MDI) formulations as well as continuous subcutaneous infusion (CSI) formulations. The co-formulations provided herein are formulated for administration by subcutaneous, intraperitoneal, intradermal, intramuscular, injection and transdermal routes. Exemplary formulations are formulated for subcutaneous administration.

The stable co-formulations provided herein are multi-dose formulations. Hence, all formulations provided herein contain an insulin (*e.g.* a fast-acting insulin such as a rapid acting insulin analog), a hyaluronan-degrading enzyme (*e.g.* a PH20), a preservative, and one or more other stabilizing excipients.

As described herein and exemplified in the Examples, it is found that due to the opposing requirements for stability of a hyaluronan-degrading enzymes, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20), and a fast-acting insulin, co-formulations cannot simply be achieved by mixing formulations of the two. For example, correct NaCl concentrations and pH are critical to the stability of co-formulations of insulin and hyaluronan-degrading enzyme (*e.g.* rHuPH20 or other soluble hyaluronidases and hyaluronan degrading enzymes). Determining the optimal NaCl concentration and pH is complicated by the opposite effect these parameters have on insulin and the exemplary hyaluronan-degrading enzyme rHuPH20. Insulin solubility is maximal at higher pH and lower NaCl concentration. These conditions, however, are detrimental to rHuPH20, which loses stability at higher pH and lower NaCl concentration. The stability of the exemplary hyaluronan-degrading enzyme rHuPH20 can be increased by increasing NaCl concentrations and lowering pH. However, such conditions have a negative effect of the solubility of insulin and insulin analogs, which precipitate at low pH and high NaCl concentration. Thus, among the objects herein is the provision of optimal NaCl concentrations and pH for stable formulations of insulin and rHuPH20 (or other soluble hyaluronidases and hyaluronan degrading enzymes) or provision of stable co-formulations that contain no NaCl or lower NaCl concentrations.

As described herein, the different stable formulations can be used for multiple drug injection (MDI) or can be used for continuous subcutaneous insulin infusion (CSII). The two modes of administration have differing requirements for stability. In particular, co-formulations for CSII need to be stable under accelerated (or stress) conditions, such as elevated temperatures and under agitation, while co-formulations for MDI, which can be stored at refrigerated or ambient temperatures until use, do not need to be stable at elevated temperatures and under agitation. Thus, as described elsewhere herein, the excipients or concentrations of excipients that promote stability under each of these storage conditions are not necessarily the same. For example, further excipients or stabilizers or differing concentrations of excipients or stabilizers are required to maintain stability at or at about 32-40 °C or under agitation than is required to maintain stability of the hyaluronan-degrading enzyme and/or insulin at or

at about 20-30° C or at or at about 2-8° C. These same stabilizers may not be compatible with stability of the formulations at the lower temperatures.

For example, it is found herein that while insulin is generally not stable under high NaCl concentrations and low pH conditions when stored or used at low temperatures less than 32°C, such conditions are conducive to insulin solubility at higher temperatures of 32° C to 40° C for at least 3 days. Thus, conditions of high NaCl and low pH can be present in co-formulations for use during CSII, which is an administration therapy that requires stability at higher temperatures. It is shown herein that formulations containing low pH (*e.g.* pH 6.8) and high NaCl (*e.g.* 200 mM) are stable at elevated temperatures, and thus suitable for CSII for at least 3 days at 37° C.. Low pH (*e.g.* pH 6.8) and high NaCl (*e.g.* 200 mM) are not suitable for stability under lower storage temperatures, such as under refrigeration or ambient temperatures.

Also, the stabilizer hyaluronan (HA) is an efficient stabilizer and maintains stability of the hyaluronan-degrading enzyme at elevated temperatures for use in CSII without exhibiting any detrimental effect on insulin solubility. In this case, while hyaluronan promotes stability of the hyaluronan degrading enzyme at elevated temperature, the solubility of the insulin at refrigerated temperatures is reduced. Thus, the presence of HA in an MDI formulation for longer term storage at lower temperatures can impact insulin solubility.

It also is found herein that Lys-Lys is a particularly good stabilizer of a hyaluronan-degrading enzyme, in particular at elevated temperatures greater than 37°C. Unlike $MgCl_2$, which also is a particularly strong stabilizer of hyaluronan-degrading enzyme at elevated temperatures, Lys-Lys can be made to be compatible with insulins while maintaining solubility. For example, lower concentrations of Lys-Lys and the presence of one or more other stabilizers retains hyaluronan-degrading enzyme activity and insulin solubility under accelerated conditions such as elevated temperatures. Therefore, such Lys-Lys containing co-formulations also can be suitable for CSII applications.

Although the excipients or concentrations of excipients for a stable MDI or CSII formulation are not necessarily the same, the MDI formulations provided herein can be used to generate stable CSII formulations. Thus, in some examples, the MDI

co-formulations, which are stable at refrigerated and ambient temperatures but not necessarily elevated temperatures and under stress, are diluted with a diluent that has a lower pH and higher salt concentration. This produces a formulation with a lower pH and higher salt concentration compared to the MDI formulation, and which is therefore stable at elevated temperatures and stress conditions (*e.g.* under agitation) and suitable for CSII. Typically, such CSII co-formulations are not stored at refrigerated temperatures due to the insolubility of insulin in compositions with low pH and high salt concentration.

Typically, the compounds are formulated into pharmaceutical compositions using techniques and procedures well known in the art (*see e.g.*, Ansel *Introduction to Pharmaceutical Dosage Forms*, Fourth Edition, 1985, 126). Pharmaceutically acceptable compositions are prepared in view of approvals for a regulatory agency or other agency prepared in accordance with generally recognized pharmacopeia for use in animals and in humans. The formulation should suit the mode of administration.

The co-formulations can be provided as a pharmaceutical preparation in liquid form as solutions, syrups or suspensions. In liquid form, the pharmaceutical preparations can be provided as a concentrated preparation to be diluted to a therapeutically effective concentration before use. Generally, the preparations are provided in a dosage form that does not require dilution for use. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). In another example, pharmaceutical preparations can be presented in lyophilized form for reconstitution with water or other suitable vehicle before use.

The volume of the co-formulations provided herein can be any volume suitable for the container in which it is provided. In some examples, the co-formulations are provided in a vial, syringe, pen, reservoir for a pump or a closed loop system, or any other suitable container. For example, the co-formulations provided herein are between or about between 0.1 mL to 500 mL, such as 0.1 mL to 100 mL, 1 mL to 100 mL, 0.1 mL to 50 mL, such as at least or about at least or about or 0.1 mL,

1 mL, 2 mL, 3 mL, 4 mL, 5 mL, 10 mL, 15 mL, 20 mL, 30 mL, 40 mL, 50 mL or more.

As described herein below, in some examples, the co-formulations are prepared as concentrated formulations of insulin and hyaluronan degrading enzymes, which are subsequently diluted with an appropriate diluent for use. In such instances, the concentrated co-formulations can be specifically formulated for long term storage at, for example, from or from about 2° C to or to about 8° C. Upon dilution, the co-formulation can be used directly for MDI applications. On the other hand, since the requirements for multi-dose formulations used in MDI or for CSII therapy can be different, the components of the diluent can be chosen to ensure stability of the diluted co-formulation for applications of the co-formulation at elevated temperatures or under agitation. For example, as discussed above and further below, the diluent can contain, for example, a requisite amount or level of components or stabilizing agents that is compatible with stability of the co-formulation at elevated temperatures or under stress conditions (*e.g.* agitation), which are characteristic conditions of CSII therapy. Thus, when the concentrated co-formulation of insulin and hyaluronan-degrading enzyme is diluted with the diluent, the new diluted co-formulation is stable at, for example, elevated temperatures such as at least or about at least 32° C to 40° C, such as about or 37° C or other stress conditions (*e.g.* agitation), for example, for use in CSII therapy.

Provided below is a description of the components that are provided in the stable co-formulations herein. The particular balance of requirements to maximize stability of both proteins as contained in the co-formulations provided herein now renders administration with a multi-dose injectable formulation and with a CSII system (*e.g.* closed-pump administration) of the co-formulation achievable. A description of each of the components or conditions, such as excipients, stabilizers or pH, is provided below.

1. Components of Stable Co-Formulations

Provided herein are stable co-formulations that contain a therapeutically effective amount of a hyaluronan-degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20). The co-formulations also contain a therapeutically effective amount of a fast-acting insulin, such as a rapid-acting (*e.g.* fast-acting)

insulin analog. In examples of co-formulations provided herein, the co-formulations further contain NaCl at a concentration of between or about between 50 mM to 200 mM, such as 80-140 mM, a pH of between or about between 6.5 to 8.0, for example, 6.5 to 7.8 or 6.8 to 7.8 such as between or about between 6.5 to 7.5 or 7.0 to 7.6, a buffering agent that maintains the pH range, an anti-microbially effective amount of a preservative or mixture of preservatives, and a stabilizing agent in an amount that, over the course of storage (temperature and time), retains at least 50% of the hyaluronan-degrading enzyme activity and retains at least 90% of the insulin purity, recovery and/or potency. For example, the co-formulations provided herein contain 0.01 % to 0.5 % surfactant as a stabilizing agent. The co-formulations can optionally contain additional stabilizing agents or an anti-oxidation agent. In some examples herein, the co-formulations is stable for at least 6 months at a temperature of from or from about 2 °C to or to about 8 °C and at least 14 days (*i.e.* 2 weeks) at a temperature of from or about 20 °C to or to about 30 °C. Such co-formulations can be used for multi-dose injection (MDI) use. In other examples, the co-formulations provided herein are stable under accelerated conditions such as elevated temperatures greater than or about greater than 32° C such as 35° C to 40° C, in particular greater than at or about or 37° C or 40° C and/or agitation conditions for at least 3 hours, and generally at least 3 days. Such co-formulations can be used for continuous subcutaneous insulin infusion (CSII) methods.

Also provided herein are co-formulations that do not contain NaCl or contain a lesser amount of NaCl, such as less than 140 mM NaCl, and generally 0 mM to 100 mM NaCl, for example, 0 mM to 50 mM, 10 mM to 40 mM, 20 mM to 30 mM, such as at least or about at least or 30 mM NaCl. In such examples, it is found herein that Lys-Lys can be included in an amount to stabilize the hyaluronan-degrading enzyme and insulin, even in the absence of NaCl. Optionally, NaCl can be included in such formulations, for example, as a tonicity modifier. This can be required, for example, if the concentration of Lys-Lys is 50 mM of Lys-Lys or less.

Thus, in some examples of co-formulations provided herein, the co-formulations contain a therapeutically effective amount of a hyaluronan-degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20). The co-formulations also contain a therapeutically effective amount of a fast-acting insulin,

such as a rapid-acting (*e.g.* fast-acting) insulin analog. In examples of co-formulations provided herein, the co-formulations further contain Lys-Lys at a concentration of between or about between 50 mM to 120 mM, such as 50 to 80 mM, 80 mM to 100 mM or 100 mM to 120 mM, a pH of between or about between 6.5 to 8.0, for example, 6.5 to 7.8 or 6.8 to 7.8 such as between or about between 6.5 to 7.5 or 7.0 to 7.6, a buffering agent that maintains the pH range, an anti-microbially effective amount of a preservative or mixture of preservatives, and a stabilizing agent in an amount that, over the course of storage (temperature and time), retains at least 50% of the hyaluronan-degrading enzyme activity and retains at least 90% of the insulin purity, recovery and/or potency. For example, the co-formulations provided herein contain 0.0005% to 1.0% (*e.g.* 0.0005% to 0.005%) surfactant as a stabilizing agent. The co-formulations can optionally contain additional stabilizing agents, tonicity modifiers, an anti-oxidation agent and/or other excipients. For example, the co-formulations contain NaCl as a concentration of less than 140 mM, such as between or about between 0 mM to 100 mM, for example between or about between 0 mM to 50 mM, 10 mM to 40 mM or 20 mM to 30 mM. In some examples herein, the co-formulations is stable for at least 6 months at a temperature of from or from about 2 °C to or to about 8 °C and at least 14 days (*i.e.* 2 weeks) at a temperature of from or about 20 °C to or to about 30 °C. Such co-formulations can be used for multi-dose injection (MDI) use. In other examples, the co-formulations provided herein are stable under accelerated conditions such as elevated temperatures greater than or about greater than 32° C such as 35° C to 40° C, in particular greater than at or about or 37° C or 40° C and/or agitation conditions for at least 3 hours, and generally at least 3 days, Such co-formulations can be used for continuous subcutaneous insulin infusion (CSII) methods.

a. Fast-Acting Insulin

The co-formulations provided herein contain a therapeutically effective amount of a fast-acting insulin, such as a rapid acting insulin analog. The insulin can be any fast-acting insulin as described in Section E. The fast-acting insulin can be regular insulin. In particular examples, the insulin is a fast-acting insulin that is a rapid acting insulin analog, for example, insulin lispro, insulin aspart or insulin glulisine. For example, the therapeutically effective amount can be an amount

between or about between 10 Units/mL to 1000 U/mL, 100 U/mL to 1000 U/mL, or 500 U/mL to 1000 U/mL, such as at least or about at least 10 U/mL, 20 U/mL, 30 U/mL, 40 U/mL, 50 U/mL, 60 U/mL, 70 U/mL, 80 U/mL, 90 U/mL, 100 U/mL, 150 U/mL, 200 U/mL, 250 U/mL, 300 U/mL, 350 U/mL, 400 U/mL, 450 U/ml, 500 U/mL or 1000 U/mL. For example, the co-formulations provided herein contain a fast-acting insulin, such as a rapid acting insulin analog (*e.g.* insulin lispro, insulin aspart or insulin glulisine) in an amount that is at least or at least about or is or is about 100 U/mL.

In the stable co-formulations provided herein, the stability of the insulin, including insulin analogs, in the formulations is a function of the recovery, purity and/or activity of the insulin under storage at various temperatures (*e.g.* 2° C-8° C, 20° C-30° C or elevated temperatures of at least or about 32° C to 40° C) and times (*e.g.* hours, days, weeks or months) or use conditions (*e.g.* agitation) as described herein. Assays to assess these parameters are discussed below. The formulations provided herein retain insulin recovery, purity and/or activity such that the formulations are suitable for therapeutic use as described herein. For example, in the formulations provided herein, the insulin purity (*e.g.* as assessed by RP-HPLC or other similar method) over time and under storage or use conditions as described herein is at least 85 % of the purity of insulin in the formulation prior to storage or use, for example, at least 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or more. Generally, for insulin purity (*e.g.* by RP-HPLC) the target acceptable specification is at least or about 90 % purity or about or greater than 90 % purity. In other examples, insulin purity can be assessed as a function of aggregation of the insulin, for example, using non-denaturing or denaturing size exclusion chromatography (SEC). In such examples, in the formulations provided herein, over time and under storage or use conditions as described herein the insulin in the formulation contains less than 2 % high molecular weight (HMWt) insulin species by peak area, for example, less than 1.9 %, 1.8 %, 1.7 %, 1.6 %, 1.5 %, 1.4 %, 1.3 %, 1.2 %, 1.1 %, 1.0 % or less. Over time (*e.g.* hours, days, weeks or month) and under storage (*e.g.* at various temperatures and time) or use (*e.g.* agitation) conditions as described herein, the insulin in the formulations provided herein retains greater than or about 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or more of its

recovery or activity. Thus, in a solution formulated with 100 Units/mL insulin, at least or about 90 U/mL, 91 U/mL, 92 U/mL, 93 U/mL, 94 U/mL, 95 U/mL, 96 U/mL, 97 U/mL, 98 U/mL or 99 U/mL remains over time of hours, days, weeks or months under storage or use at temperatures of 2°C-8°C, 20°C-30° C or elevated temperatures of at least or about 32° C to 40° C or under conditions of agitation as described herein.

b. Hyaluronan-Degrading Enzyme

The co-formulations provided herein contain a therapeutically effective amount of a hyaluronan-degrading enzyme, such as any described in Section C, for example a hyaluronidase such as a PH20 (*e.g.* rHuPH20). The amount of hyaluronan degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20), in the co-formulations provided herein is an amount that is sufficient to render the composition super-fast acting. For example, the amount is functionally equivalent to at least or about at least 30 Units/mL. For example, the co-formulations provided herein contain a hyaluronan-degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20) in an amount between or about between 30 Units/mL to 20,000 U/mL, 300 U/mL to 15,000 U/mL, 300 U/mL to 10,000 U/mL, 300 U/mL to to 5,000 U/mL, 300 U/mL to 3000 U/mL, 300 U/mL to 2000 U/mL, 600 U/mL to 20,000 U/mL, 600 U/mL to 15,000 U/mL, 600 U/mL to 10,000 U/mL, 600 U/mL to 6000 U/mL, 600 U/mL to 4000 U/mL, 600 U/mL to 2000 U/mL, 600 U/mL to 1000 U/mL, 60 U/mL to 600 U/mL, or 100 U/mL to 300 U/mL, such as at least or about at least 30 U/mL, 35 U/mL, 40 U/mL, 50 U/mL, 100 U/mL, 200 U/mL, 300 U/mL, 400 U/mL, 500 U/mL, 600 U/mL, 700 U/mL, 800 U/mL, 900 U/mL, 1000 U/ml, 2000 U/mL, 3000 U/mL, 4000 U/mL, 5000 U/mL, 6000 U/mL, 7000 U/mL, 8000 U/mL, 9000 U/mL, 10,000 U/mL, 12,000 U/mL, 15,000 U/mL or 20,000 U/mL. For example, the co-formulations provided herein contain a PH20 (*e.g.* rHuPH20) that is in an amount that is at least 100 U/mL to 1000 U/mL, for example at least or about at least or about or 600 U/mL.

In the co-formulations provided herein the stability of a hyaluronan-degrading enzyme, including a hyaluronidase such as a PH20 (*e.g.* rHuPH20), in the formulations is a function of the recovery and/or activity of the enzyme under storage at various temperatures (*e.g.* 2°C-8°C, 20°C-30°C or elevated temperatures of at least

or about 32° C to 40° C) and times (*e.g.* hours, days, weeks or months) or use conditions (*e.g.* agitation) as described herein. Assays to assess these parameters are discussed below. The formulations provided herein retain hyaluronidase recovery and/or activity such that the formulations are suitable for therapeutic use as described herein. In the stable co-formulations provided herein, the activity of the hyaluronan degrading enzyme, such as a hyaluronidase, for example a PH20, typically is greater than or about 50%, such as greater than or at least 55%, 60 %, 65 %, 70 %, 80 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 % or more of the activity of enzyme in the formulation prior to storage or use. Generally, for hyaluronidase activity the target acceptable specification for stability is at least 62 % of the initial activity of the enzyme for hours, days, weeks or months under storage or use at temperatures of 2°C-8°C, 20°C-30°C or elevated temperatures of at least or about 32° C to 40° C or under conditions of agitation as described herein. Thus, for example, in a solution formulated with 600 U/mL of a hyaluronan-degrading enzyme, for example rHuPH20, at least or about at least 360 Units/mL, 365 U/mL, 370 U/mL, 375 U/mL, 380 U/mL, 390 U/mL, 420 U/mL, 480 U/mL, 540 U/mL, 546 U/mL, 552 U/mL, 558 U/mL, 564 U/mL, 570 U/mL, 576 U/mL, 582 U/mL, 588 U/mL, 594 U/mL or more activity is retained over time and under storage or use conditions. For example, in the stable co-formulations provided herein, over time and under storage or use conditions (*e.g.* agitation), at least 375 U/mL of hyaluronan-degrading enzyme activity is retained. In other examples, stability can be assessed as function of recovery of the enzyme, for example, using RP-HPLC. In such examples, in the formulations provided herein the hyaluronidase enzyme recovery is from between or about between 60 % to 140 %. For example, in the formulations provided herein the hyaluronidase enzyme recovery is from between or about between 3-7 μg/mL.

c. Preservative

For use as a multi-dose formulation, the co-formulations provided herein contain a preservative(s). As discussed above, preservatives can have a deleterious effect on the solubility of insulin and the stability and activity of hyaluronan degrading enzymes, such as a PH20 (*e.g.* rHuPH20), while at the same time stabilizing the hexameric insulin molecules and being necessary as an anti-microbial agent in multidose formulations. Thus, one of the objects herein is to identify the type

and concentration of preservative(s) that can be used in stable co-formulations of insulin, including rapid acting insulin analogs, and hyaluronan degrading enzymes, such as soluble hyaluronidases (*e.g.* rHuPH20).

The one or more preservatives present in the co-formulation cannot substantially destabilize the hyaluronan degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20), so that it loses its activity over storage conditions (*e.g.* over time and at varied temperature) as described herein. Further, these preservatives must be present in a sufficient concentration to stabilize the insulin hexamers and exert the required anti-microbial effect, but not be so concentrated as to decrease solubility of the insulin. Importantly, the preservatives must be present in a sufficient concentration to provide the anti-microbial requirements of, for example, the United States Pharmacopoeia (USP) and the European Pharmacopoeia (EP). Table 22, in Example 7E below, sets forth these requirements, including the minimum EP anti-microbial requirements (EPA) and the preferred EP anti-microbial requirements (EPB). Typically, formulations that meet EP (EPA or EPB) anti-microbial requirements contain more preservative than those formulated only to meet USP anti-microbial requirements.

Hence, the co-formulations provided herein contain preservative(s) in an amount that exhibits anti-microbial activity by killing or inhibiting the propagation of microbial organisms in a sample of the composition as assessed in an antimicrobial preservative effectiveness test (APET). One of skill in the art is familiar with the antimicrobial preservative effectiveness test and standards to be meet under the USP and EPA or EPB in order to meet minimum requirements. In general, the antimicrobial preservative effectiveness test involves challenging a composition, *e.g.*, a co-formulation provided herein, with prescribed inoculums of suitable microorganisms, *i.e.*, bacteria, yeast and fungi, storing the inoculated preparation at a prescribed temperature, withdrawing samples at specified intervals of time and counting the organisms in the sample (see, Sutton and Porter, (2002) *PDA Journal of Pharmaceutical Science and Technology* 56(11);300-311; The United States Pharmacopeial Convention, Inc., (effective January 1, 2002), *The United States Pharmacopeia 25th Revision*, Rockville, MD, Chapter <51> Antimicrobial Effectiveness Testing; and European Pharmacopoeia, Chapter 5.1.3, Efficacy of

Antimicrobial Preservation). The microorganisms used in the challenge generally include three strains of bacteria, namely *E. coli* (ATCC No. 8739), *Pseudomonas aeruginosa* (ATCC No. 9027) and *Staphylococcus aureus* (ATCC No. 6538), yeast (*Candida albicans* ATCC No. 10231) and fungus (*Aspergillus niger* ATCC No. 16404), all of which are added such that the inoculated composition contains 10^5 or 10^6 colony forming units (cfu) of microorganism per mL of composition. The preservative properties of the composition are deemed adequate if, under the conditions of the test, there is a significant fall or no increase, as specified in Table 6, below, in the number of microorganisms in the inoculated composition after the times and at the temperatures prescribed. The criteria for evaluation are given in terms of the log reduction in the number of viable microorganism as compared to the initial sample or the previous timepoint.

Table 6. USP and EP requirements for antimicrobial effectiveness testing

USP	**Criteria for passage**
Bacteria	Not less than 1.0 log reduction from the initial calculated count at 7 days, not less than 3.0 log reduction from the initial count at 14 days, and no increase from the 14 days count at 28 days. No increase is defined as not more than 0.5 $\log_{10}$ unit higher than the previous measured value.
Yeast or mold	No increase from the initial calculated count at 7, 14 and 28 days. No increase is defined as not more than 0.5 $\log_{10}$ unit higher than the previous measured value.
EPA	**Criteria for passage**
Bacteria	2 log reduction in the number of viable microorganisms against the value obtained for the inoculum at 6 hours, a 3 log reduction in the number of viable microorganisms against the value obtained for the inoculum at 24 hours and no recovery at 28 days.
Yeast or mold	2 log reduction in the number of viable microorganisms against the value obtained for the inoculum at 7 days and no increase at 28 days. No increase is defined as not more than 0.5 $\log_{10}$ unit higher than the previous measured value.
EPB	**Criteria for passage**
Bacteria	1 log reduction in the number of viable microorganisms against the value obtained for the inoculum at 24 hours, a 3 log reduction in the number of viable microorganisms against the value obtained for the inoculum at 7 days and no increase at 28 days. No increase is defined as not more than 0.5 $\log_{10}$ unit higher than the previous measured value.
Yeast or mold	1 log reduction in the number of viable microorganisms against the value obtained for the inoculum at 7 days and no increase at 28 days. No increase is defined as not more than 0.5 $\log_{10}$ unit higher than the

	previous measured value.

Specifically, the composition, for example, the co-formulation, is aliquoted into at least 5 containers, one each for each of the bacteria or fungi (*Escherichia coli* (ATCC No. 8739), *Pseudomonas aeruginosa* (ATCC No. 9027), *Staphylococcus aureus* (ATCC No. 6538), *Candida albicans* (ATCC No. 10231) and *Aspergillus niger* (ATCC No. 16404)). Each container is then inoculated with one of the test organisms to give an inoculum of 10^5 or 10^6 microogranisms per mL of the composition, with the inoculum not exceeding 1 % of the volume of the composition. The inoculated compositions are maintained at a temperature between 20 and 25 °C for a period of 28 days, and samples removed at 6 hours, 24 hours, 7 days, 14 days and 28 days, depending upon the criteria set forth in Table 6 above. The number of viable microorganisms (cfu) in each sample is determined by plate count or membrane filtration. Finally, the cfu for each sample is compared to either the inoculum or the previous sample and log reduction is determined.

Under USP standards, the rate or level of the anti-microbial activity of preservatives in samples inoculated with the microbial organisms is at least a 1.0 $\log_{10}$ unit reduction of bacterial organisms at 7 days following inoculation; at least a 3.0 $\log_{10}$ unit reduction of bacterial organisms at 14 days following inoculation; and at least no further increase, *i.e.*, not more than a 0.5 $\log_{10}$ unit increase, in bacterial organisms from day 14 to day 28 following inoculation of the composition with the microbial inoculum. For fungal organisms according to USP standards, the rate or level of the anti-microbial activity of preservatives in samples inoculated with the microbial organisms is at least no increase from the initial amount after 7, 14 and 28 days following inoculation of the composition with the microbial inoculum. Under EPB, or minimum EP standards, the rate or level of the anti-microbial activity of preservatives in samples inoculated with the microbial organisms is at least 1 $\log_{10}$ unit reduction of bacterial organisms at 24 hours following inoculation; at least a 3 $\log_{10}$ unit reduction of bacterial organisms at 7 days following inoculation; and at least no further increase, *i.e.*, not more than a 0.5 $\log_{10}$ unit increase, in bacterial organisms 28 days following inoculation of the composition with the microbial inoculum. EPA standards require at least a 2 $\log_{10}$ unit reduction of bacterial organisms at 6 hours following inoculation, with at least a 3 $\log_{10}$ unit reduction of

bacterial organisms at 24 hours following inoculation, and no recovery of microbial organisms 28 days after inoculation. For fungal organisms according to minimum EPB standards, the rate or level of the anti-microbial activity of preservatives in samples inoculated with the microbial organisms is at least 1 $\log_{10}$ unit reduction of fungal organisms at 14 days following inoculation and no increase in fungal organisms at 28 days following inoculation of the composition, and increased EPA standards require a 2 $\log_{10}$ unit reduction at 7 days following inoculation and no increase in fungal organisms at 28 days following inoculation of the composition.

Non-limiting examples of preservatives that can be included in the co-formulations provided herein include, but are not limited to, phenol, meta-cresol (m-cresol), methylparaben, benzyl alcohol, thimerosal, benzalkonium chloride, 4-chloro-1-butanol, chlorhexidine dihydrochloride, chlorhexidine digluconate, L-phenylalanine, EDTA, bronopol (2-bromo-2-nitropropane-1,3-diol), phenylmercuric acetate, glycerol (glycerin), imidurea, chlorhexidine, sodium dehydroacetate, ortho-cresol (o-cresol), para-cresol (p-cresol), chlorocresol, cetrimide, benzethonium chloride, ethylparaben, propylparaben or butylparaben and any combination thereof. For example, co-formulations provided herein can contain a single preservative. In other examples, the co-formulations contain at least two different preservatives or at least three different preservatives. For example, co-formulations provided herein can contain two preservatives such as L-phenylalanine and m-cresol, L-phenylalanine and methylparaben, L-phenylalanine and phenol, m-cresol and methylparaben, phenol and methylparaben, m-cresol and phenol or other similar combinations. In one example, the preservative in the co-formulation contains at least one phenolic preservative. For example, the co-formulation contains phenol, m-cresol or phenol and m-cresol.

In the co-formulations provided herein, the total amount of the one or more preservative agents as a percentage (%) of mass concentration (w/v) in the formulation can be, for example, between from or between about from 0.1% to 0.4%, such as 0.1% to 0.3%, 0.15% to 0.325%, 0.15% to 0.25%, 0.1% to 0.2%, 0.2% to 0.3%, or 0.3% to 0.4%. Generally, the co-formulations contain less than 0.4% (w/v) preservative. For example, the co-formulations provided herein contain at least or about at least 0.1%, 0.12%, 0.125%, 0.13%, 0.14%, 0.15%, 0.16%, 0.17%, 0.175%,

0.18%, 0.19%, 0.2%, 0.25%, 0.3%, 0.325%, 0.35% but less than 0.4% total preservative.

Exemplary preservatives used in the stable co-formulations of insulin and hyaluronan degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20) are phenol and m-cresol. In some examples, the percentage (%) of mass concentration (w/v) of phenol in the co-formulation is greater than the percentage (%) of mass concentration (w/v) of m-cresol. This is due, at least in part, to the more detrimental effects of m-cresol on the stability of the hyaluronan-degrading enzyme (*e.g.* rHuPH20) in solution, particularly at elevated temperatures, compared to phenol (see *e.g.* Example 7). Thus, in the co-formulations provided herein, the ratio as a percentage of mass concentration of phenol:meta-cresol is greater than or is about 1:1, 1.1:1, 1.2:1, 1.3:1, 1.4:1, 1.5:1, 1.6:1, 1.7:1, 1.8:1, 1.9:1, 2:1, 2.1:1, 2.2:1, 2.3:1, 2.4:1, 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1 or more.

In some examples, the stable co-formulations provided herein contain between or between about 0.1% to 0.25% phenol, and between or about between 0.05% to 0.2% m-cresol, such as between or about between 0.10% to 0.2% phenol and between or about between 0.06% to 0.18% m-cresol or between or about between 0.1% to 0.15% phenol and between or about between 0.08% to 0.15% m-cresol. For example, stable co-formulations provided herein contain or contain about 0.1% phenol and 0.075% m-cresol; 0.1% phenol and 0.15% m-cresol; 0.125% phenol and 0.075% m-cresol; 0.13% phenol and 0.075% m-cresol; 0.13% phenol and 0.08% m-cresol; 0.15% phenol and 0.175% m-cresol; or 0.17% phenol and 0.13% m-cresol.

d. NaCl

Examples of stable co-formulations provided herein of insulin and a hyaluronan-degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20) can contain NaCl as a stabilizing agent. In co-formulations provided herein that contain NaCl as a stabilizing agent, the co-formulation can have a NaCl concentration of between or about between 50 mM to 200 mM, such as between or abut between 80 mM to 140 mM, 80 mM to 120 mM, 80 mM to 100 mM, 100 mM to 140 mM or 120 mM to 140 mM. For example, provided herein are co-formulations of insulin and a hyaluronan-degrading enzyme that contain about or at least or 50 mM, 60 mM, 70 mM, 80 mM, 85mM, 90 mM, 95 mM, 100 mM, 105 mM, 110 mM,

115 mM, 120 mM, 125 mM, 130 mM, 135 mM, 140 mM, 150 mM, 160 mM, 170 mM, 180 mM, 190 mM or 200 mM NaCl.

In addition, it is found herein that while insulins generally are not sufficiently soluble at high NaCl concentration and low pH conditions, in which the activity of a hyaluronan-degrading enzyme is optimal, the solubility of insulin is less affected by NaCl under accelerated conditions of elevated temperature. Thus, co-formulations that are stable under accelerated conditions (e.g. elevated temperature or agitation), such as those used for CSII therapy, typically contain a higher NaCl concentration than formulations that are stable under temperatures less than 32°C.

It also is understood that in the co-formulations provided herein, the particular pH in the co-formulation can be a function of the NaCl concentrations, and vice versa. For example, in co-formulations containing a pH of or of about 7.2, for example 7.2 ± 0.2 or lower, the co-formulations generally contain a salt concentration of or of about between 100 mM to 140 mM. In co-formulation containing a pH or of about 7.3, for example 7.3 ± 0.2 or higher, the co-formulations generally contain a salt concentration of or of about between 50 to 100 mM NaCl.

Also, as set forth in the Examples herein, insulin and insulin analogs each have different solubility requirements, which is influenced by the level of NaCl and pH in the formulation. Generally, insulin and insulin solubility favors high pH and low salt. For example, regular insulin forms precipitates within 1 week at high NaCl concentrations greater than 80 mM and at a low pH 7.0. But, regular insulin does not form precipitates over any time tested greater than 15 months with NaCl concentrations of 80 mM or less and a high pH 7.6. Similarly, insulin analogs Lispro and Aspart also exhibit salt- and pH-dependent effects on solubility with precipitates generally forming at salt concentrations greater than 80 mM and at low pH of 7.2 or 7.0. In contrast, at low salt concentrations of 80 mM or less and at high pH of 7.4 or 7.6, the insulins exhibit greater stability and little to no precipitation over time. Insulin Glulisine is the most soluble. Thus, the least soluble insulins tolerate less salt compared to the most soluble insulins. Thus, because of the differing apparent solubility of different insulins, the salt concentration for the formulations provided herein can depend on the type of insulin in the formulation, as solubility of insulin is directly related to the tolerance for salt.

It is within the level of one of skill in the art, in view of the description herein, to empirically assess the solubility and stability of insulin and hyaluronan-degrading enzymes herein as a function of the NaCl concentration, the particular insulin and the required stability parameters of the particular formulation.

e. pH

Provided herein are stable co-formulations of insulin and a hyaluronan-degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20) that have a pH of between or of about between 6.5 to 8.0, for example, 6.5 to 7.8 or 6.8 to 7.8 such as between or about between 6.5 to 7.5 or 7.0 to 7.6. Reference to pH herein is based on measurement of pH at room temperature. It is understood that the pH can change during storage over time, but typically will remain between or between about pH 6.5 to 8.0, for example between or about between 6.8 to or to about 7.8. For example, the pH can vary by ± 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2, 1.3, 1.4, 1.5 or more. Thus, it is understood that reference to a co-formulation that has a pH of about or at least pH 7.0, 7.1, 7.2, 7.3, 7.4 or 7.6 includes co-formulations that have a pH of or of about or at least 7.0 ± 0.2, 7.1 ± 0.2, 7.2 ± 0.2, 7.3 ± 0.2, 7.4 ± 0.2, 7.5 ± 0.2 or 7.6 ± 0.2 when prepared.

Since both salt and pH are opposing parameters that influence the solubility of insulin and the activity of a hyaluronan-degrading enzyme, their inclusion in the co-formulation is accordingly balanced. Thus, for example, generally, in the formulations provided herein, the lower the salt concentration, the higher the pH. In another example of the co-formulations provided herein, the higher the salt concentration, the lower the pH. It is within the level of one of skill in the art to empirically test the pH and salt requirements in co-formulations so as to achieve a desired stability and to retain activity of a hyaluronan-degrading enzyme and solubility of an insulin as described herein. For example, optimal pH and salt requirements can be obtained by formulation techniques known to those skilled in the art and exemplified herein. For example, optimal pH and salt concentrations can be determined by assessing activity or recovery of a hyaluronan-degrading enzyme and solubility, aggregation or recovery of an insulin under differing pH or salt conditions using various methods known to one of skill in the art, for example, as described in Section H.2.

For example, as discussed elsewhere herein, it is found herein that while insulins generally are not sufficiently soluble at high salt concentration and low pH conditions, in which the activity of a hyaluronan-degrading enzyme is optimal, the solubility of insulin is less affected by low pH conditions under accelerated conditions of elevated temperature. Thus, co-formulations that are stable under accelerated conditions (e.g. elevated temperature or agitation), such as those used for CSII therapy, typically contain lower pH than formulations that are stable under temperatures less than 32°C.

If necessary, pH can be adjusted using acidifying agents to lower the pH or alkalizing agents to increase the pH. Exemplary acidifying agents include, but are not limited to, acetic acid, citric acid, sulfuric acid, hydrochloric acid, monobasic sodium phosphate solution, and phosphoric acid. Exemplary alkalizing agents include, but are not limited to, dibasic sodium phosphate solution, sodium carbonate, or sodium hydroxide.

f. Buffers

Any buffer can be used in co-formulations provided herein so long as it does not adversely affect the stability of the co-formulation, and supports the requisite pH range required. Examples of particularly suitable buffers include Tris, succinate, acetate, phosphate buffers, citrate, aconitate, malate and carbonate. Those of skill in the art, however, will recognize that formulations provided herein are not limited to a particular buffer, so long as the buffer provides an acceptable degree of pH stability, or "buffer capacity" in the range indicated. Generally, a buffer has an adequate buffer capacity within about 1 pH unit of its pK (Lachman *et al.* 1986). Buffer suitability can be estimated based on published pK tabulations or can be determined empirically by methods well known in the art. The pH of the solution can be adjusted to the desired endpoint within the range as described above, for example, using any acceptable acid or base.

Buffers that can be included in the co-formulations provided herein include, but are not limited to, Tris (Tromethamine), histidine, phosphate buffers, such as dibasic sodium phosphate, and citrate buffers. Generally, the buffering agent is present in an amount herein to maintain the pH range of the co-formulation between or about between 6.5 to 8.0, for example between or about between 6.8 to 7.8 such as

between or about between 7.0 to 7.6. Such buffering agents can be present in the co-formulations at concentrations between or about between 1 mM to 100 mM, such as 10 mM to 50 mM or 20 mM to 40 mM, such as at or about 30 mM. For example, such buffering agents can be present in the co-formulations in a concentration of or about or at least 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, 15 mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, 55 mM, 60 mM, 65 mM, 70 mM, 75 mM, or more.

Exemplary of the buffers in the co-formulations herein are non-metal binding buffers such as Tris, which reduce insulin precipitation compared to metal-binding buffers, such as phosphate buffers. The inclusion of Tris as a buffer in the co-formulations provided herein has additional benefits. For example, the pH of a solution that is buffered with Tris is affected by the temperature at which the solution is held. Thus, when the insulin and hyaluronan-degrading enzyme co-formulations are prepared at room temperature at pH 7.3, upon refrigeration, the pH increases to approximately pH 7.6. Such a pH promotes insulin solubility at a temperature where insulin is otherwise likely to be insoluble. Conversely, at increased temperatures, the pH of the formulation decreases to approximately pH 7.1, which promotes hyaluronan-degrading enzyme stability at a temperature at which the enzyme is otherwise likely to become unstable. Thus, the solubility and stability of insulin and a hyaluronan-degrading enzyme, such as a hyaluronidase for example PH20 (*e.g.* rHuPH20) is maximized when the co-formulations contains Tris as a buffer compared to other buffers. Further, because Tris is a positive ion, the addition of NaCl into the solution as a counter ion is not required. This also is beneficial to the overall stability of the co-formulation because NaCl at high concentrations is detrimental to insulin solubility.

For example, Tris is included in the co-formulations provided herein at a concentration of or about 10 mM to 50 mM, such as, for example, 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM or 50 mM. In particular examples, the co-formulations contain or contain about 20 mM to 30 mM Tris, such as 21 mM, 22 mM, 23 mM, 24 mM, 25 mM, 26 mM, 27 mM, 28 mM, 29 mM or 30 mM Tris. In

particular examples, the co-formulations provided herein contain Tris at a concentration of or about 30 mM.

g. Lys-Lys

In examples herein, the co-formulations contain a divalent cation, and in particular lysyl-lysine (dilysine; Lys-Lys), or salt, derivative, analogue or mimetic thereof, sufficient to stabilize the hyaluronan-degrading enzyme in the co-formulation. For example, the divalent cation Lys-Lys exhibits less effects on insulin solubility than $MgCl_2$. The Lys-Lys is provided in an amount that, when combined with the preservatives, and other stabilizers at the appropriate pH, as discussed above, result in a stable co-formulation such that hyaluronan-degrading activity is retained and effects on insulin solubility is minimized as described herein above.

For example, Lys-Lys can be included in the co-formulations provide herein in an amount between or about between 50 mM to 120 mM, such as between or about between 50 to 80 mM, 80 to 100 mM or 100 to 120 mM. For example, Lys-Lys can be included in the co-formulations provided herein in an amount that is at least or at least about or is 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, 100 mM, 110 mM or 120 mM.

Typically, the higher concentration of Lys-Lys the better the stability of the co-formulated containing a PH20 and insulin or insulin analogs. The particular amount of Lys-Lys in the formulation, however, can be a function of the particular insulin. For example, to achieve similar stability in a co-formulation, the insulin analog glulisine requires the least amount of Lys-Lys (*e.g.* 50 to 105 mM), followed by the insulin analogs aspart and lispro (*e.g.* 80 to 100 mM), with regular insulin requiring the highest amount (*e.g.* 100 to 120 mM). It is within the level of one of skill in the art, in view of the description herein, to empirically assess the solubility and stability of insulin and hyaluronan-degrading enzymes herein as a function of the Lys-Lys concentration, the particular insulin and the required stability parameters of the particular formulation.

In one example, co-formulations containing regular insulin generally contain 100 to 120 mM Lys-Lys, such as at least or about at least or 100 mM, 105 mM, 110 mM, 115 mM or 120 mM. In another example, co-formulations containing insulin aspart or insulin lispro contain 80 to 120 mM Lys-Lys, such as at least or about at

least or 80 mM, 85 mM, 90 mM, 95 mM or 100 mM. In a further example, co-formulations containing insulin glulisine contain 50 to 105 mM Lys-Lys, such as at least or about at least or 50 mM, 55 mM, 60 mM, 65 mM, 70 mM, 75 mM, 80 mM, 85 mM, 90 mM, 95 mM, 100 mM or 105 mM.

Typically, in examples herein where the co-formulations contain Lys-Lys, the addition of NaCl as a stabilizer is not required to maintain stability of the components. In some cases, tonicity modifiers are required for tonicity reasons. For example, the amount of Lys-Lys in the co-formulation is less than 50 mM/mL, a tonicity modifier can be required. It is within the level of one of skill in the art to determine whether a tonicity modifier should be included in the co-formulation. As discussed below, exemplary tonicity modifiers include, but are not limited to, glycerin, NaCl, amino acids, polyalcohols, trehalose, and other salts and/or sugars. Hence, in some examples, stable co-formulations provided herein that contain Lys-Lys can optionally also contain NaCl. In such examples, the NaCl is generally less than 140 mM, and typically less than 100 mM, 90 mM, 80 mM, 70 mM, 50 mM, 40 mM, 30 mM, 20 mM, 10 mM or less. The particular amount can of tonicity modifier can be empirically determined in order to retain enzyme activity and/or tonicity.

h. Additional Exemplary Excipients or Stablizers

The co-formulations provided herein optionally can contain other components that, when combined with the preservatives, salt and stabilizers at the appropriate pH, as discussed above, result in a stable co-formulation. Other components include, for example, one or more tonicity modifiers, one or more anti-oxidation agents, zinc or other stabilizer.

For example, the stability of hyaluronan degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20) is greatly reduced where the co-formulations contain low NaCl, high pH, the presence of preservatives and are stored at elevated temperatures (*e.g.* 20° C to 30° C or higher). Similarly, insulin stability also can be affected by these and other parameters. Such instability can be countered to some extent by the additions of one or more stabilizers. Generally, the formulations provided herein contain a stabilizer or stabilizers in an amount that, over the course of storage (temperature and time), at least 50% of the initial activity (e.g. 375 U/mL) of hyaluronan-degrading enzyme activity is retained.

Included among the types of stabilizers that can be contained in the formulations provided herein are amino acids, amino acid derivatives, amines, sugars, polyols, salts and buffers, surfactants, and other agents. The co-formulations provided herein contain at least one stabilizer. For example, the co-formulations provided herein contain at least one, two, three, four, five, six or more stabilizers. Hence, any one or more of an amino acids, amino acid derivatives, amines, sugars, polyols, salts and buffers, surfactants, and other agents can be included in the co-formulations herein. Generally, the co-formulations herein contain at least contain a surfactant and an appropriate buffer. Optionally, the co-formulations provided herein can contain other additional stabilizers.

Exemplary amino acid stabilizers, amino acid derivatives or amines include, but are not limited to, L-Arginine, Glutamine, glycine, Lysine, Methionine, Proline, Lys-Lys, Gly-Gly, Trimethylamine oxide (TMAO) or betaine. Exemplary of sugars and polyols include, but are not limited to, glycerol, sorbitol, mannitol, inositol, sucrose or trehalose. Exemplary of salts and buffers include, but are not limited to, magnesium chloride, sodium sulfate, Tris such as Tris (100 mM), or sodium Benzoate. Exemplary surfactants include, but are not limited to, poloxamer 188 (*e.g.* Pluronic® F68), polysorbate 80 (PS80), polysorbate 20 (PS20). Other preservatives include, but are not limited to, hyaluronic acid (HA), human serum albumin (HSA), phenyl butyric acid, taurocholic acid, polyvinylpyrolidone (PVP) or zinc.

i. Surfactant

In some examples, the co-formulations provided herein contain one or more surfactants. Such surfactants inhibit aggregation of the hyaluronan-degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20) and minimize absorptive loss. The surfactants generally are non-ionic surfactants. Surfactants that can be included in the co-formulations herein include, but are not limited to, partial and fatty acid esters and ethers of polyhydric alcohols such as of glycerol, or sorbitol, poloxamers and polysorbates. For example, exemplary surfactants in the co-formulations herein include any one or more of poloxamer 188 (PLURONICS® such as PLURONIC® F68), TETRONICS®, polysorbate 20, polysorbate 80, PEG 400, PEG 3000, Tween® (*e.g.* Tween® 20 or Tween® 80), Triton® X-100, SPAN®, MYRJ®, BRIJ®, CREMOPHOR®, polypropylene glycols or polyethylene glycols.

In some examples, the co-formulations herein contain poloxamer 188, polysorbate 20, polysorbate 80, generally poloxamer 188 (pluronic F68). The co-formulations provided herein generally contain at least one surfactant, such as 1, 2 or 3 surfactants.

In the co-formulations provided herein, the total amount of the one or more surfactants as a percentage (%) of mass concentration (w/v) in the formulation can be, for example, between from or between about from 0.0005% to 1.0%, such as between from or between about from 0.0005% to 0.005%, 0.001% to 0.01%, 0.01% to 0.5%, such as 0.01% to 0.1% or 0.01% to 0.02%. Generally, the co-formulations contain at least 0.0005%, 0.005%, 0.05% or 0.01% surfactant and contain less than 1.0%, such as less than 0.5% or less than 0.1% surfactant. For example, the co-formulations provided herein can contain at or about 0.0005%, 0.0001%, 0.005%, 0.001%, 0.005%, 0.01%, 0.015%, 0.02%, 0.025%, 0.03%, 0.035%, 0.04%, 0.045%, 0.05%, 0.055%, 0.06%, 0.065%, 0.07%, 0.08%, or 0.09%. In particular examples, the co-formulations provided herein contain or contain about 0.01% to or to about 0.05% surfactant.

As shown in the Examples herein, stability and enzymatic activity of a hyaluronan-degrading enzyme (*e.g.* rHuPH20) is generally not affected between and among different surfactants or concentrations of surfactant. Nevertheless, it is found herein that oxidation of the enzyme is increased with increasing levels of surfactant. Also, the surfactant poloxamer 188 causes less oxidation than the polysorbates. Hence, the co-formulations herein generally contain poloxamer 188. Thus, although surfactants are able to stabilize a hyaluronan-degrading enzyme, the inclusion of surfactants in the co-formulations provided herein can result in oxidation of the hyaluronan-degrading enzyme at high concentrations. Thus, generally lower concentrations of surfactant are used in the co-formulations herein, for example, as a percentage (%) of mass concentration (w/v) of less than 1.0 % and generally between or about between 0.0005% to 0.1%, such as between or about between 0.01 % or 0.05 %. Also, as provided herein below, optionally an anti-oxidation agent can be included in the formulation to reduce or prevent oxidation.

Exemplary co-formulations provided herein contain poloxamer 188. Poloxamer 188 has a higher critical micelle concentration (cmc). Thus, use of poloxamer 188 can reduce the formation of micelles in the formulation, which can in turn reduce the effectiveness of the preservatives. Thus, among the co-formulations

provided herein are those that contain or contain about 0.01 % or 0.05 % poloxamer 188.

In other examples, exemplary co-formulations provided herein contain polysorbate 20. For example, co-formulations provided herein contain 0.0005% to 0.1%, such as 0.0005% to 0.01%, such as at least or about at least or 0.001% polysorbate 20.

ii. Tonicity modifier

For example, tonicity modifiers can be included in the formulation provided herein to produce a solution with the desired osmolarity. The co-formulations provided herein have an osmolarity of between or about between 245 mOsm/kg to 305 mOsm/kg. For example, the osmolarity is or is about 245 mOsm/kg, 250 mOsm/kg, 255 mOsm/kg, 260 mOsm/kg, 265 mOsm/kg, 270 mOsm/kg, 275 mOsm/kg, 280 mOsm/kg, 285 mOsm/kg, 290 mOsm/kg, 295 mOsm/kg, 300 mOsm/kg or 305 mOsm/kg. In some examples, the co-formulations of an insulin and a hyaluronan-degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20) have an osmolarity of or of about 275 mOsm/kg.

Tonicity modifiers include, but are not limited to, glycerin, NaCl, amino acids, polyalcohols, trehalose, and other salts and/or sugars. For example, NaCl can be included in the co-formulations provided herein at a concentration of between or about between 0 mM to 200 mM, such as generally 30 mM to 100 mM, 50 mM to 160 mM, for example 50 mM to 120 mM or 80 mM to 140 mM, or 50 mM to 200 mM. Typically, when used as a tonicity modifier, for example in co-formulations containing Lys-Lys, NaCl is provided in a concentration of less than 140 mM, and generally less than 130 mM, 120 mM, 110 mM, 100 mM, 90 mM, 80 mM, 70 mM, 60 mM, 50 mM, 40 mM, 30 mM, 20 mM, 10 mM or less. The particular amount can be empirically determined in order to retain enzyme activity, insulin solubility and/or tonicity.

iii. Glycerin

In other instances, glycerin (glycerol) is included in the co-formulations. For example, co-formulations provided herein typically contain less than 60 mM glycerin, such as less than 55 mM, less than 50 mM, less than 45 mM, less than 40 mM, less than 35 mM, less than 30 mM, less than 25 mM, less than 20 mM, less than 15 mM,

10 mM or less. The amount of glycerin typically depends on the amount of NaCl present: the more NaCl present in the co-formulation, the less glycerin is required to achieve the desired osmolarity. Thus, for example, in co-formulations containing higher NaCl concentrations, such as those formulated with insulins with higher apparent solubility (*e.g.* insulin glulisine), little or no glycerin need be included in the formulation. In contrast, in co-formulations containing slightly lower NaCl concentrations, such as those formulated with insulins with lower apparent solubility (*e.g.* insulin aspart), glycerin can be included. For example, co-formulations provided herein that contain insulin aspart contain glycerin at a concentration less than 50 mM, such as 20 mM to 50 mM, for example at or about 50 mM. In co-formulations containing an even lower NaCl concentration, such as those formulated with insulins with the lowest apparent solubility (*e.g.* insulin lispro or regular insulin), glycerin is included at a concentration of or of about, for example, 40 mM to 60 mM.

iv Antioxidants

The co-formulations provided herein also can contain antioxidants to reduce or prevent oxidation, in particular oxidation of the hyaluronan-degrading enzyme. For example, the examples herein show that oxidation can be effected by high concentrations of surfactant or hyaluronan oligomers. Exemplary antioxidants include, but are not limited to, cysteine, tryptophan and methionine. In particular examples, the anti-oxidant is methionine. The co-formulations provided herein containing an insulin and a hyaluronan-degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20) can include an antioxidant at a concentration from between or from about between 5 mM to or to about 50 mM, such as 5 mM to 40 mM, 5 mM to 20 mM or 10 mM to 20 mM. For example, methionine can be provided in the co-formulations herein at a concentration from between or from about between 5 mM to or to about 50 mM, such as 5 mM to 40 mM, 5 mM to 20 mM or 10 mM to 20 mM. For example, an antioxidant, for example methionine, can be included at a concentration that is or is about 5 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, 15 mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 21 mM, 22 mM, 23 mM, 24 mM, 25 mM, 26 mM, 27 mM, 28 mM, 29 mM, 30 mM, 35 mM, 40 mM, 45 mM or 50 mM. In some examples, the co-formulations contain 10 mM to 20 mM methionine, such as or about 10 mM or 20 mM methionine.

v. Zinc

In some instances, zinc is included in the co-formulations as a stabilizer for insulin hexamers. For example, formulations containing regular insulin, insulin lispro or insulin aspart typically contain zinc, whereas formulations containing insulin glulisine do not contain zinc. Zinc can be provided, for example, as zinc oxide, zinc acetate or zinc chloride. Zinc can be present in a composition provided herein at between or about between 0.001 to 0.1 mg per 100 units of insulin (mg/100 U), 0.001 to 0.05 mg per 100U or 0.01 to 05 mg per 100 U. For example, the co-formulations provided herein can contain zinc at or about 0.002 milligrams per 100 units of insulin (mg/100 U), 0.005 mg/100 U, 0.01 mg/100 U, 0.012 mg/100 U, 0.014 mg/100 U, 0.016 mg/100 U, 0.017 mg/100 U, 0.018 mg/100 U, 0.02 mg/100 U, 0.022 mg/100 U, 0.024 mg/100 U, 0.026 mg/100 U, 0.028 mg/100 U, 0.03 mg/100 U, 0.04 mg/100 U, 0.05 mg/100 U, 0.06 mg/100 U, 0.07 mg/100 U, 0.08 mg/100 U or 0.1 mg/100 U.

vi. Amino acid stabilizer

The co-formulation provided herein also can contain an amino acid stabilizer, which contributes to the stability of the preparation. The stabilizer can be a non-polar and basic amino acids. Exemplary non-polar and basic amino acids include, but are not limited to, alanine, histidine, arginine, lysine, ornithine, isoleucine, valine, methionine, glycine and proline. For example, the amino acid stabilizer is glycine or proline, typically glycine. The stabilizer can be a single amino acid or it can be a combination of 2 or more such amino acids. The amino acid stabilizers can be natural amino acids, amino acid analogues, modified amino acids or amino acid equivalents. Generally, the amino acid is an L-amino acid. For example, when proline is used as the stabilizer, it is generally L-proline. It is also possible to use amino acid equivalents, for example, proline analogues. The concentration of amino acid stabilizer, for example glycine, included in the co-formulation ranges from 0.1 M to 1 M amino acid, typically 0.1 M to 0.75 M, generally 0.2 M to 0.5 M, for example, at least at or about 0.1 M, 0.15 M, 0.2 M, 0.25 M, 0.3 M, 0.35 M, 0.4 M, 0.45 M, 0.5 M, 0.6 M, 0.7 M, 0.75 M or more. The amino acid, for example glycine, can be used in a form of a pharmaceutically acceptable salt, such as hydrochloride, hydrobromide, sulfate, acetate, etc. The purity of the amino acid, for example glycine, should be at least 98 %, at least 99 %, or at least 99.5 % or more.

vii. Hyaluronidase Inhibitor

In some examples of co-formulations provided herein, stability of a hyaluronan-degrading enzyme and a fast-acting insulin at a temperature of from or about 20 °C to or to about 30 °C for at least 14 days (*i.e.* 2 weeks) as described herein above can be increased by including a hyaluronidase inhibitor. Such an inhibitor is generally not suitable for formulations stored at 2° C to 8° C, since, as observed with hyaluronan (HA) herein, it can cause insulin to aggregate at lower temperatures. In some examples, a hyaluronidase inhibitor can be chosen that is suitable for use at 2° C to 8° C.

In particular, hyaluronidase inhibitors are included in a co-formulation to stabilize the hyaluronan-degrading enzyme to the effects of phenolic preservatives. In particular examples, the hyaluronidase inhibitor is one that reacts with insulin or hyaluronan-degrading enzyme in an associative and non-covalent manner, and does not form covalent complexes with insulin or a hyaluronan-degrading enzyme. The hyaluronidase inhibitor is provided at least at its equilibrium concentration. One of skill in the art is familiar with various classes of hyaluronidase inhibitors (*see e.g.* Girish *et al.* (2009) *Current Medicinal Chemistry*, 16:2261-2288, and references cited therein). One of skill in the art knows or can determine by standard methods in the art the equilibrium concentration of a hyaluronidase inhibitor in a reaction or stable composition herein. The choice of hyaluronidase inhibitor will depend on the particular hyaluronan-degrading enzyme used in the composition. For example, hyaluronan is an exemplary hyaluronidase inhibitor for use in the stable compositions herein when the hyaluronan-degrading enzyme is a PH20.

Exemplary hyaluronidase inhibitors for use as stabilizing agents herein include, but are not limited to, a protein, glycosaminoglycan (GAG), polysaccharides, fatty acid, lanostanoids, antibiotics, anti-nematodes, synthetic organic compounds or a plant-derived bioactive component. For example, a hyaluronidase plant-derived bioactive component can be an alkaloid, antioxidant, polyphenol, flavonoids, terpenoids and anti-inflammatory drugs. Exemplary hyaluronidase inhibitors include, for example, serum hyaluronidase inhibitor, *Withania somnifera* glycoprotein (WSG), heparin, heparin sulfate, dermatan sulfate, chitosans, β-(1,4)-galacto-oligosaccharides, sulphated verbascose, sulphated planteose, pectin, poly(styrene-4-sulfonate), dextran

sulfate, sodium alginate, polysaccharide from *Undaria pinnatifida*, mandelic acid condensation polymer, eicosatrienoic acid, nervonic acid, oleanolic acid, aristolochic acid, ajmaline, reserpine, flavone, desmethoxycentauredine, quercetin, apigenin, kaempferol, silybin, luteolin, luteolin-7-glucoside, phloretin, apiin, hesperidin, sulphonated hesperidin, calycosin-7-O-β-D-glucopyranoside, sodium flavone-7-sulphate, flavone 7-fluoro-4'-hydroxyflavone, 4'-chloro-4,6-dimethoxychalcone, sodium 5-hydroxyflavone 7-sulphate, myricetin, rutin, morin, glycyrrhizin, vitamin C, D-isoascorbic acid, D-saccharic 1,4-lactone, L-ascorbic acid-6-hexadecanoate (Vcpal), 6-O-acylated vitamin C, catechin, nordihydroguaiaretic acid, curcumin, N-propyl gallate, tannic acid, ellagic acid, gallic acid, phlorofucofuroeckol A, dieckol, 8,8'-bieckol, procyanidine, gossypol, celecoxib, nimesulide, dexamethasone, indomethcin, fenoprofen, phenylbutazone, oxyphenbutazone, salicylates, disodium cromoglycate, sodium aurothiomalate, transilist, traxanox, ivermectin, linocomycin and spectinomycin, sulfamethoxazole and trimerthoprim, neomycin sulphate, 3α-acetylpolyporenic acid A, (25S)-(+)-12α-hydroxy-3α-methylcarboxyacetate-24-methyllanosta-8,24(31)-diene-26-oic acid, lanostanoid, polyporenic acid c, PS53 (hydroquinone-sulfonic acid-formaldehyde polymer), polymer of poly (styrene-4-sulfonate), VERSA-TL 502, 1-tetradecane sulfonic acid, mandelic acid condensation polymer (SAMMA), 1,3-diacetylbenzimidazole-2-thione, N-monoacylated benzimidazol-2thione, N,N'-diacylated benzimidazol-2-thione, alkyl-2-phenylindole derivate, 3-propanoylbenzoxazole-2-thione, N-alkylated indole derivative, N-acylated indole derivate, benzothiazole derivative, N-substituted indole-2- and 3-carboxamide derivative, halogenated analogs (chloro and fluoro) of N-substituted indole-2- and 3-carboxamide derivative, 2-(4-hydroxyphenyl)-3-phenylindole, indole carboxamides, indole acetamides, 3-benzolyl-1-methyl-4-phenyl-4-piperidinol, benzoyl phenyl benzoate derivative, l-arginine derivative, guanidinum HCL, L-NAME, HCN, linamarin, amygdalin, hederagenin, aescin, CIS-hinokiresinol and 1,3-di-p-hydroxyphenyl-4-penten-1-one.

In some examples, the stabilizing agent that is a hyaluronidase inhibitor is a polysaccharide of N-acetylglucosamine and glurcuronic acid. In another example, the stabilizing agent that is a hyaluronidase inhibitor is an amine sugar with a negatively

charged sugar. In further examples, the stabilizing agent that is a hyaluronidase inhibitor is an aminomethyl indole or an ascorbic acid derivative.

Exemplary co-formulations provided herein contain a stabilizing agent that is hyaluronan (hyaluronic acid; HA). Hyaluronic acid (HA, also known as hyaluronan and hyaluronate) is the natural substrate for hyaluronan degrading enzymes such as a hyaluronidase, for example a PH20, including rHuPH20. HA is a non-sulfated glycosaminoglycan that is widely distributed throughout connective, epithelial, and neural tissues. It is a polymer of up to 25,000 disaccharide units, themselves composed of D-glucuronic acid and D-N-acetylglucosamine. The molecular weight of HA ranges from about 5 kDa to 200,000 kDa. By catalyzing the hydrolysis of hyaluronan, rHuPH20 (and other hyaluronidases and hyaluronan degrading enzymes) lowers the viscosity of hyaluronan, thereby increasing tissue permeability and increasing the absorption rate of fluids administered parenterally.

As demonstrated herein, hyaluronic acid (HA) is an efficient stabilizer of hyaluronan degrading enzymes in the presence of otherwise destabilizing agents and conditions, such as, for example, low salt, high pH, the presence of preservatives and elevated temperatures. In particular, HA appears to reduce or negate the negative affect that higher pH and/or elevated temperatures typically have on rHuPH20 and other soluble hyaluronidases and hyaluronan degrading enzymes, particularly in the presence of phenolic preservatives. For example, as shown in the studies described below (see *e.g.* Example 7.D and Example 17), rHuPH20 stability increases significantly when HA oligomers (4-16mers) are included in the co-formulations with insulin. Increasing concentrations of HA have increasing stabilizing properties. For example, after 1 week at 30 °C at pH 7.1 with 1 mg/mL HA and 75 mM NaCl, the activity of the rHuPH20 in the rHuPH20/insulin co-formulation decreased from 600 U/mL to 341 U/mL (*i.e.* retained 57 % of the original activity). When the HA concentration was increased to 10 mg/mL, the activity of the rHuPH20 only decreased from 600 U/mL to 510 U/mL (*i.e.* retained 85% of the original activity). Further, HA reduces or negates the destabilizing effect that a high pH has on rHuPH20. For example, after 1 week at 30 °C at pH 7.1 with 5.5 mg/mL HA and 100 mM NaCl, 68 % of the original rHuPH20 remained. This percentage was essentially unchanged when the pH was increased to 7.5. A similar positive impact of HA on rHuPH20

stability of rHuPH20 was observed at elevated temperatures (see *e.g.* Example 17). Thus, it is determined herein that HA can be included in formulations of insulin and rHuPH20 (or other soluble hyaluronidases and hyaluronan degrading enzymes) to effectively stabilize rHuPH20.

Thus, provided herein are co-formulations containing HA. Any size HA can be used in the compositions as a stabilizer. In some examples, the HA is a disaccharide, composed of D-glucuronic acid and D-N-acetylglucosamine. In other examples, the HA is an oligosaccharide, such as a tetrasaccharide, containing 2 repeating disaccharide units, or alternatively, the HA used in the co-formulations provided herein can contain multiple repeating disaccharide units, such as 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more disaccharide units. In other example, the HA used in the co-formulations provided herein has a molecular weight that is from or from about 5 kDa to or to about 5,000 kDa; from or from about 5 kDa to or to about 1,000 kDa; from or from about 5 kDa to or to about 500 kDa; or from or from about 5 kDa to or to about 200 kDa. Exemplary HA oligosaccharides for use in the co-formulations herein have a molecular weight of or of about 6.4 kDa, 74.0 kDa. or 234.4 kDa. For example, included among the compositions provided herein of insulin and a hyaluronan degrading enzyme, such as a hyaluronidase (*e.g.* rHuPH20), are those that contain HA having a molecular weight of at least or about 5 kDa, 6 kDa, 7 kDa, 8 kDa, 9 kDa, 10 kDa, 15 kDa, 20 kDa, 30 kDa, 40 kDa, 50 kDa, 60 kDa, 70 kDa, 80 kDa, 90 kDa, 100 kDa, 120 kDa, 140 kDa, 160 kDa, 180 kDa, 200 kDa, 220 kDa, 240 kDa, 260 kDa, 280 kDa, 300 kDa, 350 kDa, 400 kDa, 450 kDa, or 500 kDa. In one example, the molecular weight of the HA in the co-formulation is less than 10 kDa.

Provided herein, therefore, are co-formulations of insulin and a hyaluronan-degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20) that contain an HA oligosaccharide. The co-formulations contain 1 mg/mL to 20 mg/mL HA, such as at least or about 1 mg/mL, 2 mg/mL, 3 mg/mL, 4 mg/mL, 5 mg/mL, 6 mg/mL, 7 mg/mL, 8 mg/mL, 9 mg/mL, 10 mg/mL, 11 mg/mL, 12 mg/mL, 13 mg/mL, 14 mg/mL, 15 mg/mL, 16 mg/mL, 17 mg/mL, 18 mg/mL, 19 mg/mL or 20 mg/mL or more HA. Exemplary stable co-formulations of insulin and rHuPH20 include from or from about 8 mg/mL to or to about 12 mg/mL HA, such as, for example 10 mg/mL or

about 10 mg/mL. In some examples, the molar ratio of HA to hyaluronan degrading enzyme is or is about 100,000:1, 95,000:1, 90,000:1, 85,000:1, 80,000:1, 75,000:1, 70,000:1, 65,000:1, 60,000:1, 55,000:1, 50,000:1, 45,000:1, 40,000:1, 35,000:1, 30,000:1, 25,000:1, 20,000:1, 15,000:1, 10,000:1, 5,000:1, 1,000:1, 900:1, 800:1, 700:1, 600:1, 500:1, 400:1, 300:1, 200:1, or 100:1 or less.

viii. Nicotinic compound

In some examples, a nicotinic compound is used as a stabilizing agent. Nicotinic compounds include, but are not limited to, nicotinamide, nicotinic acid, niacin, niacinamide, vitamin B3 and/or salts thereof and/or any combination thereof. In particular applications, the stabilizing agent can include a nicotinic compound and an amino acid or amino acids (*see e.g.* International published PCT Appl. No. WO2010149772). For example, the amino acid can be arginine, glutamic acid and/or salts thereof or combinations thereof.

ix. Other Excipients or Agents

Optionally, the co-formulations can include carriers such as a diluent, adjuvant, excipient, or vehicle with which the co-formulation is administered. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin. Such compositions will contain a therapeutically effective amount of the compound, generally in purified form or partially purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, and sesame oil. Water is a typical carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions also can be employed as liquid carriers, particularly for injectable solutions.

For example, pharmaceutically acceptable carriers used in parenteral preparations include aqueous vehicles, nonaqueous vehicles, antimicrobial agents, isotonic agents, buffers, antioxidants, local anesthetics, suspending and dispersing agents, emulsifying agents, sequestering or chelating agents and other pharmaceutically acceptable substances. Examples of aqueous vehicles include Sodium Chloride Injection, Ringers Injection, Isotonic Dextrose Injection, Sterile

Water Injection, Dextrose and Lactated Ringers Injection. Nonaqueous parenteral vehicles include fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil and peanut oil. Antimicrobial agents in bacteriostatic or fungistatic concentrations can be added to parenteral preparations packaged in multiple-dose containers, which include phenols or cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride. Isotonic agents include sodium chloride and dextrose. Buffers include phosphate and citrate. Antioxidants include sodium bisulfate. Local anesthetics include procaine hydrochloride. Suspending and dispersing agents include sodium carboxymethylcellulose, hydroxypropyl methylcellulose and polyvinylpyrrolidone. Emulsifying agents include Polysorbate 80 (Tween 80). A sequestering or chelating agent of metal ions include EDTA. Pharmaceutical carriers also include ethyl alcohol, polyethylene glycol and propylene glycol for water miscible vehicles and sodium hydroxide, hydrochloric acid, citric acid or lactic acid for pH adjustment.

Compositions can contain along with an active ingredient: a diluent such as lactose, sucrose, dicalcium phosphate, or carboxymethylcellulose; a lubricant, such as magnesium stearate, calcium stearate and talc; and a binder such as starch, natural gums, such as gum acacia, gelatin, glucose, molasses, polyvinylpyrrolidone, celluloses and derivatives thereof, povidone, crospovidones and other such binders known to those of skill in the art.

For example, an excipient protein can be added to the co-formulation that can be any of a number of pharmaceutically acceptable proteins or peptides. Generally, the excipient protein is selected for its ability to be administered to a mammalian subject without provoking an immune response. For example, human serum albumin is well-suited for use in pharmaceutical formulations. Other known pharmaceutical protein excipients include, but are not limited to, starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, and ethanol. The excipient is included in the formulation at a sufficient concentration to prevent adsorption of the protein to the holding vessel or vial. The concentration of the excipient will vary according to the nature of the excipient and the concentration of the protein in the co-formulation.

A composition, if desired, also can contain minor amounts of wetting or emulsifying agents, or pH buffering agents, for example, acetate, sodium citrate, cyclodextrin derivatives, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, and other such agents.

2. Exemplary Stable Co-Formulations

a. Exemplary Multi-Dose Injection (MDI) co-formulations

Provided herein are stable co-formulations of a fast acting insulin, such as a rapid acting (fast-acting) insulin analog, and a hyaluronan degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20) that are stable for at least 6 months at a temperature of from or from about 2 °C to or to about 8 °C and at least 14 days (*i.e.* 2 weeks) at a temperature of from or about 20 °C to or to about 30 °C. Exemplary of the MDI co-formulations are those that are stable for at least or about 6, 7, 8, 9, 10, 15, 20, 24, 30, 36, 42, 48, 54, 60 or more months at a temperature of from or from about 2 °C to or to about 8 °C, and for at least or about 14, 15, 20, 25, 28, 30, 35, 40, 45 or 50 or more days at a temperature of from or about 20 °C to or to about 30 °C.

For example, the formulations provided herein are stable at or at about 2-8° C for at least one year, for example at least 12 months, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 20 months, 21 months, 22 months, 23 months, 24 months, 25 months, 26 months, 27 months, 28 months, 29 months, 30 months, 31 months, 32 months, 33 months, 34 months, 35 months, 36 months or more. In particular, the formulations provided herein are stable at or at about 2-8° C for at least 24 months.

In other examples, the formulations provided herein are stable for at least one week at or at about 20-30° C, such as at or about 22° C, 23° C, 24° C, 25° C, 26° C, 27° C, 28° C, 29° C or 30° C, for at least one week. For example, formulations provided herein are stable at or at about 20-30° C for at least 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, one month, 2 months, 3 months, 4 months, 5 months, 6 months or more. In particular, the formulations provided herein are stable at or at about 20-30° C, such as at or about 25° C or 30° C for at least one month.

In some examples, a stable co-formulation provided herein contains 100 U/mL to 1000 U/mL of a hyaluronan-degrading enzyme such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20), and in particular at or about or at least 600 U/mL; 10 U/mL to 1000 U/mL of a fast-acting insulin, and in particular at least or about 100 U/mL; NaCl at a concentration between or about between 50 mM to 200 mM; a pH of between or about between 6.8 to 7.8, such as between or about between 7.0 to 7.6; a buffering agent that maintains the pH range of between or about between 6.8 to 7.8 or 7.0 to 7.6; an anti-microbially effective amount of a preservative or a mixture of preservatives of 0.1 % to 0.4 % preservative as a mass concentration (w/v); and a stabilizing agent in an amount that, over the course of storage (temperature and time), at least 50% of the initial hyaluronan-degrading enzyme activity, such as at least or about at least 375 U/mL of hyaluronan-degrading enzyme activity is retained. With respect to the buffering agent, any buffering agent can be used that can be included in an amount to maintain the pH range of the co-formulation between or about between 6.8 to 7.8 such as between or about between 7.0 to 7.6. Typically, Tris is included in the co-formulations provided herein at a concentration of or about 10 mM to 50 mM, such as, for example, 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM or 50 mM. In particular examples, the co-formulations contain or contain about 20 mM to 30 mM Tris, such as 21 mM, 22 mM, 23 mM, 24 mM, 25 mM, 26 mM, 27 mM, 28 mM, 29 mM or 30 mM Tris. In particular examples, the co-formulations provided herein contain Tris at a concentration of or about 30 mM.

For example, exemplary of such formulations contain 100 U/mL to 1000 U/mL of a hyaluronan-degrading enzyme such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20), and in particular at or about or at least 600 U/mL; 10 U/mL to 1000 U/mL of a fast-acting insulin, and in particular at least or about 100 U/mL; NaCl at a concentration of between or about between 80-140 mM; a pH of between or about between 7.0 to 7.6; a buffering agent that maintains the pH range of between or about between 7.0 to 7.6; 0.1 % to 0.4 % preservative as a mass concentration (w/v); and a stabilizing agent in an amount that, over the course of storage (temperature and time), at least 50% of the initial hyaluronan-degrading enzyme activity is retained, such as at least or about at least 375 U/mL of hyaluronan-degrading enzyme activity is retained. For example, the co-formulations provided herein contain 1 mM to 100 mM of a

buffering agent (*e.g.* Tris). For example, the co-formulations provided herein contain 0.01 % to 0.5 % surfactant. Exemplary co-formulations provided herein also can contain less than 60 mM glycerin (glycerol) and 5 mM to or to about 50 mM of an antioxidant.

The following stable formulations are exemplary only and provide a platform from which minor adjustments can be made. It is understood that very small changes in the concentrations of the various excipients and other components (*e.g.* ± 15 % of the stated concentrations), or small changes in pH, can be made while retaining some if not all of the insulin solubility and stability and hyaluronan degrading enzyme stability. Further changes also can be made by adding or removing excipients. For example, the type of stabilizing surfactant can be changed. For example, the exemplary co-formulations herein contain 100 U/mL to 1000 U/mL of a hyaluronan-degrading enzyme such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20), and in particular at least or about at least or about 600 U/mL of a hyaluronan-degrading enzyme such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20); 10 U/mL to 1000 U/mL of a fast-acting insulin, and in particular at least or about at least or about 100 U/mL of a fast-acting insulin; from or from about 10 mM to or to about 50 mM Tris (*e.g.* from or from about 20 mM to 40 mM Tris, such as at least or about at least 20 mM, 25 mM, 30 mM, 35 mM or 40 mM); from or from about 80 mM to or to about 140 mM NaCl (*e.g.* at least or about at least 80 mM, 90 mM, 100 mM, 110 mM 120 mM, 130 mM, 140 mM, 150 mM or 160 mM NaCl); from or from about 5 mM to or to about 50 mM methionine (*e.g.* at least or about at least 5 mM, 10 mM, 20 mM, 30 mM, 40 mM or 50 mM methionine); from or from about 0 mM to or to about 50 mM glycerin (*e.g.* at least or about at least 5 mM, 10 mM, 20 mM, 30 mM, 40 mM or 50 mM glycerin); from or from about 0.01 % to or to about 0.5 % poloxamer 188, such as 0.01% to 0.05% (*e.g.* at least or about at least 0.01%, 0.02%, 0.03%, 0.04% or 0.05% poloxamer 188); from or from about 0.1% to or to about 0.25% phenol (*e.g.* at least or about at least 0.1%, 0.12%, 0.125%, 0.13%, 0.14%, 0.15%, 0.16% or 0.17% phenol); and from or from about 0.05% to or to about 0.2% m-cresol (*e.g.* at least or about at least 0.075%, 0.08%, 0.09%, 0.1%, 0.12%, 0.13%, 0.14%, 0.15%, 0.16% or 0.17% m-cresol). The formulations are prepared with a pH from or from about 7.0 to or to about 7.6 (*e.g.* at least or about at least pH 7.0, 7.1, 7.2, 7.3, 7.4, 7.5 or 7.6). In

further examples, zinc is included at a concentration of or about 0.017 mg/100 U, 0.018 mg/100 U, 0.02 mg/100 U, 0.022 mg/100 U or 0.024 mg/100 U insulin.

As discussed above, the concentrations of the various components in the formulations can be increased or decreased depending upon the particular properties of the insulin. For example, formulations of insulins with higher apparent solubility, such as insulin aspart, typically contain a higher concentration of NaCl and a lower concentration of glycerin compared to formulations of insulins with lower apparent solubility, such as insulin lispro. Depending on the NaCl concentration, the particular pH of the formulation also can vary between different insulins.

For example, included among stable co-formulations provided herein are stable co-formulations of an insulin and a hyaluronan-degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20) that contains between or about between 50 to 120 mM NaCl, for example 50 mM to 100 mM, such as 50 mM to 90 mM or 80 mM to 100 mM. Such co-formulations include those that contain insulin analogs insulin lispro. In other examples, stable co-formulations provided herein are stable co-formulations of an insulin and a hyaluronan-degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20) that contains between or about between 80 mM to 160 mM NaCl, such as 100 mM to 140 mM for example 120 mM. Such co-formulations include those that contain insulin aspart. For example provided herein are co-formulations of rHuPH20 and insulin aspart or insulin lispro that contain or contain about 80 mM or 100 mM NaCl.

In another example, included among stable co-formulations provided herein are stable co-formulations of an insulin and a hyaluronan-degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20) that contains between or about between 80 mM to 200 mM, for example, 100 mM to 150 mM, such as 130 mM to 150 mM, 120 mM to 140 mM or 110 mM to 130 mM. Such co-formulations include those that contain the insulin analog glulisine. In some examples, the co-formulations containing, for example insulin glulisine, have a salt (NaCl) concentration of or of about 80 mM, 90 mM, 100 mM, 110 mM, 120 mM, 121 mM, 122 mM, 123 mM, 124 mM, 125 mM, 126 mM, 127 mM, 128 mM, 129 mM, 130 mM, 131 mM, 132 mM, 133 mM, 134 mM, 135 mM, 136 mM, 137 mM, 138 mM, 139 mM, 140 mM, 150 mM, 160 mM, 170 mM, 180 mM, 190 mM or 200 mM. For example provided herein

are co-formulations of rHuPH20 and insulin glulisine that contain or contain about 120 mM or 140 mM NaCl.

In examples of co-formulations provided herein are co-formulations of an insulin, such as insulin aspart, and rHuPH20 that have a pH of or of about 7.2, for example 7.2 ± 0.2. In other examples, the co-formulations of an insulin, such as insulin lispro, and rHuPH20 have a pH of or of about 7.4, for example 7.4 ± 0.2. In further examples, the co-formulations of an insulin, such as insulin glulisine, and rHuPH20 have a pH of or of about 7.3 or 7.4, for example 7.3 ± 0.2 or 7.4 ± 0.2.

Exemplary of the co-formulations provided herein that contain a hyaluronan degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20), and insulin lispro are those that contain from or about 25 mM to or to about 35 mM Tris (*e.g.* at or about 30 mM); from or from about 70 mM to or to about 100 mM NaCl (*e.g.* at or about 80 mM or 100 mM NaCl); from or from about 10 mM to or to about 30 mM methionine (*e.g.* at or about 10 mM or 20 mM methionine); from or from about 40 mM to or to about 60 mM glycerin (*e.g.* at or about 50 mM glycerin); from or from about 0.005 % to or to about 0.05 % poloxamer 188 (*e.g.* at or about 0.01 % poloxamer 188); from or from about 0.017 mg zinc/100 U insulin to or to about 0.024 mg zinc/100 U insulin (*e.g.* 0.017 mg zinc/100 U insulin, 0.018 mg/100 U, 0.02 mg/100 U, 0.022 mg/100 U or 0.024 mg zinc/100 U insulin); from or from about 0.08 % to or to about 0.17 % phenol (*e.g.* 0.1 %, 0.125 % or 0.13 % phenol); and from or from about 0.07% to or to about 0.17 % m-cresol (*e.g.* 0.075 %, 0.08 %, 0.13 % or 0.15 % m-cresol). For example, the co-formulations can contain at or about 0.1% phenol and 0.015 % m-cresol; at or about 0.125 % phenol and 0.075% m-cresol; at or about 0.13 % phenol and 0.075 % m-cresol; at or about 0.13 % phenol and 0.08 % m-cresol; or at or about 0.17% phenol and 0.13 % m-cresol. Such formulations of insulin lispro and a hyaluronan degrading enzyme, such as a soluble hyaluronidase (*e.g.* rHuPH20), are prepared with a pH of or about 7.0 to or to about 7.5 (typically a pH of or about pH 7.2).

Exemplary of the co-formulations provided herein that contain a hyaluronan degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20), and insulin aspart are those that contain from or from about 25 mM to or to about 35 mM Tris (*e.g.* at or about 30 mM); from or from about 70 mM to or to about 100 mM

NaCl (*e.g.* at or about 80 mM or 100 mM NaCl); from or from about 10 mM to or to about 30 mM methionine (*e.g.* at or about 10 mM or 20 mM methionine); from or from about 40 mM to or to about 60 mM glycerin (*e.g.* at or about 50 mM glycerin); from or from about 0.005% to or to about 0.05 % poloxamer 188 (*e.g.* at or about 0.01% poloxamer 188); from or from about 0.017 mg zinc/100 U insulin to or to about 0.024 mg zinc/100 U insulin (*e.g.* 0.017 mg zinc/100 U insulin, 0.018 mg/100 U, 0.02 mg/100 U, 0.022 mg/100 U or 0.024 mg zinc/100 U insulin); from or from about 0.08 % to or to about 0.17 % phenol (*e.g.* 0.1%, 0.125% or 0.13% phenol); and from or from about 0.07 % to or to about 0.17% m-cresol (*e.g.* 0.075 %, 0.08 %, 0.13 % or 0.15 % m-cresol). For example, the co-formulations can contain at or about 0.1 % phenol and 0.015 % m-cresol; at or about 0.125 % phenol and 0.075 % m-cresol; at or about 0.13 % phenol and 0.075 % m-cresol; at or about 0.13 % phenol and 0.08 % m-cresol; or at or about 0.17 % phenol and 0.13 % m-cresol. Such formulations of insulin aspart and a hyaluronan degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20) are prepared with a pH of or about 7.0 to or to about 7.6 (typically a pH of or about pH 7.4).

Exemplary of the co-formulations provided herein that contain a hyaluronan degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20), and insulin glulisine are those that contain from or from about 25 mM to or to about 35 mM Tris (*e.g.* at or about 30 mM); from or from about 100 mM to or to about 150 mM NaCl (*e.g.* at or about 100 mM or 140 mM NaCl); from or from about 10 mM to or to about 30 mM methionine (*e.g.* at or about 10 mM or 20 mM methionine); from or from about 40 mM to or to about 60 mM glycerin (*e.g.* at or about 50 mM glycerin); from or from about 0.005 % to or to about 0.05 % poloxamer 188 (*e.g.* at or about 0.01 % poloxamer 188); from or from about 0.08% to or to about 0.17% phenol (*e.g.* 0.1 %, 0.125 % or 0.13 % phenol); and from or from about 0.07 % to or to about 0.17% m-cresol (*e.g.* 0.075 %, 0.08 %, 0.13 % or 0.15 % m-cresol). For example, the co-formulations can contain at or about 0.1 % phenol and 0.015 % m-cresol; at or about 0.125 % phenol and 0.075 % m-cresol; at or about 0.13 % phenol and 0.075 % m-cresol; at or about 0.13 % phenol and 0.08 % m-cresol; or at or about 0.17 % phenol and 0.13 % m-cresol. Such formulations of insulin glulisine and a hyaluronan

degrading enzyme, such as a hyaluronidase (*e.g.* rHuPH20) are prepared with a pH of or about 7.0 to or to about 7.6 (typically a pH of or about pH 7.4).

b. Exemplary Continuous Subcutaneous Insulin Infusion (CSII) Co-formulations

Provided herein are stable co-formulations that are stable in the presence of accelerated or stress conditions such as elevated temperatures greater than or about greater than 32° such as 35° C to 40° C, in particular greater than at or about or 37° C or 40° C and/or agitation conditions for at least 3 hours, 4 hours, 5 hours, 6 hours, 12 hours, 1 day, 2 days, 3 days, 4 days, at least 5 days, at least 6 days or at least 7 days, and generally at least 3 hours or at least 3 days. These stable co-formulations are suitable for administration by continuous subcutaneous insulin infusion (CSII).

As discussed above, the concentration, amount or level of components that confer stability of co-formulations herein for at least 6 months at temperatures of from or from about 2 °C to or to about 8 °C and at least 14 days (*i.e.* 2 weeks) at a temperature of from or about 20 °C to or to about 30 °C generally are not sufficient to confer stability of the co-formulation under stress conditions such as elevated temperature. Generally, such co-formulations are stable under such stress conditions (e.g. elevated temperature) for less than 24 hours, and generally less than 8 hours, which can substantially impair their use in multi-dose applications where such conditions exist. For example, CSII therapy is associated with continuous infusion of formulations by a pump or other device that is worn outside and near to the body for 24 hours a day for 2 to 3 days. The insulin formulation or co-formulation is injected through a needle into the abdominal wall or thigh, which injection can be controlled by a programmed pump so that the insulin formulation or co-formulation is infused continuously. Therefore, co-formulations used for CSII therapy are subjected to elevated body temperatures of at least or about or greater than 37° C and agitation conditions.

For example, a hyaluronan-degrading enzyme is particularly unstable at elevated temperatures greater than 32°C, and typically greater than 37° C or 40° C. It also is found herein that although insulin crystallizes at 2° C to 8° C at high salt concentrations and low pH, it does not crystallize at high salt concentrations and low pH at higher temperatures of 32° C to 40° C. Accordingly, the opposing requirement

of high salt concentration and low pH required by a hyaluronan-degrading enzymes (*e.g.* PH20) to maintain its stability at high temperatures of 32° C to 40° C is more compatible at higher temperatures for at least a short period of time of at least 3 days. Also, the same high salt and low pH formulations confer similar stability between and among the insulin analogs, despite differences in apparent solubility that affect stability of insulin at the lower temperatures.

The stable co-formulations that are stable under stress conditions, for example for use in CSII therapy, generally contain the same components as other co-formulations provided here. Such co-formulations, however, differ in that the co-formulations that are stable under stress conditions generally contain a higher salt concentration, a lower pH and/or the presence of one or more other excipients that sufficiently stabilize the hyaluronan-degrading enzyme and/or insulin generally for at least 2 to 3 days at elevated temperatures greater than or about greater than 32° such as 35° C to 40° C, in particular greater than at or about or 37° C or 40° C and/or agitation conditions. For example, co-formulations provided herein that are stable at stress conditions of elevated temperatures or agitation generally contain a hyaluronidase inhibitor, such as a hyaluronidase substrate (e.g. hyaluronan) as an excipient.

In one example, the co-formulations provided herein that are stable at stress conditions of elevated temperatures or agitation contain a higher salt concentration and a lower pH than co-formulations provided above in Section E.1.a. For example, provided herein are co-formulations that are stable under stress conditions (*e.g.* elevated temperature of 32° C to 40° C or agitation) for at least 3 days or 3 hours that contain 120 mM NaCl to 200 mM NaCl and pH of 6.5 to 7.5. As discussed above, however, insulin solubility, particularly at refrigerated temperatures, decreases in these reduced pH and increased salt conditions. Thus such formulations typically are not stored at refrigerated or ambient temperatures prior to use.

In another example, the co-formulations provided herein that are stable at stress conditions of elevated temperatures (*e.g.* 32° C to 40° C) for at least 3 days or agitation for at least 3 hours contain a hyaluronidase inhibitor to stabilize the hyaluronan-degrading enzyme in the co-formulation. Any of the hyaluronidase inhibitors described above can be used in a co-formulation herein that is stable at

stress conditions of elevated temperatures (*e.g.* 32° C to 40° C) for at least 3 days or agitation for at least 3 hours. In particular examples, the hyaluronidase inhibitor is a hyaluronidase substrate, for example, a hyaluronan.

As shown in the Examples herein with the hyaluronidase inhibitor hyaluronan, the presence of a hyaluronidase inhibitor stabilizes PH20 activity, particularly in the presence of preservatives especially at elevated temperatures, such as under stress conditions of temperatures of 32° C to 40° C. Since HA oligomers are the substrate/product of the enzymatic reaction of a hyaluronan-degrading enzyme with hyaluronan, the hyaluronan oligomers can bind to the enzyme active site and cause the stabilizing effect. Nevertheless, it is also found that over time under stress conditions of elevated temperatures of 32° C to 40° C, such as greater than 1 week or 2 weeks at 37° C, the presence of a hyaluronidase inhibitor, such as HA, in the co-formulation can result in degradation of insulin, thereby resulting in covalent HA-insulin analog adducts. For example, the presence of high concentrations of HA in the co-formulations provided herein has been shown by reverse-phase high performance liquid chromatography (RP-HPLC) to cause degradation of insulin Aspart® after 1 week at 37° C and insulin Glulisine® after 2 weeks at 30° C. Liquid chromatography-mass spectrometry (LC-MS) analysis indicated that some of the degradation products are covalent HA-insulin analog glycation adducts formed by reaction of insulin with the reducing end of the HA. For example, one peak was determined to be the product of insulin Aspart® and a HA 7mer while another peak was the product of insulin Aspart® and a HA 2mer.

The presence of a hyaluronidase inhibitor, such as HA, also can have effects on the precipitation and color change of the co-formulation. Hence, while HA improves the stability of hyaluronan-degrading enzyme at stress conditions of elevated temperatures of 32° C to 40° C, it also can have effects on insulin degradation, precipitation and color change of the co-formulation. It is within the level of one of skill in the art to monitor these conditions within desired safety and pharmacologic parameters and guidelines. Generally, stable co-formulations provided herein that contain a hyaluronidase inhibitor, such as HA, are stable at elevated temperatures, such as under stress conditions of temperatures of 32° C to 40° C for at least 3 hours but no more than 7 days due to effects on these parameters.

In some examples provided herein, a hyaluronidase inhibitor is used that is not capable of forming covalent complexes with insulin or a hyaluronan-degrading enzymes. Hence, non-covalent inhibitors that act by associative binding are contemplated in the formulations herein. For example, provided herein are co-formulations that contain HA with a reacted reducing end so that it is no longer possible to form glycation adducts with insulin. For example, in some examples, the HA used in the co-formulations provided herein has been modified by reductive amination. Reductive amination involves formation of a Schiff base between an aldehyde and amine, which is then reduced to form the more stable amine. The reducing end of a sugar, *i.e.*, HA, exists as an equilibrium mixture of the cyclic hemiacetal form and the open chain aldehyde form. Under suitable conditions known of one of skill in the art, amine groups will condense with the sugar aldehyde to form an iminium ion which can be reduced to an amine, with a reducing agent such as sodium cyanoborohydride (see, *e.g.*, Gildersleeve *et al.*, (2008) *Bioconjug Chem* 19(7):1485-1490). The resulting HA is unreactive to the insulin and unable to form insulin glycation adducts.

In particular, provided herein is a stable co-formulation composition that is stable for at least 3 days at a temperature from or from about 32°C to 40°C and/or is stable for at least 3 hours under agitation containing 100 U/mL to 1000 U/mL of a hyaluronan-degrading enzyme such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20), and in particular at or about or at least 600 U/mL; 10 U/mL to 1000 U/mL of a fast-acting insulin, and in particular at least or about 100 U/mL; NaCl at a concentration between or about between 120 mM to 200 mM; a pH of between or about between 6.5 to 7.5; an anti-microbially effective amount of a preservative or mixture of preservatives; and one or more further stabilizing agent or agents, such as a hyaluronidase inhibitor, such that at least 50% of the initial hyaluronan-degrading enzyme activity, such as at least or about at least 375 U/mL of hyaluronan-degrading enzyme activity is retained. For example, the co-formulation can contain HA at a concentration of between or about between 1 mg/mL to 20 mg/mL. The stable co-formulations also can contain a buffering agent to maintain the pH range of between or about between pH 6.5 (*e.g.* Tris) in an amount that is between or about between 1 mM to 100 mM; an anti-microbially effective amount of a preservative or a mixture

of preservatives, for example, a phenolic preservative (*e.g.* phenol and/or m-cresol) in a total amount as a percentage (%) of mass concentration (w/v) in the formulation that is or is between 0.1% and 0.4%; a surfactant (e.g. poloxamer 188) as a % of mass concentration (w/v) of between or about between 0.005% to 1.0%; and optionally a further stabilizing agent.

For example, co-formulations provided herein that are stable under stress conditions (*e.g.* elevated temperature of 32°C to 40°C or agitation) for at least 3 days or 3 hours contain 120 mM to 200 mM, such as 150 mM NaCl to 200 mM NaCl or 160 mM NaCl to 180 mM NaCl, for example at or about 120 mM, 130 mM, 140 mM, 150 mM, 155 mM, 160 mM, 165 mM, 170 mM, 175 mM, 180 mM, 185 mM, 190 mM, 195 mM or 200 mM NaCl. Also, the co-formulations provided herein that are stable under stress conditions (*e.g.* elevated temperature of 32°C to 40°C or agitation) for at least 3 days or 3 hours contain a pH of 6.5 to 7.5 or 6.5 to 7.2, such as or about a pH of 6.5 ± 0.2, 6.6 ± 0.2, 6.7 ± 0.2, 6.8 ± 0.2, 6.9 ± 0.2, 7.0 ± 0.2, 7.1 ± 0.2, 7.2 ± 0.2, 7.3 ± 0.2, 7.4 ± 0.2 or 7.5 ± 0.2.

In examples herein, co-formulations provided herein that are stable at stress conditions of elevated temperatures (*e.g.* 32° C to 40° C) or agitation for at least 3 days or 3 hours contain hyaluronan (hyaluronic acid; HA) that has a molecular weight of 5 kDa to 5,000 kDa, 5 kDa to or to about 1,000 kDa, 5 kDa to or to about 200 kDa, or 5 kDa to or to about 50 kDa. In particular, the molecular weight of HA is less than 10 kDa. The HA can be an oligosaccharide, composed of disaccharides, such as a 2mer to 30mer or a 4mer to 16mer. The co-formulations of insulin and a hyaluronan-degrading enzyme such as a hyaluronidase, for example, a PH20 (*e.g.* rHuPH20) contain HA at a concentration of between or about between 1 mg/mL to 20 mg/mL, such as at least or about 1 mg/mL, 2 mg/mL, 3 mg/mL, 4 mg/mL, 5 mg/mL, 6 mg/mL, 7 mg/mL, 8 mg/mL, 9 mg/mL, 10 mg/mL, 11 mg/mL, 12 mg/mL, 13 mg/mL, 14 mg/mL, 15 mg/mL, 16 mg/mL, 17 mg/mL, 18 mg/mL, 19 mg/mL or 20 mg/mL or more HA. Exemplary stable co-formulations include from or from about 8 mg/mL to or to about 12 mg/mL HA, such as, for example 10 mg/mL or about 10 mg/mL. In some examples, the molar ratio of HA to hyaluronan degrading enzyme is or is about 100,000:1, 95,000:1, 90,000:1, 85,000:1, 80,000:1, 75,000:1, 70,000:1, 65,000:1, 60,000:1, 55,000:1, 50,000:1, 45,000:1, 40,000:1, 35,000:1, 30,000:1, 25,000:1,

20,000:1, 15,000:1, 10,000:1, 5,000:1, 1,000:1, 900:1, 800:1, 700:1, 600:1, 500:1, 400:1, 300:1, 200:1, or 100:1 or less.

Since the co-formulations that are stable at elevated temperatures (*e.g.* 32° C to 40° C) or agitation, such as is desired for CSII formulations, have a pH that is decreased and the salt concentration increased as compared to the co-formulations set forth above in Section E.1.a, they can be prepared or derived therefrom. This can be achieved, for example, by diluting a co-formulation such as any provided in Section E.1.a that is suitable for MDI with a stabilizing diluent that has a low pH and a high salt concentration. For example, the diluent can be a high NaCl solution with buffer at lower pH and preservatives. For example, the diluent can contain 10 mM to 50 mM Tris or other similar buffer; 120 mM to 200 mM NaCl; 0.1 % to 0.4 % preservative. The diluent can be prepared at a pH of between or about between 6.5 to 7.8. Hence, a stable co-formulation provided herein that is stable at 2° C to 8° C or 20° C to 30° C can be provided and mixed with diluent to provide a co-formulation that is stable under stress conditions of elevated temperatures (*e.g.* 32° C to 40° C) for at least 3 days or agitation for at least 3 hours.

For example, any of the above MDI co-formulations in Section E.1.a can be diluted with a stabilizing diluent resulting in a CSII formulation with lower insulin concentration, a pH of from or from about 6.8 to or to about 7.0 (such as or about 6.8, 6.9 or 7.0) and a NaCl concentration of from or from about 150 mM to or to about 200 mM.

In other examples, the stable MDI co-formulation can be provided as a modified high concentration MDI formulation containing higher insulin concentrations and higher PH20 concentrations and lower NaCl (between or about between 80 mM to 150 mM) and lower buffering capacity to provide acceptable tonicity and lower pH after mixing with the stabilizing excipient diluent. For example, the higher insulin concentration can be, for example, 120 to 500 Units, such as 150, 200 or 500 Units (U) and the higher PH20 concentration can be 6 to 25 μg/mL, such as 6 to 25 6, 7.5, 10 or 25 μg/mL. Dilution of a modified high concentration MDI formulation with a stabilizing diluent can provide a CSII formulation with lower pH (*e.g.* 6.5 to 7.2) and increased NaCl (140 mM to 200 mM) than any of the MDI co-formulations provided in Section E.1.a above.

In a further example, any of the MDI formulations provided herein in Section E.1.a can be prepared and stored in lyophilized form. Immediately prior to use under stress conditions, the lyophilized product can be diluted with stabilizing diluent containing lower pH (*e.g.* 6.5 to 7.8) and increased NaCl (120 mM to 200 mM) resulting in a CSII formulation with lower pH (*e.g.* 6.5 to 7.8) and increased NaCl (120 mM to 200 mM) than any of the MDI co-formulations provided in Section E.1.a above.

As shown in the examples herein, however, hyaluronan is not suitable for use with formulations stored at 2° C to 8° C, since it causes insulin to aggregate at lower temperatures. Thus, in examples above where the CSII stable formulation is generated from dilution of an MDI formulation and the MDI co-formulation or modified concentrated MDI co-formulation does not contain a hyaluronidase inhibitor, a hyaluronidase inhibitor can be included in the stabilizing diluent in order to provide the appropriate concentration of hyaluronidase inhibitor to maintain stability of the co-formulation under stress conditions of elevated temperatures (*e.g.* 32° C to 40° C) for at least 3 days or agitation for at least 3 hours.

c. Exemplary Lys-Lys Co-formulations

Provided herein are stable co-formulations containing that contain a therapeutically effective amount of a hyaluronan-degrading enzyme, such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20), a therapeutically effective amount of a fast-acting insulin, such as a rapid-acting (*e.g.* fast-acting) insulin analog, and an amount of Lys-Lys to render the co-formulation stable. Typically, the co-formulations are multi-dose formulations and also contain a microbially effective amount of one or more preservatives. The co-formulations also can contain one or more other stabilizer or excipients. Such co-formulations are stable for at least 6 months at a temperature of from or from about 2° C to or to about 8° C and at least 14 days (*i.e.* 2 weeks) at a temperature of from or about 20° C to or to about 30° C. In particular, such co-formulations are stable under accelerated conditions such as elevated temperatures greater than or about greater than 32° such as 35° C to 40° C, in particular greater than at or about or 37° C or 40° C and/or agitation conditions for at least 3 hours, and generally at least 3 days, The co-formulations can be used for

multi-dose injection (MDI) use or for continuous subcutaneous insulin infusion (CSII) methods.

Exemplary stable Lys-Lys containing formulations are described below. The following stable formulations are exemplary only and provide a platform from which minor adjustments can be made. It is understood that very small changes in the concentrations of the various excipients and other components (*e.g.* ± 15 % of the stated concentrations), or small changes in pH, can be made while retaining some if not all of the insulin solubility and stability and hyaluronan degrading enzyme stability. Further changes also can be made by adding or removing excipients.

For example, co-formulations provided herein contain: 100 U/mL to 1000 U/mL of a hyaluronan-degrading enzyme such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20), and in particular at or about or at least 600 U/mL; 10 U/mL to 1000 U/mL of a fast-acting insulin, and in particular at least or about 100 U/mL; the co-formulations further contain Lys-Lys at a concentration of between or about between 50 mM to 120 mM, such as 50 to 80 mM, 80 mM to 100 mM or 100 mM to 120 mM, a pH of between or about between 6.5 to 8.0, for example, 6.5 to 7.8 or 6.8 to 7.8 such as between or about between 6.5 to 7.5, 6.8 to 7.4 or 7.0 to 7.6, a buffering agent that maintains the pH range, an anti-microbially effective amount of a preservative or mixture of preservatives, and a stabilizing agent in an amount that, over the course of storage (temperature and time), retains at least 50% of the hyaluronan-degrading enzyme activity and retains at least 90% of the insulin purity, recovery and/or potency. For example, the co-formulations provided herein contain 0.0005% to 1.0% (*e.g.* 0.0005% to 0.005%) surfactant as a stabilizing agent. The co-formulations can optionally contain additional stabilizing agents, tonicity modifiers, an anti-oxidation agent and/or other excipients. For example, the co-formulations contain NaCl as a concentration of less than 140 mM, such as between or about between 0 mM to 100 mM, for example between or about between 0 mM to 50 mM, 10 mM to 40 mM or 20 mM to 30 mM.

In one example, an exemplary formulation contains: 100 U/mL to 1000 U/mL, of a hyaluronan-degrading enzyme such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20), and in particular at least or about at least or about 600 U/mL of a hyaluronan-degrading enzyme such as a hyaluronidase for example a PH20 (*e.g.*

rHuPH20); 10 U/mL to 1000 U/mL of insulin glulisine, and in particular at least or about 100 U/mL from or from about 50 mM to or to about 105 mM Lys-Lys (*e.g.* at least or about at least 50 mM, 60 mM, 70 mM, 80 mM, 90 mM or 100 mM); 0 mM to or to about 50 mM methionine (*e.g.* between or about between 5 mM to 20 mM, such as at least or about at least 5 mM, 10 mM, 20 mM, 30 mM, 40 mM or 50 mM methionine); and from or from about 0.0005 % to or to about 0.005 % polysorbate 20, such as 0.001% to 0.005% (*e.g.* at least or about at least 0.0005%, 0.0001%, 0.005% or 0.001% polysorbate 20); and preservative(s) that include phenol at a percentage (%) of mass concentration (w/v) of between or between about between 0.01% to 0.25% and m-cresol at a % w/v of between or between about 0.05% to 0.2%. The formulations are prepared with a pH from or from about 6.8 to 7.4, (*e.g.* at least or about at least pH 6.8, 6.9, 7.0, 7.1, 7.2, 7.3 or 7.4). In further examples, NaCl is included at a concentration less than 140 mM. For example, NaCl is included in a concentration of less than 100 mM, such as at least or about at least 0 mM to 100 mM, for example at least or about at least 5 mM, 10 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM or 90 mM.

In another example, an exemplary formulation contains: 100 U/mL to 1000 U/mL, of a hyaluronan-degrading enzyme such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20), and in particular at least or about at least or about 600 U/mL of a hyaluronan-degrading enzyme such as a hyaluronidase for example a PH20 (*e.g.* rHuPH20); 10 U/mL to 1000 U/mL of insulin lispro or aspart, and in particular at least or about 100 U/mL from or from about 80 mM to or to about 100 mM Lys-Lys (*e.g.* at least or about at least 80 mM, 85 mM, 90 mM, 95 mM or 100 mM); 0 mM to or to about 50 mM methionine (*e.g.* between or about between 5 mM to 20 mM, such as at least or about at least 5 mM, 10 mM, 20 mM, 30 mM, 40 mM or 50 mM methionine); from or from about 0.0005 % to or to about 0.005 % polysorbate 20, such as 0.001% to 0.005% (*e.g.* at least or about at least 0.0005%, 0.0001%, 0.005% or 0.001% polysorbate 20); and preservative(s) that include phenol at a percentage (%) of mass concentration (w/v) of between or between about between 0.01% to 0.25% and m-cresol at a % w/v of between or between about 0.05% to 0.2% phenol. The formulations are prepared with a pH from or from about 6.8 to 7.4, (*e.g.* at least or about at least pH 6.8, 6.9, 7.0, 7.1, 7.2, 7.3 or 7.4). In further examples, NaCl is

included at a concentration less than 140 mM. For example, NaCl is included in a concentration of less than 100 mM, such as at least or about at least 0 mM to 100 mM, for example at least or about at least 5 mM, 10 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM or 90 mM.

G. Dosage and Administration

The compositions provided herein that are stable formulations of a hyaluronan-degrading enzyme can be formulated as pharmaceutical compositions for single or multiple dosage administration. The co-formulations of a hyaluronan degrading enzyme and a fast-acting insulin are formulated as pharmaceutical compositions for multiple dosage administration. The formulations and co-formulations can be formulated by any suitable route, such as, for example, parenteral administration, including subcutaneous, intramuscular, intraperitoneal, intravenous, and intradermal administration. Typically, the formulations or co-formulations provided herein are administered subcutaneously.

Therapeutically effective doses can be determined empirically by testing the formulations or co-formulations in known *in vitro* and *in vivo* systems and also can be individualized for each subject based upon such factors as metabolism, food intake and severity of the disease. The concentration of a hyaluronan-degrading enzyme and/or selected insulin in the formulation or co-formulation depends on, for example, absorption, inactivation and excretion rates of the complex, the physicochemical characteristics of the complex, the dosage schedule, and amount administered as well as other factors known to those of skill in the art. For example, for co-formulations with insulin, it is understood that the precise dosage of treatment is a function of the blood glucose levels in a subject, and can be determined empirically using known algorithms or by extrapolation from *in vivo* or *in vitro* test data, past experience of the subject, carbohydrate counting to determine the carbohydrate content in a meal and, therefore, the estimated prandial blood glucose increase and subsequent requirement for insulin. It is to be noted that concentrations and dosage values can vary with each subject treated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the formulations, and that the concentration ranges set forth herein

are exemplary only and are not intended to limit the scope thereof. The amount of a selected insulin to be administered for the treatment of a diabetic condition can be determined by standard clinical techniques. In addition, *in vitro* assays and animal models can be employed to help identify optimal dosage ranges.

Hence, the precise dosage, which can be determined empirically, can depend on the particular hyaluronan-degrading enzyme and/or insulin contained in the formulations or co-formulations, the regime and dosing schedule, the route of administration, the type of diabetes to be treated, the seriousness of the disease and the subject being treated. Generally, insulin is provided in an amount that achieves glycemic control. For example, to achieve post prandial glycemic control, diabetic subjects typically are administered a bolus injection of or about 0.05 U of fast-acting insulin per kg body weight (U/kg) to 1.0 U/kg 30 minutes to 5 minutes prior to a meal, when insulin is delivered without a hyaluronan degrading enzyme. It is understood that this dose can be increased or decreased as appropriate based upon, for example, the metabolism of a particular subject, the content of the meal, and blood glucose levels. It is further understood that the time at which the insulin is delivered for post prandial glycemic control can be changed to be closer to or further from the time of ingestion of a meal, and, in some cases, can be changed such that the insulin is delivered at the time of the meal or after the meal.

Fast-acting insulins typically are administered at doses of between 0.05 Units/kg to 0.25 Units /kg, such as, for example, 0.10 Units/kg, although the particular dose varies. Due to the improved pharmacokinetic and pharmacodynamic properties of insulin co-formulated with hyaluronan degrading enzymes (such as rHuPH20), the provide co-formulations can be administered at lower doses compared to the fast-acting insulin administered in the absence of a hyaluronan degrading enzyme. The degree to which the amount of a fast-acting insulin can be lowered by administering it as a co-formulation with a hyaluronan degrading enzyme depends on, for example, the type of diabetes the patient has and the type of insulin contained in the co-formulation. Typically, the reduction in the amount of fast-acting insulin administered to Type 2 diabetic patients when administered as co-formulation with hyaluronan degrading enzyme is greater than the reduction in the amount of fast-acting insulin administered to Type 1 diabetic patients when administered as a co-

formulation with a hyaluronan degrading enzyme. For example, in instances where a Type 1 diabetic patient and Type 2 diabetic patient are both administered 0.20 U/kg of fast-acting insulin to control postprandial glucose levels, the Type 1 diabetic patient can be administered 0.15 U/kg of fast-acting insulin co-formulated with a hyaluronan degrading enzyme to achieve the same or better glycemic control, and the Type 2 diabetic patient can be administered 0.10 U/kg fast-acting insulin co-formulated with a hyaluronan degrading enzyme to achieve the same or better glycemic control.

Exemplary dosage ranges for parenteral, such as subcutaneous, administration of insulin using the co-formulations with a hyaluronan degrading enzyme provided herein to control postprandial blood glucose levels are from at or about 0.05 U/kg to 0.50 U/kg, such as 0.05 U/kg, 0.06 U/kg, 0.07 U/kg, 0.08 U/kg, 0.09 U/kg, 0.10 U/kg, 0.11 U/kg, 0.12 U/kg, 0.13 U/kg, 0.14 U/kg, 0.15 U/kg, 0.20U/kg, 0.25 U/kg, 0.30 U/kg, 0.40 U/kg, 0.50 U/kg or 1.0 U/kg. The particular dosage depends upon the disease and individual.

The co-formulations of insulin and a hyaluronan degrading enzyme provided herein also can be administered to diabetic subjects to effect glycemic control throughout the day and night, in addition to postprandial glycemic control. Typically, dosages of insulin administered to provide continuous glycemic control are less than those required to achieve postprandial glycemic control. Dosages can, however, be increased or decreased based on blood glucose levels. Exemplary dosage ranges for parenteral, such as subcutaneous, administration of insulin administered as a co-formulation with a hyaluronan degrading enzyme to provide continuous glycemic control are from at or about 0.001 U/kg to 0.30 U/kg, such as 0.001 U/kg, 0.005 U/kg, 0.01 U/kg, 0.02 U/kg, 0.05 U/kg to 0.30 U/kg, such as 0.05 U/kg, 0.06 U/kg, 0.07 U/kg, 0.08 U/kg, 0.09 U/kg, 0.10 U/kg, 0.11 U/kg, 0.12 U/kg, 0.13 U/kg, 0.14 U/kg, 0.15 U/kg, 0.20U/kg, 0.25 U/kg, 0.30 U/kg, 0.40 U/kg, 0.50 U/kg or 1.0 U/kg. The particular dosage depends upon the disease, the time of administration, and the individual. If necessary, dosage can be empirically determined.

It is understood that the precise dosage and duration of treatment is a function of the diabetes being treated and can be determined empirically using known testing protocols or by extrapolation from *in vivo* or *in vitro* test data. It is to be noted that dosage values also can vary with the severity of the diabetes and other factors, such as

metabolism, food intake, and body weight of the subject. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or use of compositions and combinations containing them. The compositions can be administered every minute, every several minutes, hourly, daily, weekly, monthly, yearly or once, depending upon the subject and the diabetic state. Generally, dosage regimens are chosen to limit toxicity and/or other negative effects, such as excess insulin. It should be noted that the attending physician would know how to and when to terminate, interrupt or adjust therapy to lower dosage. Conversely, the attending physician would also know how to and when to adjust treatment to higher levels if the clinical response is not adequate (precluding toxic side effects).

Mode of administration

a. Syringes or Vials

The formulations or co-formulations provided herein can be parentally administered to a subject using one or more of several modes of administration, including, but not limited to, syringes, vials or other containers suitable for single dose or multiple dose formulations. For example, single-use syringes, including insulin syringes, can be used to administer discrete injections, *e.g.* bolus injections, of the compositions. Syringes useful for administrations of the compositions provided herein include insulin syringes, which can be designed to hold standard concentrations of insulin preparations, including 100 U/ml concentrations of insulin preparations, and have markings in insulin units for ease of administration.

b. Insulin pen

An insulin pen is a delivery system that can be used to administer the co-formulations provided herein. Insulin pens include those with replaceable cartridges filled with the composition to be administered and those with non-replaceable cartridges. Insulin pens with non-replaceable cartridges are typically disposed of when the cartridge has been emptied. Insulin pens enable dosing in, for example, half unit, one unit or two unit increments, which are generally measured using a dosing

dial or other mechanism to set the dose (*see e.g.* U.S. Pat. Nos. 5,947,934, 6,074,372, 6,110,149, 6,524,280, 6,582,404). The co-formulation is then delivered by way of a fine needle attached to the pen. Insulin pens are well known in the art and include those described elsewhere, including, but not limited to, those described in U.S. Pat. Nos. 5,947,934, 4,973,318, 5,462,535, 5,599,323, 5,626,566, 5,984,906, 6,074,372, 6,110,149, 6,302,869, 6,379,339 and 7,241,278). Other similar dosing devices, such as for example, those described in U.S. Pat. Nos. 5,947,934, 6,074,372, 6,110,149 and 6,379,339 also can be used to administer the compositions provided herein, either as a co-formulation of insulin and hyaluronan degrading enzyme or separately as an insulin composition and a hyaluronan degrading enzyme composition. In some examples, the insulin pen or similar device also contains a sensor or monitor than can measure the blood glucose level of the subject (*see e.g.* WO2003047426).

Insulin pens and similar delivery devices that can be used, or modified to be used, to deliver the co-formulations provided herein are well known in the art and include, but are not limited to, those marketed under the trademarks Autopen® (Owen Mumford, Inc.), Disetronic Pen (Disetronic Medical Systems), Humalog® Pen (Eli Lilly and Company), Humalog® Mix 75/25 Pen (Eli Lilly and Company), Humulin® 70/30 Pen (Eli Lilly and Company), Humulin® N Pen (Eli Lilly and Company), Novolog® FlexPen (Novo Nordisk), NovoPen® 3 (Novo Nordisk), NovoPen® 4 (Novo Nordisk), NovoPen® Junior (Novo Nordisk), Novolog® Mix 70/30 FlexPen (Novo Nordisk), InDuo® (Novo Nordisk), Novolin® InnoLet® (Novo Nordisk), Innovo® (Novo Nordisk), OptiPen® (Sanofi-Aventis) OptiPen® Pro2 (Sanofi-Aventis), OptiSet® (Sanofi-Aventis) and SoloSTAR® (Sanofi-Aventis).

c. Insulin pumps and other insulin delivery devices

The co-formulations provided herein can be administered to a diabetic subject using an insulin delivery device, such as an insulin pump or other similar continuous infusion device. Insulin delivery devices typically contain at least one disposable reservoir containing an insulin formulation, a pump (including any controls, software, processing modules and/or batteries) and a disposable infusion set, including a cannula or needle for subcutaneous injection and a tube connecting the cannula or needle to the insulin reservoir. For use with the stable co-formulations provided herein, the insulin delivery device can contain a reservoir containing the co-

formulated insulin and hyaluronan degrading enzyme. The co-formulations can be administered continuously or in bolus injections. Further, an insulin delivery device user has the ability to influence the profile of the insulin by shaping the bolus. For example, a standard bolus can be administered, which is an infusion similar to a discrete injection in that all of the dose is pumped immediately. An extended bolus is a slow infusion over time that avoids a high initial dose and extends the action of the composition. A combination bolus containing both a standard bolus and an extended bolus also can be administered using an insulin pump or other continuous delivery system. Insulin delivery devices are known in the art and described elsewhere, including, but not limited to, in U.S. Pat. Nos. 6,554,798, 6,641,533, 6,744,350, 6,852,104, 6,872,200, 6,936,029, 6,979,326, 6,999,854, 7,025,713 and 7,109,878. Insulin delivery devices also can be connected to a glucose monitor or sensor, and/or can contain a means to calculate the recommended insulin dose based upon blood glucose levels, carbohydrate content of a meal, or other input. Further insulin delivery devices can be implantable or can be external to the subject.

d. Continuous Infusion Pump systems

An insulin delivery device for use with the co-formulations herein includes an insulin pump or other similar device capable of continuous subcutaneous insulin infusion. Insulin delivery devices, including open loop and closed loop systems, typically contain at least one disposable reservoir containing an insulin co-formulation, a pump (including any controls, software, processing modules and/or batteries) and a disposable infusion set, including a cannula or needle for subcutaneous injection and a tube connecting the cannula or needle to the insulin reservoir. Closed loop delivery devices additionally include a glucose monitor or sensor. The insulin delivery device can contain a reservoir containing a super-fast acting insulin co-formulation of insulin and a hyaluronan degrading enzyme.

The insulin co-formulations can be administered continuously and/or in bolus injections. Users set the pump to give a steady trickle or "basal" amount of insulin formulation continuously throughout the day. Pumps also release additional ("bolus") doses of insulin formulation at meals and at times when blood sugar is too high based on user input. Frequent blood glucose monitoring is essential to determine insulin dosages and to ensure that insulin is delivered appropriately. This can be achieved by

manual monitoring, a separate or contained glucose monitor. Further, an insulin delivery device user has the ability to influence the profile of the insulin by shaping the bolus. For example, a standard bolus can be administered, which is an infusion similar to a discrete injection in that all of the dose is pumped immediately. An extended bolus is a slow infusion over time that avoids a high initial dose and extends the action of the composition. A combination bolus containing both a standard bolus and an extended bolus also can be administered using an insulin pump or other continuous delivery system.

Insulin delivery devices are known in the art and described elsewhere, including, but not limited to, in U.S. Pat. Nos. 6,554,798, 6,641,533, 6,744,350, 6,852,104, 6,872,200, 6,936,029, 6,979,326, 6,999,854, 7,025,713 and 7,109,878. Insulin delivery devices also can be connected to a glucose monitor or sensor, *e.g.* , a closed-loop system, and/or can contain a means to calculate the recommended insulin dose based upon blood glucose levels, carbohydrate content of a meal, or other input. Further insulin delivery devices can be implantable or can be external to the subject. The use of external insulin infusion pumps requires careful selection of individuals, meticulous monitoring, and thorough education and long term ongoing follow-up. This care is generally provided by a multidisciplinary team of health professionals with specific expertise and experience in the management of individuals on insulin pump treatment.

i. Open loop systems

Open loop systems can be used with the co-formulations provided herein. Open loop systems typically contain at least one disposable reservoir containing an insulin formulation, a pump (including any controls, software, processing modules and/or batteries) and a disposable infusion set, including a cannula or needle for subcutaneous injection and a tube connecting the cannula or needle to the insulin reservoir. The open loop system infuses in small (basal) doses every few minutes and large (bolus) doses that the patient sets manually. But, an open loop system does not generally contain a glucose monitor or sensor and therefore cannot respond to changes in the patient's serum glucose levels. Various methods and devices used to measure blood glucose levels are known to one of skill in the art. The conventional technique used by many diabetics for personally monitoring their blood glucose level includes

the periodic drawing of blood, the application of that blood to a test strip, and the determination of the blood glucose level using calorimetric, electrochemical, or photometric detection. A variety of devices have been developed for continuous or automatic monitoring of analytes, such as glucose, in the blood stream or interstitial fluid. Some of these devices use electrochemical sensors which are directly implanted into a blood vessel or in the subcutaneous tissue of a patient. Exemplary methods and devices for monitoring glucose levels include, but are not limited to, those described in U.S. Pat. Nos. 5,001,054, 5,009,230,5,713,353, 6,560,417, 6,574,490, 6,892,085, 6,958,809, 7,299,081, 7,774,145, 7,826,879, 7,857,760 and 7,885,699, which are incorporated herein by reference.

Insulin delivery systems, such as insulin pumps, are known in the art and can be used in the open loop systems. Exemplary open loop insulin delivery devices (such as those described above) include, but are not limited to, those described in U.S. Pat. Nos. 4,562,751, 4,678,408, 4,685,903, 4,373,527, 4,573,994, 6,554,798, 6,641,533, 6,744,350, 6,852,104, 6,872,200, 6,936,029, 6,979,326, 6,999,854, 7,109,878, 7,938,797 and 7,959,598, which are incorporated by reference herein. These and similar systems, easily identifiable by one of skill in the art, can be used to deliver the co-formulations provided herein. The insulin delivery devices typically contain one or more reservoirs, which generally are disposable, containing an insulin preparation, such as a co-formulation of a fast acting insulin and hyaluronan degrading enzyme described herein. In some examples, the co-formulations are delivered using an infusion tube and a cannula or needle. In other examples, the infusion device is attached directly to the skin and the co-formulations flow from the infusion device, through a cannula or needle directly into the body without the use of a tube. In further examples, the infusion device is internal to the body and an infusion tube optionally can be used to deliver the co-formulations.

ii. Closed Loop Systems

Closed loop systems, sometimes referred to as an artificial pancreas, are of particular interest for use with the co-formulations provided herein. Closed loop systems refer to systems with an integrated continuous glucose monitor, an insulin pump or other delivery system and controller that includes a mathematical algorithm that constantly calculates the required insulin infusion for glycemic control based

upon real time measurements of blood glucose levels. Such systems, when optimized, can facilitate constant and very tight glycemic control, similar to the natural insulin response and glycemic control observed in a healthy non-diabetic subject. To be effective, however, closed loop systems require both a reliable and accurate continuous glucose monitor, and delivery of an insulin with a very fast action. For example, delays in insulin absorption and action associated with subcutaneous delivery of fast-acting insulins can lead to large postprandial glycemic excursions (Hovorka *et al.* (2006) *Diabetic Med.* 23:1-12). The delay because of insulin absorption, insulin action, interstitial glucose kinetics, and the transport time for *ex vivo*-based monitoring systems, such as those based on the microdialysis technique, can result in an overall 100 minute or more time lag from the time of insulin delivery to the peak of its detectable glucose-lowering effect (Hovorka *et al.* (2006) *Diabetic Med.* 23:1-12). Thus, once administered, insulin will continue to increase its measurable effect for nearly 2 hours. This can complicate effective lowering of glucose concentration following meal ingestion using a closed-loop system. First, a glucose increase has to be detected. However, this typically happens only after an approximate 10–40 minute lag. The system must determine that a meal has been digested and administer an appropriate insulin dose. The ability of the system to compensate subsequently for a 'misjudged' insulin dose is compromised by long delays and the inability to 'withdraw' insulin once administered. Such problems can, at least in part, be overcome by using the co-formulations of a fast-acting insulin and hyaluronan degrading enzyme, such as those provided herein, which can exhibit an increased rate and level of absorption and an associated improvement in the pharmacodynamics (*see e.g.* US20090304665 and WO2009134380). Co-formulations of fast-acting insulin and a hyaluronan degrading enzyme have a reduced t_{max} (*i.e.* achieve maximal concentration faster) than fast-acting insulins alone and begin controlling blood glucose levels faster than fast-acting insulins alone. This increased rate of absorbance and onset of action reduces the lag between insulin action and glucose monitoring and input, resulting in a more effective closed loop system that can more tightly control blood glucose levels, reducing glycemic excursions.

Closed loop systems are well known in the art and have been described elsewhere, including, but not limited to, U.S. Pat. Nos. 5,279,543, 5,569,186, 6,558,351, 6,558,345, 6,589,229, 6,669,663, 6,740,072, 7,267,665 and 7,354,420, which are incorporated by reference herein. These and similar systems, easily identifiable by one of skill in the art, can be used to deliver the co-formulations provided herein. Closed loops systems include a sensor system to measure blood glucose levels, a controller and a delivery system. This integrated system is designed to model a pancreatic beta cell (β-cell), such that it controls an infusion device to deliver insulin into a subject in a similar concentration profile as would be created by fully functioning human β-cells when responding to changes in blood glucose concentrations in the body. Thus, the system simulates the body's natural insulin response to blood glucose levels and not only makes efficient use of insulin, but also accounts for other bodily functions as well since insulin has both metabolic and mitogenic effects. Further, the glycemic control achieved using a closed loop system is achieved without requiring any information about the size and timing of a meal, or other factors. The system can rely solely on real time blood glucose measurements. The glucose sensor generates a sensor signal representative of blood glucose levels in the body, and provides the sensor signal to the controller. The controller receives the sensor signal and generates commands that are communicated to the insulin delivery system. The insulin delivery system receives the commands and infuses insulin into the body in response to the commands. Provided below are descriptions of exemplary components of closed loop systems that can be used to deliver the co-formulations of a fast acting insulin and a hyaluronan degrading enzyme provided herein. It is understood that one of skill in the art can readily identify suitable closed loop systems for use with the co-formulations. Such systems have been described in the art, including but not limited to, those described in U.S. Pat. Nos. 5,279,543, 5,569,186, 6,558,351, 6,558,345, 6,589,229, 6,669,663, 6,740,072, 7,267,665 and 7,354,420. The individual components of the systems also have been described in the art, individually and in the context of a closed loops system for use in achieving glycemic control. It is understood that the examples provided herein are exemplary only, and that other closed loop systems or individual components can be used to deliver the co-formulations provided herein.

Closed loop systems contain a glucose sensor or monitor that functions continuously. Such devices can contain needle-type sensors that are inserted under the skin and attached to a small transmitter that communicates glucose data wirelessly by radiofrequency telemetry to a small receiver. In some examples, the sensor is inserted through the subject's skin using an insertion needle, which is removed and disposed of once the sensor is positioned in the subcutaneous tissue. The insertion needle has a sharpened tip and an open slot to hold the sensor during insertion into the skin (*see e.g.* U.S. Pat. Nos. 5,586,553 and 5,954,643). The sensor used in the closed loop system can optionally contain three electrodes that are exposed to the interstitial fluid (ISF) in the subcutaneous tissue. The three electrodes include a working electrode, a reference electrode and a counter electrode that are used to form a circuit. When an appropriate voltage is supplied across the working electrode and the reference electrode, the ISF provides impedance between the electrodes. An analog current signal flows from the working electrode through the body and to the counter electrode. The voltage at the working electrode is generally held to ground, and the voltage at the reference electrode can be held at a set voltage Vset, such as, for example, between 300 and 700 mV. The most prominent reaction stimulated by the voltage difference between the electrodes is the reduction of glucose as it first reacts with the glucose oxidase enzyme (GOX) to generate gluconic acid and hydrogen peroxide (H_2O_2). Then the H_2O_2 is reduced to water (H_2O) and (O^-) at the surface of the working electrode. The O^- draws a positive charge from the sensor electrical components, thus repelling an electron and causing an electrical current flow. This results in the analog current signal being proportional to the concentration of glucose in the ISF that is in contact with the sensor electrodes (*see e.g.* U.S. Pat. No. 7,354,420).

In some examples, more than one sensor is used to measure blood glucose. For example, redundant sensors can be used and the subject can be notified when a sensor fails by the telemetered characteristic monitor transmitter electronics. An indicator also can inform the subject of which sensors are still functioning and/or the number of sensors still functioning. In other examples, sensor signals are combined through averaging or other means. Further, different types of sensors can be used.

For example, an internal glucose sensor and an external glucose sensor can be used to measure blood glucose at the same time.

Glucose sensors that can be used in a closed loop system are well known and can be readily identified and, optionally, further modified, by one of skill in the art. Exemplary internal glucose sensors include, but are not limited to, those described in U.S. Pat. Nos. 5,497,772, 5,660,163, 5,791,344, 5,569,186 and 6,895,265. Exemplary of a glucose sensor that uses florescence is that described in U.S. Pat. No. 6,011,984. Glucose sensor systems also can use other sensing technologies, including light beams, conductivity, jet sampling, micro dialysis, micro-poration, ultra sonic sampling, reverse iontophoresis, or other method (*e.g.* U.S. Pat. Nos. 5,433,197 and 5,945,676, and International Pat. Pub. WO 199929230). In some examples, only the working electrode is located in the subcutaneous tissue and in contact with the ISF, and the counter and reference electrodes are located external to the body and in contact with the skin. The counter electrode and the reference electrode can be located on the surface of a monitor housing and can be held to the skin as part of a telemetered characteristic monitor. In further examples, the counter electrode and the reference electrode are held to the skin using other devices, such as running a wire to the electrodes and taping the electrodes to the skin, incorporating the electrodes on the underside of a watch touching the skin. Still further, more than one working electrode can be placed into the subcutaneous tissue for redundancy. Interstitial fluid also can be harvested from the body of a subject and flowed over an external sensor that is not implanted in the body.

The controller receives input from the glucose sensor. The controller is designed to model a pancreatic beta cell (β-cell) and provide commands to the insulin delivery device to infuse the required amount of insulin for glycemic control. The controller utilizes software with algorithms to calculate the required amount of insulin based upon the glucose levels detected by the glucose sensor. Exemplary algorithms include those that model the β-cells closely, since algorithms that are designed to minimize glucose excursions in the body, without regard for how much insulin is delivered, can cause excessive weight gain, hypertension, and atherosclerosis. Typically, the system is intended to emulate the *in vivo* insulin secretion pattern and to adjust this pattern consistent with the *in vivo* β-cell adaptation experienced by

normal healthy individuals. Control algorithms useful for closed loop systems include those utilized by a proportional-integral-derivative (PID) controller. Proportional derivative controllers and model predictive control (MPC) algorithms also can be used in some systems (Hovorka *et al.* (2006) *Diabetic Med.* 23:1-12). Exemplary algorithms include, but are not limited to, those described Hovorka *et al.* (*Diabetic Med.* (2006) 23:1-12), Shimoda *et al.*, (*Front Med Biol Eng* (1997) 8:197-211), Shichiri *et al.* (*Artif. Organs* (1998) 22:32-42), Steil *et al.* (*Diabetes Technol Ther* (2003) 5: 953– 964), Kaletz *et al.*, (*Acta Diabetol.* (1999) 36:215) and U.S. Pat. Nos. 5,279,543, 5,569,186, 6,558,351, 6,558,345, 6,589,229, 6,740,042, 6,669,663, 6,740,072, 7,267,665 and 7,354,420 and U.S. Pat. Pub. No. 20070243567.

In one example, a PID controller is utilized in the closed loop system. A PID controller continuously adjusts the insulin infusion by assessing glucose excursions from three viewpoints: the departure from the target glucose (the proportional component), the area under the curve between ambient and target glucose (the integral component), and the change in ambient glucose (the derivative component). Generally, the *in vivo* β-cell response to changes in glucose is characterized by "first" and "second" phase insulin responses. The biphasic insulin response of a β-cell can be modeled using components of a proportional, plus integral, plus derivative (PID) controller (*see e.g.* U.S. Pat. No. 7,354,420).

The controller generates commands for the desired insulin delivery. Insulin delivery systems, such as insulin pumps, are known in the art and can be used in the closed loop systems. Exemplary insulin delivery devices (such as those described above) include, but are not limited to, those described in U.S. Pat. Nos. 4,562,751, 4,678,408, 4,685,903, 4,373,527, 4,573,994, 6,554,798, 6,641,533, 6,744,350, 6,852,104, 6,872,200, 6,936,029, 6,979,326, 6,999,854, 7,025,713 and 7,109,878. The insulin delivery devices typically contain one or more reservoirs, which generally are disposable, containing an insulin preparation, such as a co-formulation of a fast acting insulin and hyaluronan degrading enzyme described herein. In some examples, the co-formulations are delivered using an infusion tube and a cannula or needle. In other examples, the infusion device is attached directly to the skin and the co-formulations flow from the infusion device, through a cannula or needle directly into the body without the use of a tube. In further examples, the infusion device is internal

to the body and an infusion tube optionally can be used to deliver the co-formulations. Closed loop systems also can contain additional components, including, but not limited to, filters, calibrators and transmitters.

H. METHODS OF PRODUCING NUCLEIC ACIDS ENCODING AN INSULIN OR HYALURONAN DEGRADING ENZYME AND POLYPEPTIDES THEREOF

Polypeptides of an insulin and hyaluronan degrading enzyme set forth herein can be obtained by methods well known in the art for protein purification and recombinant protein expression. Polypeptides also can be synthesized chemically. For example, the A-chain and B-chain of insulin can be chemically synthesized and then cross-linked by disulfide bonds through, for example, a reduction-reoxidation reaction. When the polypeptides are produced by recombinant means, any method known to those of skill in the art for identification of nucleic acids that encode desired genes can be used. Any method available in the art can be used to obtain a full length (*i.e.*, encompassing the entire coding region) cDNA or genomic DNA clone encoding a hyaluronidase, such as from a cell or tissue source. Modified or variant insulins or hyaluronan degrading enzymes can be engineered from a wildtype polypeptide, such as by site-directed mutagenesis.

Polypeptides can be cloned or isolated using any available methods known in the art for cloning and isolating nucleic acid molecules. Such methods include PCR amplification of nucleic acids and screening of libraries, including nucleic acid hybridization screening, antibody-based screening and activity-based screening.

Methods for amplification of nucleic acids can be used to isolate nucleic acid molecules encoding a desired polypeptide, including for example, polymerase chain reaction (PCR) methods. A nucleic acid containing material can be used as a starting material from which a desired polypeptide-encoding nucleic acid molecule can be isolated. For example, DNA and mRNA preparations, cell extracts, tissue extracts, fluid samples (*e.g.* blood, serum, saliva), and samples from healthy and/or diseased subjects can be used in amplification methods. Nucleic acid libraries also can be used as a source of starting material. Primers can be designed to amplify a desired polypeptide. For example, primers can be designed based on expressed sequences from which a desired polypeptide is generated. Primers can be designed based on back-translation of a polypeptide amino acid sequence. Nucleic acid molecules

generated by amplification can be sequenced and confirmed to encode a desired polypeptide.

Additional nucleotide sequences can be joined to a polypeptide-encoding nucleic acid molecule, including linker sequences containing restriction endonuclease sites for the purpose of cloning the synthetic gene into a vector, for example, a protein expression vector or a vector designed for the amplification of the core protein coding DNA sequences. Furthermore, additional nucleotide sequences specifying functional DNA elements can be operatively linked to a polypeptide-encoding nucleic acid molecule. Examples of such sequences include, but are not limited to, promoter sequences designed to facilitate intracellular protein expression, and secretion sequences, for example heterologous signal sequences, designed to facilitate protein secretion. Such sequences are known to those of skill in the art. Additional nucleotide residues sequences such as sequences of bases specifying protein binding regions also can be linked to enzyme-encoding nucleic acid molecules. Such regions include, but are not limited to, sequences of residues that facilitate or encode proteins that facilitate uptake of an enzyme into specific target cells, or otherwise alter pharmacokinetics of a product of a synthetic gene. For example, enzymes can be linked to PEG moieties.

In addition, tags or other moieties can be added, for example, to aid in detection or affinity purification of the polypeptide. For example, additional nucleotide residues sequences such as sequences of bases specifying an epitope tag or other detectable marker also can be linked to enzyme-encoding nucleic acid molecules. Exemplary of such sequences include nucleic acid sequences encoding a His tag (*e.g.*, 6xHis, HHHHHH; SEQ ID NO:54) or Flag Tag (DYKDDDDK; SEQ ID NO:55).

The identified and isolated nucleic acids can then be inserted into an appropriate cloning vector. A large number of vector-host systems known in the art can be used. Possible vectors include, but are not limited to, plasmids or modified viruses, but the vector system must be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, or plasmids such as pCMV4, pBR322 or pUC plasmid derivatives or the Bluescript vector (Stratagene, La Jolla, CA). Other expression vectors include the HZ24

expression vector exemplified herein. The insertion into a cloning vector can, for example, be accomplished by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. Insertion can be effected using TOPO cloning vectors (Invitrogen, Carlsbad, CA). If the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules can be enzymatically modified. Alternatively, any site desired can be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers can contain specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. In an alternative method, the cleaved vector and protein gene can be modified by homopolymeric tailing. Recombinant molecules can be introduced into host cells via, for example, transformation, transfection, infection, electroporation and sonoporation, so that many copies of the gene sequence are generated.

Insulin can be produced using a variety of techniques (*see e.g.* Ladisch *et al.* (1992) *Biotechnol. Prog.* 8:469-478). In some examples, nucleic acid encoding a preproinsulin or proinsulin polypeptide is inserted into an expression vector. Upon expression, the preproinsulin or proinsulin polypeptide is converted to insulin by enzymatic or chemical methods that cleave the signal sequence and/or the C peptide, resulting in the A- and B-chains that are cross-linked by disulfide bonds through, for example, a reduction-reoxidation reaction (*see e.g.* Cousens *et al.*, (1987) *Gene* 61:265-275, Chance *et al.*, (1993) *Diabetes Care* 4:147-154). In another example, the nucleic acid encoding the A-chain and B-chain of an insulin are inserted into one or two expression vectors for co-expression as a single polypeptide from one expression vector or expression as two polypeptides from one or two expression vectors. Thus, the A- and B-chain polypeptides can be expressed separately and then combined to generate an insulin, or can be co-expressed, in the absence of a C chain. In instances where the A- and B-chains are co-expressed as a single polypeptide, the nucleic acid encoding the subunits also can encode a linker or spacer between the B-chain and A-chain, such as a linker or spacer described below. The nucleic acid inserted into the expression vector can contain, for example, nucleic acid encoding the insulin B-chain, a linker, such as for example, an alanine-alanine-lysine linker, and the A-chain,

resulting in expression of, for example, "insulin B chain-Ala-Ala-Lys-insulin A chain."

In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the isolated protein gene, cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene can be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants and, when necessary, retrieving the inserted gene from the isolated recombinant DNA.

1. Vectors and cells

For recombinant expression of one or more of the desired proteins, such as any described herein, the nucleic acid containing all or a portion of the nucleotide sequence encoding the protein can be inserted into an appropriate expression vector, *i.e.*, a vector that contains the necessary elements for the transcription and translation of the inserted protein coding sequence. The necessary transcriptional and translational signals also can be supplied by the native promoter for enzyme genes, and/or their flanking regions.

Also provided are vectors that contain a nucleic acid encoding the enzyme. Cells containing the vectors also are provided. The cells include eukaryotic and prokaryotic cells, and the vectors are any suitable for use therein.

Prokaryotic and eukaryotic cells, including endothelial cells, containing the vectors are provided. Such cells include bacterial cells, yeast cells, fungal cells, Archea, plant cells, insect cells and animal cells. The cells are used to produce a protein thereof by growing the above-described cells under conditions whereby the encoded protein is expressed by the cell, and recovering the expressed protein. For purposes herein, for example, the enzyme can be secreted into the medium.

Provided are vectors that contain a sequence of nucleotides that encodes the soluble hyaluronidase polypeptide coupled to the native or heterologous signal sequence, as well as multiple copies thereof. The vectors can be selected for expression of the enzyme protein in the cell or such that the enzyme protein is expressed as a secreted protein.

A variety of host-vector systems can be used to express the protein coding sequence. These include but are not limited to mammalian cell systems infected with

virus (*e.g.* vaccinia virus, adenovirus and other viruses); insect cell systems infected with virus (*e.g.* baculovirus); microorganisms such as yeast containing yeast vectors; or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system used, any one of a number of suitable transcription and translation elements can be used.

Any methods known to those of skill in the art for the insertion of DNA fragments into a vector can be used to construct expression vectors containing a chimeric gene containing appropriate transcriptional/translational control signals and protein coding sequences. These methods can include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of nucleic acid sequences encoding protein, or domains, derivatives, fragments or homologs thereof, can be regulated by a second nucleic acid sequence so that the genes or fragments thereof are expressed in a host transformed with the recombinant DNA molecule(s). For example, expression of the proteins can be controlled by any promoter/enhancer known in the art. In a specific embodiment, the promoter is not native to the genes for a desired protein. Promoters which can be used include but are not limited to the SV40 early promoter (Bernoist and Chambon, *Nature 290*:304-310 (1981)), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto *et al. Cell 22*:787-797 (1980)), the herpes thymidine kinase promoter (Wagner *et al.*, *Proc. Natl. Acad. Sci. USA 78*:1441-1445 (1981)), the regulatory sequences of the metallothionein gene (Brinster *et al.*, *Nature 296*:39-42 (1982)); prokaryotic expression vectors such as the β-lactamase promoter (Jay *et al.*, (1981) *Proc. Natl. Acad. Sci. USA 78*:5543) or the *tac* promoter (DeBoer *et al.*, *Proc. Natl. Acad. Sci. USA 80*:21-25 (1983)); see also "Useful Proteins from Recombinant Bacteria": in Scientific American *242*:79-94 (1980)); plant expression vectors containing the nopaline synthetase promoter (Herrara-Estrella *et al.*, *Nature 303*:209-213 (1984)) or the cauliflower mosaic virus 35S RNA promoter (Gardner *et al.*, *Nucleic Acids Res. 9*:2871 (1981)), and the promoter of the photosynthetic enzyme ribulose bisphosphate carboxylase (Herrera-Estrella *et al.*, *Nature 310*:115-120 (1984)); promoter elements from yeast and other fungi such as the Gal4 promoter, the alcohol dehydrogenase promoter, the phosphoglycerol kinase promoter, the alkaline

phosphatase promoter, and the following animal transcriptional control regions that exhibit tissue specificity and have been used in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift *et al.*, *Cell 38*:639-646 (1984); Ornitz *et al.*, *Cold Spring Harbor Symp. Quant. Biol. 50*:399-409 (1986); MacDonald, *Hepatology 7*:425-515 (1987)); insulin gene control region which is active in pancreatic beta cells (Hanahan *et al.*, *Nature 315*:115-122 (1985)), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl *et al.*, *Cell 38*:647-658 (1984); Adams *et al.*, *Nature 318*:533-538 (1985); Alexander *et al.*, *Mol. Cell Biol. 7*:1436-1444 (1987)), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder *et al.*, *Cell 45*:485-495 (1986)), albumin gene control region which is active in liver (Pinckert *et al.*, *Genes and Devel. 1*:268-276 (1987)), alpha-fetoprotein gene control region which is active in liver (Krumlauf *et al.*, *Mol. Cell. Biol. 5*:1639-1648 (1985); Hammer *et al.*, *Science 235*:53-58 1987)), alpha-1 antitrypsin gene control region which is active in liver (Kelsey *et al.*, *Genes and Devel. 1*:161-171 (1987)), beta globin gene control region which is active in myeloid cells (Magram *et al.*, *Nature 315*:338-340 (1985); Kollias *et al.*, *Cell 46*:89-94 (1986)), myelin basic protein gene control region which is active in oligodendrocyte cells of the brain (Readhead *et al.*, *Cell 48*:703-712 (1987)), myosin light chain-2 gene control region which is active in skeletal muscle (Shani, *Nature 314*:283-286 (1985)), and gonadotrophic releasing hormone gene control region which is active in gonadotrophs of the hypothalamus (Mason *et al.*, *Science 234*:1372-1378 (1986)).

In a specific embodiment, a vector is used that contains a promoter operably linked to nucleic acids encoding a desired protein, or a domain, fragment, derivative or homolog, thereof, one or more origins of replication, and optionally, one or more selectable markers (*e.g.*, an antibiotic resistance gene). Exemplary plasmid vectors for transformation of *E. coli* cells, include, for example, the pQE expression vectors (available from Qiagen, Valencia, CA; see also literature published by Qiagen describing the system). pQE vectors have a phage T5 promoter (recognized by *E. coli* RNA polymerase) and a double lac operator repression module to provide tightly regulated, high-level expression of recombinant proteins in *E. coli,* a synthetic ribosomal binding site (RBS II) for efficient translation, a 6XHis tag coding sequence,

t_0 and T1 transcriptional terminators, ColE1 origin of replication, and a beta-lactamase gene for conferring ampicillin resistance. The pQE vectors enable placement of a 6xHis tag at either the N- or C-terminus of the recombinant protein. Such plasmids include pQE 32, pQE 30, and pQE 31 which provide multiple cloning sites for all three reading frames and provide for the expression of N-terminally 6xHis-tagged proteins. Other exemplary plasmid vectors for transformation of *E. coli* cells include, for example, the pET expression vectors (see, U.S. Pat. 4,952,496; available from Novagen, Madison, WI; see, also literature published by Novagen describing the system). Such plasmids include pET 11a, which contains the T7lac promoter, T7 terminator, the inducible *E. coli* lac operator, and the lac repressor gene; pET 12a-c, which contains the T7 promoter, T7 terminator, and the *E. coli* ompT secretion signal; and pET 15b and pET19b (Novagen, Madison, WI), which contain a His-Tag™ leader sequence for use in purification with a His column and a thrombin cleavage site that permits cleavage following purification over the column, the T7-lac promoter region and the T7 terminator.

Exemplary of a vector for mammalian cell expression is the HZ24 expression vector. The HZ24 expression vector was derived from the pCI vector backbone (Promega). It contains DNA encoding the Beta-lactamase resistance gene (AmpR), an F1 origin of replication, a Cytomegalovirus immediate-early enhancer/promoter region (CMV), and an SV40 late polyadenylation signal (SV40). The expression vector also has an internal ribosome entry site (IRES) from the ECMV virus (Clontech) and the mouse dihydrofolate reductase (DHFR) gene.

2. Linker Moieties

In some examples, insulin is prepared by generating the A-chain and B-chain polypeptides with a linker, such that, for example, the C-terminus of the B-chain is joined to the N-terminus of the A-chain by a short linker. The A-chain and B-chains can be expressed from a single polypeptide containing a linker, or can be expressed separately and then joined by a linker. The linker moiety is selected depending upon the properties desired. The linker moiety should be long enough and flexible enough to allow the A-chain and B-chain to mimic the natural conformation of the insulin.

Linkers can be any moiety suitable to the insulin A-chain and B-chain. Such moieties include, but are not limited to, peptidic linkages; amino acid and peptide

linkages, typically containing between one and about 60 amino acids; chemical linkers, such as heterobifunctional cleavable cross-linkers, photocleavable linkers and acid cleavable linkers.

The linker moieties can be peptides. The peptide typically has from about 2 to about 60 amino acid residues, for example from about 5 to about 40, or from about 10 to about 30 amino acid residues. Peptidic linkers can conveniently be encoded by nucleic acid and incorporated in fusion proteins upon expression in a host cell, such as *E. coli*. In one example, an alanine-alanine-lysine (AAK) (SEQ ID NO:178) linker is encoded in a nucleic acid between nucleic acid encoding the insulin B-chain and nucleic acid encoding the A-chain, such that upon expression, an "insulin B-chain-AAK-insulin A chain" polypeptide is produced. Peptide linkers can be a flexible spacer amino acid sequence, such as those known in single-chain antibody research. Examples of such known linker moieties include, but are not limited to, RPPPPC (SEQ ID NO:166) or SSPPPPC (SEQ ID NO:167), GGGGS (SEQ ID NO:168), $(GGGGS)_n$ (SEQ ID NO:169), GKSSGSGSESKS (SEQ ID NO:170), GSTSGSGKSSEGKG (SEQ ID NO:171), GSTSGSGKSSEGSGSTKG (SEQ ID NO:172), GSTSGSGKSSEGKG (SEQ ID NO:173), GSTSGSGKPGSGEGSTKG (SEQ ID NO:174), EGKSSGSGSESKEF (SEQ ID NO:175), SRSSG (SEQ ID NO:176) and SGSSC (SEQ ID NO:177).

Alternatively, the peptide linker moiety can be VM (SEQ ID NO: 179) or AM (SEQ ID NO: 180), or have the structure described by the formula: $AM(G_{2\text{ to }4}S)_XAM$ wherein X is an integer from 1 to 11 (SEQ ID NO: 181). Additional linking moieties are described, for example, in Huston *et al.*(1988) *Proc. Natl. Acad. Sci. U.S.A.* 85:5879-5883; Whitlow, M., *et al.* (1993) *Protein Engineering* 6:989-995; Newton *et al.* (1996) *Biochemistry* 35:545-553; A. J. Cumber *et al.* (1992) *Bioconj. Chem.* 3:397-401; Ladurner *et al.* (1997) *J. Mol. Biol.* 273:330-337; and U.S. Pat. No. 4,894,443.

In some examples, peptide linkers are encoded by nucleic acid and incorporated between the B-chain and A-chain upon expression in a host cell, such as *E. coli* or *S. cerevisiae*. In other examples, a peptide linker is synthesized by chemical methods. This can be performed in a separate protocol to the synthesis of one or more of the A- and B-chain, after which the components are joined, such as with the use of

heterobifunctional linkers. Alternatively, a peptide linker can be synthesized at the N- or C- terminus of one of the insulin chains, which is then linked to the other chain via the peptide linker, such as with a heterobifunctional linker.

Any linker known to those of skill in the art can be used herein to link the insulin A-chain and B-chain. Linkers and linkages that are suitable for chemically linking the chains include, but are not limited to, disulfide bonds, thioether bonds, hindered disulfide bonds, and covalent bonds between free reactive groups, such as amine and thiol groups. These bonds are produced using heterobifunctional reagents to produce reactive thiol groups on one or both of the polypeptides and then reacting the thiol groups on one polypeptide with reactive thiol groups or amine groups to which reactive maleimido groups or thiol groups can be attached on the other. Other linkers include, acid cleavable linkers, such as bismaleimideothoxy propane, acid labile-transferrin conjugates and adipic acid dihydrazide, that would be cleaved in more acidic intracellular compartments; cross linkers that are cleaved upon exposure to UV or visible light and linkers, such as the various domains, such as CH1, CH2, and CH3, from the constant region of human IgG1 (see, Batra *et al.* (1993) *Molecular Immunol.* 30:379-386). In some embodiments, several linkers can be included in order to take advantage of desired properties of each linker. Chemical linkers and peptide linkers can be inserted by covalently coupling the linker to the insulin A-chain and B-chain. The heterobifunctional agents, described below, can be used to effect such covalent coupling. Peptide linkers also can be linked by expressing DNA encoding the linker between the B-chain and A-chain.

Other linkers that can be used to join the A-chain and B-chain of insulin include: enzyme substrates, such as cathepsin B substrate, cathepsin D substrate, trypsin substrate, thrombin substrate, subtilisin substrate, Factor Xa substrate, and enterokinase substrate; linkers that increase solubility, flexibility, and/or intracellular cleavability include linkers, such as $(gly_mser)_n$ and $(ser_mgly)_n$, in which m is 1 to 6, preferably 1 to 4, more preferably 2 to 4, and n is 1 to 30, preferably 1 to 10, more preferably 1 to 4 (see, *e.g.*, International PCT application No. WO 96/06641, which provides exemplary linkers). In some embodiments, several linkers can be included in order to take advantage of desired properties of each linker.

3. Expression

Insulin and hyaluronan degrading enzyme polypeptides can be produced by any method known to those of skill in the art including *in vivo* and *in vitro* methods. Desired proteins can be expressed in any organism suitable to produce the required amounts and forms of the proteins, such as for example, needed for administration and treatment. Expression hosts include prokaryotic and eukaryotic organisms such as *E. coli*, yeast, plants, insect cells, mammalian cells, including human cell lines and transgenic animals. Expression hosts can differ in their protein production levels as well as the types of post-translational modifications that are present on the expressed proteins. The choice of expression host can be made based on these and other factors, such as regulatory and safety considerations, production costs and the need and methods for purification.

Many expression vectors are available and known to those of skill in the art and can be used for expression of proteins. The choice of expression vector will be influenced by the choice of host expression system. In general, expression vectors can include transcriptional promoters and optionally enhancers, translational signals, and transcriptional and translational termination signals. Expression vectors that are used for stable transformation typically have a selectable marker which allows selection and maintenance of the transformed cells. In some cases, an origin of replication can be used to amplify the copy number of the vector.

Soluble hyaluronidase polypeptides also can be utilized or expressed as protein fusions. For example, an enzyme fusion can be generated to add additional functionality to an enzyme. Examples of enzyme fusion proteins include, but are not limited to, fusions of a signal sequence, a tag such as for localization, *e.g.* a his_6 tag or a myc tag, or a tag for purification, for example, a GST fusion, and a sequence for directing protein secretion and/or membrane association.

a. Prokaryotic Cells

Prokaryotes, especially *E. coli*, provide a system for producing large amounts of proteins. Transformation of *E. coli* is a simple and rapid technique well known to those of skill in the art. Expression vectors for *E. coli* can contain inducible promoters, such promoters are useful for inducing high levels of protein expression and for expressing proteins that exhibit some toxicity to the host cells. Examples of inducible promoters include the lac promoter, the trp promoter, the hybrid tac

promoter, the T7 and SP6 RNA promoters and the temperature regulated λPL promoter.

Proteins, such as any provided herein, can be expressed in the cytoplasmic environment of *E. coli*. The cytoplasm is a reducing environment and for some molecules, this can result in the formation of insoluble inclusion bodies. Reducing agents such as dithiothreitol and β-mercaptoethanol and denaturants, such as guanidine-HCl and urea can be used to resolubilize the proteins. An alternative approach is the expression of proteins in the periplasmic space of bacteria which provides an oxidizing environment and chaperonin-like and disulfide isomerases and can lead to the production of soluble protein. Typically, a leader sequence is fused to the protein to be expressed which directs the protein to the periplasm. The leader is then removed by signal peptidases inside the periplasm. Examples of periplasmic-targeting leader sequences include the pelB leader from the pectate lyase gene and the leader derived from the alkaline phosphatase gene. In some cases, periplasmic expression allows leakage of the expressed protein into the culture medium. The secretion of proteins allows quick and simple purification from the culture supernatant. Proteins that are not secreted can be obtained from the periplasm by osmotic lysis. Similar to cytoplasmic expression, in some cases proteins can become insoluble and denaturants and reducing agents can be used to facilitate solubilization and refolding. Temperature of induction and growth also can influence expression levels and solubility, typically temperatures between 25° C and 37° C are used. Typically, bacteria produce aglycosylated proteins. Thus, if proteins require glycosylation for function, glycosylation can be added *in vitro* after purification from host cells.

b. Yeast Cells

Yeasts such as *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Yarrowia lipolytica, Kluyveromyces lactis* and *Pichia pastoris* are well known yeast expression hosts that can be used for production of proteins, such as any described herein. Yeast can be transformed with episomal replicating vectors or by stable chromosomal integration by homologous recombination. Typically, inducible promoters are used to regulate gene expression. Examples of such promoters include GAL1, GAL7 and GAL5 and metallothionein promoters, such as CUP1, AOX1 or

other *Pichia* or other yeast promoter. Expression vectors often include a selectable marker such as LEU2, TRP1, HIS3 and URA3 for selection and maintenance of the transformed DNA. Proteins expressed in yeast are often soluble. Co-expression with chaperonins such as Bip and protein disulfide isomerase can improve expression levels and solubility. Additionally, proteins expressed in yeast can be directed for secretion using secretion signal peptide fusions such as the yeast mating type alpha-factor secretion signal from *Saccharomyces cerevisiae* and fusions with yeast cell surface proteins such as the Aga2p mating adhesion receptor or the *Arxula adeninivoran*s glucoamylase. A protease cleavage site such as for the Kex-2 protease, can be engineered to remove the fused sequences from the expressed polypeptides as they exit the secretion pathway. Yeast also is capable of glycosylation at Asn-X-Ser/Thr motifs.

c. Insect Cells

Insect cells, particularly using baculovirus expression, are useful for expressing polypeptides such as hyaluronidase polypeptides. Insect cells express high levels of protein and are capable of most of the post-translational modifications used by higher eukaryotes. Baculovirus have a restrictive host range which improves the safety and reduces regulatory concerns of eukaryotic expression. Typical expression vectors use a promoter for high level expression such as the polyhedrin promoter of baculovirus. Commonly used baculovirus systems include the baculoviruses such as *Autographa californica* nuclear polyhedrosis virus (AcNPV), and the *Bombyx mori* nuclear polyhedrosis virus (BmNPV) and an insect cell line such as Sf9 derived from *Spodoptera frugiperda, Pseudaletia unipuncta* (A7S) and *Danaus plexippus* (DpN1). For high-level expression, the nucleotide sequence of the molecule to be expressed is fused immediately downstream of the polyhedrin initiation codon of the virus. Mammalian secretion signals are accurately processed in insect cells and can be used to secrete the expressed protein into the culture medium. In addition, the cell lines *Pseudaletia unipuncta* (A7S) and *Danaus plexippus* (DpN1) produce proteins with glycosylation patterns similar to mammalian cell systems.

An alternative expression system in insect cells is the use of stably transformed cells. Cell lines such as the Schneider 2 (S2) and Kc cells (*Drosophila melanogaster*) and C7 cells (*Aedes albopictus*) can be used for expression. The

Drosophila metallothionein promoter can be used to induce high levels of expression in the presence of heavy metal induction with cadmium or copper. Expression vectors are typically maintained by the use of selectable markers such as neomycin and hygromycin.

d. Mammalian Cells

Mammalian expression systems can be used to express proteins including soluble hyaluronidase polypeptides. Expression constructs can be transferred to mammalian cells by viral infection such as adenovirus or by direct DNA transfer such as liposomes, calcium phosphate, DEAE-dextran and by physical means such as electroporation and microinjection. Expression vectors for mammalian cells typically include an mRNA cap site, a TATA box, a translational initiation sequence (Kozak consensus sequence) and polyadenylation elements. IRES elements also can be added to permit bicistronic expression with another gene, such as a selectable marker. Such vectors often include transcriptional promoter-enhancers for high-level expression, for example the SV40 promoter-enhancer, the human cytomegalovirus (CMV) promoter and the long terminal repeat of Rous sarcoma virus (RSV). These promoter-enhancers are active in many cell types. Tissue and cell-type promoters and enhancer regions also can be used for expression. Exemplary promoter/enhancer regions include, but are not limited to, those from genes such as elastase I, insulin, immunoglobulin, mouse mammary tumor virus, albumin, alpha fetoprotein, alpha 1 antitrypsin, beta globin, myelin basic protein, myosin light chain 2, and gonadotropic releasing hormone gene control. Selectable markers can be used to select for and maintain cells with the expression construct. Examples of selectable marker genes include, but are not limited to, hygromycin B phosphotransferase, adenosine deaminase, xanthine-guanine phosphoribosyl transferase, aminoglycoside phosphotransferase, dihydrofolate reductase (DHFR) and thymidine kinase. For example, expression can be performed in the presence of methotrexate to select for only those cells expressing the DHFR gene. Fusion with cell surface signaling molecules such as TCR-ζ and $Fc_{\varepsilon}RI$-γ can direct expression of the proteins in an active state on the cell surface.

Many cell lines are available for mammalian expression including mouse, rat human, monkey, chicken and hamster cells. Exemplary cell lines include but are not

limited to CHO, Balb/3T3, HeLa, MT2, mouse NS0 (nonsecreting) and other myeloma cell lines, hybridoma and heterohybridoma cell lines, lymphocytes, fibroblasts, Sp2/0, COS, NIH3T3, HEK293, 293S, 2B8, and HKB cells. Cell lines also are available adapted to serum-free media which facilitates purification of secreted proteins from the cell culture media. Examples include CHO-S cells (Invitrogen, Carlsbad, CA, cat # 11619-012) and the serum free EBNA-1 cell line (Pham *et al.*, (2003) *Biotechnol. Bioeng.* 84:332-42.). Cell lines also are available that are adapted to grow in special mediums optimized for maximal expression. For example, DG44 CHO cells are adapted to grow in suspension culture in a chemically defined, animal product-free medium.

e. Plants

Transgenic plant cells and plants can be used to express proteins such as any described herein. Expression constructs are typically transferred to plants using direct DNA transfer such as microprojectile bombardment and PEG-mediated transfer into protoplasts, and with agrobacterium-mediated transformation. Expression vectors can include promoter and enhancer sequences, transcriptional termination elements and translational control elements. Expression vectors and transformation techniques are usually divided between dicot hosts, such as *Arabidopsis* and tobacco, and monocot hosts, such as corn and rice. Examples of plant promoters used for expression include the cauliflower mosaic virus promoter, the nopaline synthase promoter, the ribose bisphosphate carboxylase promoter and the ubiquitin and UBQ3 promoters. Selectable markers such as hygromycin, phosphomannose isomerase and neomycin phosphotransferase are often used to facilitate selection and maintenance of transformed cells. Transformed plant cells can be maintained in culture as cells, aggregates (callus tissue) or regenerated into whole plants. Transgenic plant cells also can include algae engineered to produce hyaluronidase polypeptides. Because plants have different glycosylation patterns than mammalian cells, this can influence the choice of protein produced in these hosts.

4. Purification Techniques

Method for purification of polypeptides, including insulin and hyaluronan degrading enzyme polypeptides or other proteins, from host cells will depend on the chosen host cells and expression systems. For secreted molecules, proteins are

generally purified from the culture media after removing the cells. For intracellular expression, cells can be lysed and the proteins purified from the extract. When transgenic organisms such as transgenic plants and animals are used for expression, tissues or organs can be used as starting material to make a lysed cell extract. Additionally, transgenic animal production can include the production of polypeptides in milk or eggs, which can be collected, and if necessary, the proteins can be extracted and further purified using standard methods in the art.

Proteins, such as insulin polypeptides or hyaluronan degrading enzyme polypeptides, can be purified using standard protein purification techniques known in the art including but not limited to, SDS-PAGE, size fractionation and size exclusion chromatography, ammonium sulfate precipitation and ionic exchange chromatography, such as anion exchange. Affinity purification techniques also can be utilized to improve the efficiency and purity of the preparations. For example, antibodies, receptors and other molecules that bind hyaluronidase enzymes can be used in affinity purification. Expression constructs also can be engineered to add an affinity tag to a protein such as a myc epitope, GST fusion or His_6 and affinity purified with myc antibody, glutathione resin and Ni-resin, respectively. Purity can be assessed by any method known in the art including gel electrophoresis, orthogonal HPLC methods, staining and spectrophotometric techniques.

I. Methods of Assessing Stability and Activity

Assays can be used to assess the stability of the formulations or co-formulations provided herein, including co-formulations containing a fast-acting insulin and hyaluronan degrading enzyme provided herein. Such assays can assess the stability and activity of the hyaluronan degrading enzyme and/or the stability, activity and solubility of the fast-acting insulin in the co-formulations. Such assays can be used, for example, to determine the stability of the co-formulations over time at particular storage temperatures and conditions, by assessing activity, solubility, and stability (*e.g.* formation of aggregates, etc.) prior to storage and then at various time points thereafter. The assays also can be used make minor adjustments to the formulations provided herein while retaining the stability of both active agents.

1. Insulin

The stability and solubility of insulin the co-formulations provided herein can be assessed using methods and assays well known in the art. For example, insulin stability and solubility can be assessed by visual assessment, acid clarification, optical microscopy, reversed phase high performance liquid chromatography (RP-HPLC), *in vivo* bioassays and denaturing and non-denaturing size exclusion chromatography (SEC). In one example, insulin solubility and stability is determined by visual assessment, including changes in color, clarity, presence of aggregates or clumping and material adhesion, or frosting, to the vessel containing the co-formulations provided herein. Visual changes are confirmed by acid clarification, wherein lack of dissolution after acidification confirms the presence of insoluble denatured insulin in the co-formulations provided herein. Visual changes in the insulin in the co-formulations provided herein can also be confirmed by optical microscopy and/or micrography by fluorescent backlighting. The apparent solubility of a fast acting insulin in the co-formulations provided herein can be assessed, for example, by RP-HPLC, such as described in Example 3. In the methods sets forth in Example 3, apparent solubility is measured as the percent insulin recovery following storage under various conditions and time points. Percent recovery is determined as compared to a reference sample. In addition, insulin degradation products, such as desamido insulin, can be determined by RP-HPLC. In one example, insulin stability in the co-formulations provided herein is assessed by measuring the formation of aggregates using size exclusion chromatography (*see e.g.*, Example 4). In this example, SEC is used to determine the presence of high molecular weight proteins, *i.e.*, aggregates.

Insulin activity also can be assessed using methods and assays well known in the art. For example, the ability of an insulin, including insulin compositions and co-formulations, to act as a therapeutic agent can be assessed *in vitro* or *in vivo*. For example, *in vitro* assays well known in the art can be performed to assess the ability an insulin to bind to insulin receptor. In one example, a competitive binding assay is performed in which human placental cell membranes are prepared as a source of insulin receptors and incubated with radiolabeled human insulin with or without the unlabeled insulin analog. The amount of bound radiolabeled insulin is then detected to determine the ability of the insulin analog to compete for binding and the relative

affinity of the insulin analog for the placental insulin receptor is calculated (*see e.g.* Weiss *et al.*, (2001) *J. Biol. Chem.* 276:40018-40024). Other sources of insulin receptors, including other cells that naturally or recombinantly express the insulin receptor, also can be used in such competitive binding assays (Duttaroy *et al.*, (2005) *Diabetes* 54:251-258).

The ability of insulin to stimulate glucose uptake or effect any other of its typical metabolic outcomes can be assessed *in vitro*. To measure insulin-stimulated glucose uptake, adipocytes are incubated with labeled glucose, such as 2-deoxy-D-[2,6-^{3}H]glucose or D- [U-^{14}C]glucose with or without insulin. The incorporated radioactivity is then measured to determine the amount of glucose uptake in the presence or absence of insulin (Louveau *et al.*, (2004) *J Endocrin.* 181:271-280, Duttaroy *et al.*, (2005) *Diabetes* 54:251-258). When assessing the activity of an insulin analog, the activity of human insulin also can be assessed and used for comparison. *In vitro* assays to assess glucose production in H4IIE cells in the presence of insulin also can be performed (Wang *et al.*, (2000) *J. Biochem.*, 275:14717-14721, Duttaroy *et al.*, (2005) *Diabetes* 54:251-258).

In vivo studies using diabetic or healthy animal models or human subjects also can be performed to assess the therapeutic activity of insulin, including insulin compositions and co-formulations. Insulin can be administered to animal models of diabetes to assess the effects on blood glucose levels, circulating insulin levels, and hemoglobin A1c (HbA1c), for example. Hemoglobin A1c forms when glucose attaches to hemoglobin, which occurs when blood glucose levels are elevated. HbA1c levels in a blood sample can be assessed by, for example, HPLC, ELISA, RIA or other immunoassay. Normal HbA1c values for healthy subjects are approximately 4.0-6.2 percent. The American Diabetes Association recommends that it should be below 7 % (or below 6 % in certain persons) for patients with diabetes to help prevent the complications from diabetes. Insulin levels can be measured by, for example, ELISA or RIA. Glucose levels are typically measured using a glucose sensor or analyzer.

Animal models for type I diabetes include the nonobese diabetic (NOD) mouse and the BioBreeding (BB) rat (Atkinson *et al.*, (1999) *Nature Med.* 5:601-604). Animal models for type 2 diabetes include, but are not limited to, ob/ob mice and

db/db mice, which have mutations in the leptin gene or leptin receptor, respectively, KK mice, Nagoya-Shibata-Yasuda (NSY) mice, Zucker diabetic fatty (ZDF) rats and Gato-Katazaki (GK) rats (Cefalu (2006) *ILAR Journal* 47:186-198). In other examples, healthy animals are used to test the activity of an insulin, with or without a hyaluronan degrading enzyme.

2. Hyaluronan degrading enzymes

The activity of a hyaluronan degrading enzyme can be assessed using methods well known in the art. For example, the USP XXII assay for hyaluronidase determines activity indirectly by measuring the amount of undegraded hyaluronic acid, or hyaluronan, (HA) substrate remaining after the enzyme is allowed to react with the HA for 30 min at 37° C (USP XXII-NF XVII (1990) 644-645 United States Pharmacopeia Convention, Inc, Rockville, MD). A Hyaluronidase Reference Standard (USP) or National Formulary (NF) Standard Hyaluronidase solution can be used in an assay to ascertain the activity, in units, of any hyaluronidase. In one example, activity is measured using a microturbidity assay. This is based on the formation of an insoluble precipitate when hyaluronic acid binds with serum albumin. The activity is measured by incubating hyaluronidase with sodium hyaluronate (hyaluronic acid) for a set period of time (*e.g.* 10 minutes) and then precipitating the undigested sodium hyaluronate with the addition of acidified serum albumin. The turbidity of the resulting sample is measured at 640 nm after an additional development period. The decrease in turbidity resulting from hyaluronidase activity on the sodium hyaluronate substrate is a measure of hyaluronidase enzymatic activity (*see e.g.* Example 2).

In another example, hyaluronidase activity is measured using a microtiter assay in which residual biotinylated hyaluronic acid is measured following incubation with hyaluronidase (*see e.g.* Frost and Stern (1997) *Anal. Biochem.* 251:263-269, U.S. Pat. Publication No. 20050260186). The free carboxyl groups on the glucuronic acid residues of hyaluronic acid are biotinylated, and the biotinylated hyaluronic acid substrate is covalently coupled to a microtiter plate. Following incubation with hyaluronidase, the residual biotinylated hyaluronic acid substrate is detected using an avidin-peroxidase reaction, and compared to that obtained following reaction with hyaluronidase standards of known activity. Other assays to measure hyaluronidase

activity also are known in the art and can be used in the methods herein (*see e.g.* Delpech *et al.*, (1995) *Anal. Biochem.* 229:35-41; Takahashi *et al.*, (2003) *Anal. Biochem.* 322:257-263).

The ability of a hyaluronan degrading enzyme to act as a spreading or diffusing agent also can be assessed. For example, trypan blue dye can be injected subcutaneously with or without a hyaluronan degrading enzyme into the lateral skin on each side of nude mice. The dye area is then measured, such as with a microcaliper, to determine the ability of the hyaluronan degrading enzyme to act as a spreading agent (U.S. Pat. Pub. No. 20060104968). The effect of co-administration of hyaluronidase with another agent, such as an insulin, on the pharmacokinetic and pharmacodynamic properties of that agent also can be assessed *in vivo* using animal model and/or human subjects, such as in the setting of a clinical. The functional activity of a hyaluronan degrading enzyme that is not a hyaluronidase can be compared to a hyaluronidase using any of these assays. This can be done to determine a functionally equivalent amount of a hyaluronan degrading enzyme. For example, the ability of a hyaluronan degrading enzyme to act as a spreading or diffusing agent can be assessed by injecting it into the lateral skin of mice with trypan blue, and the amount required to achieve the same amount of diffusion as, for example, 100 units of a Hyaluronidase Reference Standard, can be determined. The amount of hyaluronan degrading enzyme required is, therefore, functionally equivalent to 100 hyaluronidase units.

The stability of hyaluronan degrading enzymes in a composition, such as in the co-formulations provided herein, also can be assessed using other methods and assays known in the art. For example, stability can be assessed by determining hyaluronidase activity as described above and in Example 2, visual inspection as described above, percent recovery and protein purity, over time, as measured by reversed phase high performance liquid chromatography (RP-HPLC) (see, *e.g.*, Example 3), and apparent melting temperature. Protein purity, as determined by RP-HPLC, is the percent of the main hyaluronan degrading enzyme in the co-formulation, for example, rHuPH20, as compared to all of the hyaluronidase species present. Percent recovery is the relative percentage of the hyaluronidase in the co-formulation over time and at various storage conditions, as compared to a reference sample. In

one example, the melting temperature of the hyaluronidase in the co-formulations provided herein, is determined by measuring the hydrodynamic radius of particles by dynamic light scattering (see, *e.g.*, Example 7.B). An increase in particle size and a decrease in the melting temperature indicates denaturation and subsequent aggregation of the hyaluronidase. Hyaluronidase stability in the co-formulations provided herein can be determined by measuring the oxidation of the hyaluronidase, such as the rHuPH20, by RP-HPLC. Percent oxidation is a measure of sum of the peak areas of the major (ox-1) and minor (ox-2) peaks (see, *e.g.*, Example 10.B). Other methods known to one of skill in the art that can be used to determine the stability of the hyaluronidase in the co-formulations provided herein, include polyacrylamide gel electrophoresis (PAGE), immunoblotting, nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry, circular dichroism (CD) and dye-based fluorescence assays.

J. Therapeutic uses

The co-formulations of a fast acting insulin and hyaluronan degrading enzyme described herein can be used for treatment of any condition for which a fast-acting insulin is employed. The co-formulations can be administered subcutaneously to treat any condition that is amenable to treatment with insulin. This section provides exemplary therapeutic uses of fast-acting insulin. The therapeutic uses described below are exemplary and do not limit the applications of the co-formulations described herein. Therapeutic uses include, but are not limited to, treatment for type 1 diabetes mellitus, type 2 diabetes mellitus, gestational diabetes, and for glycemic control in critically ill patients. For example, the co-formulations of a fast acting insulin and hyaluronan degrading enzyme can be administered subcutaneously in discrete doses, such as via a syringe or insulin pen, prior to a meal as prandial insulin therapy in subjects with diabetes to achieve glycemic control. The co-formulations also can be administered subcutaneously or intraperitonally using an insulin pump or in the context of a closed loop system to continuously control blood glucose levels throughout the day and night and/or to control post-prandial glycemic excursions. It is within the skill of a treating physician to identify such diseases or conditions.

As discussed above, particular dosages and treatment protocols are typically individualized for each subject. If necessary, a particular dosage and duration and

treatment protocol can be empirically determined or extrapolated. For example, exemplary doses of fast-acting insulin without a hyaluronan degrading enzyme can be used as a starting point to determine appropriate dosages of the co-formulations provided herein. Dosage levels can be determined based on a variety of factors, such as body weight of the individual, general health, age, the activity of the specific compound employed, sex, diet, metabolic activity, blood glucose concentrations, time of administration, rate of excretion, drug combination, the severity and course of the disease, and the patient's disposition to the disease and the judgment of the treating physician. In particular, blood glucose levels, such as measured by a blood glucose sensor, can be measured and used to determine the amount of insulin and a hyaluronan degrading enzyme to be administered to achieve glycemic control. Algorithms are known in the art that can be used to determine a dose based on the rate of absorption and level of absorption of the co-formulations of a fast acting insulin and a hyaluronan degrading enzyme provided herein, and also based upon blood glucose levels. Dosages of insulin for post-prandial glycemic control also can be calculated or adjusted, for example, by determining the carbohydrate content of a meal (see, *e.g.*, Bergenstal *et al.,* (2008) *Diabetes Care* 31:1305-1310, Lowe *et al.,* (2008) *Diabetes Res. Clin. Pract.* 80:439-443, Chiesa *et al.*,(2005) *Acta Biomed.* 76:44-48).

1. Diabetes Mellitus

Diabetes mellitus (or diabetes) is characterized by an impaired glucose metabolism. Blood glucose is derived from carbohydrates absorbed in the gut and produced in the liver. Increasing blood glucose levels stimulate insulin release. The postprandial glucose influx can be 20 to 30 times higher than the hepatic production of glucose observed between meals. Early phase insulin release, lasting 10 minutes or thereabouts, suppresses hepatic glucose production and precedes a longer (late) phase of release, which lasts two hours or more and covers mealtime carbohydrate influx. Between meals, a low continuous insulin level, basal insulin, covers ongoing metabolic requirements, in particular to regulate hepatic glucose output as well as glucose utilization by adipose tissue, muscle tissue and other target sites. Patients with diabetes present with elevated blood glucose levels (hyperglycemia). Diabetes can be classified into two major groups: type 1 diabetes and type 2 diabetes. Type 1

diabetes, or insulin dependent diabetes mellitus (IDDM), is characterized by a loss of the insulin-producing β-cell of the islets of Langerhans in the pancreas, leading to a deficiency of insulin. The primary cause of the β-cell deficiency is T-cell mediated autoimmunity. Type 2 diabetes, or non-insulin dependent diabetes mellitus (NIDDM), occurs in patients with an impaired β-cell function. These patients have insulin resistance or reduced insulin sensitivity, combined with reduced insulin secretion. Type 2 diabetes may eventually develop into type 1 diabetes. Also included in diabetes is gestational diabetes. Patients with diabetes can be administered insulin to both maintain basal insulin levels and to prevent glycemic excursions, such as following a meal.

a. Type 1 diabetes

Type 1 diabetes is a T-cell dependent autoimmune disease characterized by infiltration of the islets of Langerhans, the endocrine unit of the pancreas, and destruction of β-cells, leading to a deficiency in insulin production and hyperglycemia. Type 1 diabetes is most commonly diagnosed in children and young adults but can be diagnosed at any age. Patients with type 1 diabetes can present with, in addition to low insulin levels and high blood glucose levels, polyuria, polydipsia, polyphagia, blurred vision and fatigue. Patients can be diagnosed by presenting with fasting plasma glucose levels at or above 126 mg/dL (7.0 mmol/l), plasma glucose levels at or above 200 mg/dL (11.1 mmol/l) two hours after a 75 g oral glucose load, such as in a glucose tolerance test, and/or random plasma glucose levels at or above 200 mg/dL (11.1 mmol/l).

The primary treatment for patients with type 1 diabetes is administration of insulin as replacement therapy, which is typically performed in conjunction with blood glucose monitoring. Without sufficient replacement insulin, diabetic ketoacidosis can develop, which can result in coma or death. Patients can be administered subcutaneous injections of fast-acting insulin using, for example, a syringe or insulin pen, or an insulin pump to maintain appropriate blood glucose levels throughout the day and also to control post-prandial glucose levels. In some instances, an insulin pump, including in the context of a closed loop system, can be used to deliver insulin intraperitoneally. Thus, patients with type 1 diabetes can be administered the co-formulations of a fast acting insulin and hyaluronan degrading

enzyme described herein subcutaneously or intraperitoneally via syringe, insulin pen, or insulin pump, or any other means useful for delivering insulin, to more rapidly control blood glucose and insulin levels.

b. Type 2 diabetes

Type 2 diabetes is associated with insulin resistance and, in some populations, also by insulinopenia (loss of β-cell function). In type 2 diabetes, phase 1 release of insulin is absent, and phase 2 release is delayed and inadequate. The sharp spike of insulin release occurring in healthy subjects during and following a meal is delayed, prolonged, and insufficient in amount in patients with type 2 diabetes, resulting in hyperglycemia. Patients with type 2 diabetes can be administered insulin to control blood glucose levels (Mayfield *et al.* (2004) *Am Fam Physican* 70:489-500). This can be done in combination with other treatments and treatment regimes, including diet, exercise and other anti-diabetic therapies (*e.g.* sulphonylureas, biguanides, meglitinides, thiazolidinediones and alpha-glucosidase inhibitors). Thus, patients with type 2 diabetes can be administered the co-formulations of a fast acting insulin and hyaluronan degrading enzyme described herein subcutaneously or intraperitoneally via syringe, insulin pen, or insulin pump, or any other means useful for delivering insulin, to more rapidly control blood glucose and insulin levels.

c. Gestational diabetes

Pregnant women who have never had diabetes before but who have high blood glucose levels during pregnancy are diagnosed with gestational diabetes. This type of diabetes affects approximately 1-14% of all pregnant women, depending upon the population studied (Carr *et al.,* (1998) *Clinical Diabetes* 16). While the underlying cause remains unknown, it appears likely that hormones produced during pregnancy reduce the pregnant woman's sensitivity to insulin. The mechanism of insulin resistance is likely a postreceptor defect, since normal insulin binding by insulin-sensitive cells has been demonstrated. The pancreas releases 1.5–2.5 times more insulin in order to respond to the resultant increase in insulin resistance. Patients with normal pancreatic function are able to meet these demands. Patients with borderline pancreatic function have difficulty increasing insulin secretion and consequently produce inadequate levels of insulin. Gestational diabetes thus results when there is

delayed or insufficient insulin secretion in the presence of increasing peripheral insulin resistance.

Patients with gestational diabetes can be administered insulin to control blood glucose level. Thus, patients with gestational diabetes can be administered the co-formulations of a fast acting insulin and hyaluronan degrading enzyme described herein subcutaneously via syringe, insulin pen, insulin pump or artificial pancreas, or any other means, to more rapidly control blood glucose and insulin levels.

2. Insulin therapy for critically ill patients

Hyperglycemia and insulin resistance occurs frequently in medically and/or surgically critically ill patients and has been associated with increased morbidity and mortality in both diabetic and non-diabetic patients and in patients with traumatic injury, stroke, anoxic brain injury, acute myocardial infarction, post-cardiac surgery, and other causes of critical illness (McCowen *et al.* (2001) *Crit. Clin. Care* 17:107-124). Critically ill patients with hyperglycemia have been treated with insulin to control blood glucose levels. Such treatment can reduce morbidity and mortality amongst this group (Van den Berghe *et al.* (2006) *N. Eng. J Med.* 354:449-461). Insulin is typically administered intravenously to the patient, such as by injection with a syringe by a medical practitioner or by infusion using an insulin pump. In some examples, algorithms and software are used to calculate the dose. Thus, critically ill patients with hyperglycemia can be administered a co-formulation of a fast acting insulin and hyaluronan degrading enzyme described herein to control blood glucose levels, thereby alleviating the hyperglycemia and reducing morbidity and mortality.

K. Combination Therapies

Any of the co-formulations of a fast acting insulin and hyaluronan degrading enzyme described herein can be administered in combination with, prior to, intermittently with, or subsequent to, other therapeutic agents or procedures including, but not limited to, other biologics and small molecule compounds. For any disease or condition, including all those exemplified above, for which a fast-acting insulin is indicated or has been used and for which other agents and treatments are available, the co-formulations can be used in combination therewith. Depending on the disease or condition to be treated, exemplary combinations include, but are not limited to, combination with anti-diabetic drugs, including, but not limited to, sulfonylureas,

biguanides, meglitinides, thiazolidinediones, alpha-glucosidase inhibitors, peptide analogs, including glucagon-like peptide (GLP) analogs and, gastric inhibitory peptide (GIP) analogs and DPP-4 inhibitors. In another example, the co-formulations of a fast acting insulin and hyaluronan degrading enzyme described herein can be administered in combination with, prior to, intermittently with, or subsequent to, with one or more other insulins, including fast-acting insulin, and basal-acting insulins.

L. Articles of Manufacture and Kits

The co-formulations of a fast acting insulin and hyaluronan degrading enzyme provided herein can be packaged as articles of manufacture containing packaging material, a pharmaceutical composition which is effective for controlling blood glucose levels, such as in diabetic or critically subjects, and a label that indicates that the co-formulations are to be used for controlling blood glucose levels.

The articles of manufacture provided herein contain packaging materials. Packaging materials for use in packaging pharmaceutical products are well known to those of skill in the art. See, for example, U.S. Pat. Nos. 5,323,907, 5,052,558 and 5,033,252, each of which is incorporated herein in its entirety. Examples of pharmaceutical packaging materials include, but are not limited to, blister packs, bottles, tubes, inhalers, pumps, bags, vials, containers, syringes, bottles, and any packaging material suitable for a selected formulation and intended mode of administration and treatment.

The co-formulations of a fast acting insulin and hyaluronan degrading enzyme also can be provided as kits. Kits can include a co-formulation described herein and an item for administration. The kits also can include additional pharmaceutical compositions. In one example, the kits can include one or more of the co-formulations provided herein and one or more other insulin compositions, such as for example, slow acting or intermediate-acting insulins, including crystalline insulins, or any combination thereof. The co-formulations of a fast acting insulin and hyaluronan degrading enzyme can be supplied with a device for administration, such as a syringe, an insulin pen, a pump, or a reservoir that is inserted into an insulin pen, a pump or other delivery device. The kit can, optionally, include instructions for application including dosages, dosing regimens and instructions for modes of administration.

Kits also can include a co-formulation described herein and an item for diagnosis. For example, such kits can include a glucose monitor or sensor.

M. EXAMPLES

The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

Example 1

Insulin and Insulin Analog Stock Preparation

A. Regular Insulin

For regular insulin, the powder (Organon Insulin API, Recombinant Human insulin SIHR 143) was weighed and mixed with a proper amount of water until the solution contained about 10-25 mg/mL insulin. 1 M HCl was added to the cloudy mixture to a final concentration of 20 mM HCl. The solution was gently mixed with a stir bar until the insulin was completely dissolved and 250 mM Tris, pH 10.7 (Trizma, Cat. No. T6066, Sigma) was added to a final Tris concentration of 20 mM. The pH was adjusted using 1 M NaOH and then water was added, such that the insulin was formulated as described in each of the individual examples below. This insulin contains approximately 13 μg/mL zinc.

B. Insulin Analogs

For the insulin analogs (either insulin Aspart or insulin Lispro), 12 vials (10 mL each) of commercial product (Insulin Lispro: Eli Lilly Humalog® (insulin Lispro) 100 U/mL, Lot A572364; Insulin Aspart: Novo Nordisk, NovoRapid® (insulin Aspart), Lot XS60195; Insulin Glulisine: Apidra® insulin) were pooled and concentrated using an Amicon Ultracel-10 K (Insulin Lispro) or 3K (Insulin Aspart) column concentrator until the final concentration was about 5 times the original concentration. The insulin analogs were precipitated by addition of 1 M sodium acetate, pH 5.3 and 30 mM zinc chloride ($ZnCl_2$, EMD, Cat No. ZX0065-1) at 1/10 of the protein solution volume. The solutions were placed on ice for 30 minutes followed by centrifugation at 5600 rpm for 20 minutes in an Avanti J-E Centrifuge with JS-5.3 swinging bucket rotor (Beckman Coulter). The supernatant was decanted and the pellet was resuspended and washed with 20 mM sodium acetate, 2 mM zinc chloride, pH 5.5 solution. The resuspended solution was centrifuged as described above. The washing step was repeated a total of 5 times. A final wash was

performed with 20 mM sodium acetate, pH 5.5 to remove all traces of zinc chloride. The resulting protein paste was dissolved with water containing 20 mM HCl. After complete dissolution, 250 mM Tris, pH 10.7 was added to a final Tris concentration of 20 mM. The pH of the resulting solution was adjusted such that the insulin analog was formulated as described in each of the individual examples below and the protein concentration was adjusted to about 15-20 mg/mL. An insulin analog prepared in this way typically had a yield of about 90 %, with a residual preservative concentration at less than 100 times the starting material.

Example 2

Determination of hyaluronidase activity of rHuPH20

Hyaluronidase activity of rHuPH20 (obtained by expression and secretion in CHO cells of a nucleic acid encoding amino acids 36-482 of SEQ ID NO:1) was determined using a turbidimetric assay. In the first two assay (A and B), the hyaluronidase activity of rHuPH20 was measured by incubating soluble rHuPH20 with sodium hyaluronate (hyaluronic acid) and then precipitating the undigested sodium hyaluronate by addition of acidified serum albumin. In the third assay (C), rHuPH20 hyaluronidase activity was measured based on the formation of an insoluble precipitate when hyaluronic acid (HA) binds with cetylpyridinium chloride (CPC). In all assays containing 600 U/mL rHuPH20 (5 μg/mL), the acceptance criteria was enzymatic activity above 375 U/mL.

A. Microturbidity Assay

In this assay, the hyaluronidase activity of rHuPH20 was measured by incubating soluble rHuPH20 with sodium hyaluronate (hyaluronic acid) for a set period of time (10 minutes) and then precipitating the undigested sodium hyaluronate with the addition of acidified serum albumin. The turbidity of the resulting sample was measured at 640 nm after a 30 minute development period. The decrease in turbidity resulting from enzyme activity on the sodium hyaluronate substrate was a measure of the soluble rHuPH20 hyaluronidase activity. The method was performed using a calibration curve generated with dilutions of a soluble rHuPH20 assay working reference standard, and sample activity measurements were made relative to this calibration curve. Dilutions of the sample were prepared in Enzyme Diluent

Solutions. The Enzyme Diluent Solution was prepared by dissolving 33.0 ± 0.05 mg of hydrolyzed gelatin in 25.0 mL of 50 mM PIPES Reaction Buffer (140 mM NaCl, 50 mM PIPES, pH 5.5) and 25.0 mL of Sterile Water for Injection (SWFI; Braun, product number R5000-1) and diluting 0.2 mL of a 25 % Human Serum Albumin (US Biologicals) solution into the mixture and vortexing for 30 seconds. This was performed within 2 hours of use and stored on ice until needed. The samples were diluted to an estimated 1-2 U/mL. Generally, the maximum dilution per step did not exceed 1:100 and the initial sample size for the first dilution was not be less than 20 µL. The minimum sample volumes needed to perform the assay were: In-process Samples, FPLC Fractions: 80 µL; Tissue Culture Supernatants:1 mL; Concentrated Material 80 µL; Purified or Final Step Material: 80 µL. The dilutions were made in triplicate in a Low Protein Binding 96-well plate, and 30 µL of each dilution was transferred to Optilux black/clear bottom plates (BD BioSciences).

Dilutions of known soluble rHuPH20 with a concentration of 2.5 U/mL were prepared in Enzyme Diluent Solution to generate a standard curve and added to the Optilux plate in triplicate. The dilutions included 0 U/mL, 0.25 U/mL, 0.5 U/mL, 1.0 U/mL, 1.5 U/mL, 2.0 U/mL, and 2.5 U/mL. "Reagent blank" wells that contained 60 µL of Enzyme Diluent Solution were included in the plate as a negative control. The plate was then covered and warmed on a heat block for 5 minutes at 37 °C. The cover was removed and the plate was shaken for 10 seconds. After shaking, the plate was returned to the plate to the heat block and the MULTIDROP 384 Liquid Handling Device was primed with the warm 0.25 mg/mL sodium hyaluronate solution (prepared by dissolving 100 mg of sodium hyaluronate (LifeCore Biomedical) in 20.0 mL of SWFI. This was mixed by gently rotating and/or rocking at 2-8 °C for 2-4 hours, or until completely dissolved). The reaction plate was transferred to the MULTIDROP 384 and the reaction was initiated by pressing the start key to dispense 30 µL sodium hyaluronate into each well. The plate was then removed from the MULTIDROP 384 and shaken for 10 seconds before being transferred to a heat block with the plate cover replaced. The plate was incubated at 37 °C for 10 minutes.

The MULTIDROP 384 was prepared to stop the reaction by priming the machine with Serum Working Solution and changing the volume setting to 240 µL. (25 mL of Serum Stock Solution [1 volume of Horse Serum (Sigma) was diluted with

9 volumes of 500 mM Acetate Buffer Solution and the pH was adjusted to 3.1 with hydrochloric acid] in 75 mL of 500 mM Acetate Buffer Solution). The plate was removed from the heat block and placed onto the MULTIDROP 384 and 240 µL of serum Working Solutions was dispensed into the wells. The plate was removed and shaken on a plate reader for 10 seconds. After a further 15 minutes, the turbidity of the samples was measured at 640 nm and the hyaluronidase activity (in U/mL) of each sample was determined by fitting to the standard curve.

Specific activity (Units/mg) was calculated by dividing the hyaluronidase activity (U/ml) by the protein concentration (mg/mL).

B. Turbidity Assay for rHuPH20 Enzymatic Activity

Samples were diluted with Enzyme Diluent [66 mg gelatin hydrolysate (Sigma #G0262) dissolved in 50 mL Phosphate Buffer (25 mM phosphate, pH 6.3, 140 mM NaCl) and 50 mL deionized (DI) water] to achieve an expected enzyme concentration of between 0.3 and 1.5 U/mL.

Each of two test tubes labeled Standard 1, 2, 3, 4, 5, or 6, and duplicate test tubes for each sample to be analyzed (labeled accordingly) were placed in a block heater at 37 °C. The volumes of Enzyme Diluent shown in the following table were added in duplicate to the Standard test tubes. 0.50 mL HA Substrate Solution [1.0 mL of 5 mg/mL hyaluronic acid (ICN # 362421) in DI water, 9 mL DI water, 10 mL Phosphate Buffer] was dispensed into all the Standard and Sample test tubes. Volumes of 1.5 U/mL USP Hyaluronidase Standard (USP # 31200) in Enzyme Diluent were dispensed into duplicate Standard test tubes as indicated in the Table 7 below. When all the Standard test tubes had been completed, 0.50 mL of each sample was dispensed into each of the duplicate Sample test tubes. After a 30-minute incubation at 37 °C, 4.0 mL of Serum Working Solution {50 mL Serum Stock Solution [1 volume horse serum (donor herd, cell culture tested, hybridoma culture tested, USA origin), 9 volumes 500 mM Acetate Buffer, adjust to pH 3.1, allow to stand at room temperature 18-24 hours, store at 4 °C] plus 150 mL 500 mM Acetate Buffer} was added to the Standard test tubes, which were then removed from the block heater, mixed and placed at room temperature. The Sample test tubes were processed in this manner until all of the Standard and Sample test tubes were processed.

A "blank" solution was prepared by combining 0.5 mL Enzyme Diluent, 0.25 mL DI water, 0.25 mL Phosphate Buffer and 4.0 mL Serum Working Solution. The solution was mixed and an aliquot transferred to a disposable cuvette. This sample was used to zero the spectrophotometer at 640 nm.

After a 30-minute incubation at room temperature an aliquot from each Standard test tube was transferred in turn to a disposable cuvette and the absorbance at 640 nm was measured. This procedure was repeated for the duplicate Sample test tubes.

A linear calibration curve was constructed by plotting the hyaluronidase concentration (U/mL) versus the observed absorbance. Linear regression analysis was used to fit the data (excluding the data for the 0.0 U/mL calibration standard) and to determine the slope, intercept and correlation coefficient (r^2). A standard curve regression equation and the observed sample absorbance were used to determine the sample concentrations.

Table 7. Dilutions for Enzyme Standards

Standard	U/mL	mL Enzyme Diluent	mL 1.5 U/mL USP Hyaluronidase
1	0.0	0.50	0
2	0.3	0.40	0.10
3	0.6	0.30	0.20
4	0.9	0.20	0.30
5	1.2	0.10	0.40
6	1.5	0	0.50

C. Turbidity Assay for rHuPH20 Enzymatic Activity

The turbidimetric method for the determination of hyaluronidase activity and enzyme concentration was based on the formation of an insoluble precipitate when hyaluronic acid (HA) binds with cetylpyridinium chloride (CPC). The activity was measured by incubating hyaluronidase with hyaluronan for a set period of time (30 minutes) and then precipitating the undigested hyaluronan by the addition of CPC. The turbidity of the resulting sample is measured at 640 nm and the decrease in turbidity resulting from enzyme activity on the HA substrate was a measure of the hyaluronidase potency. The method is run using a calibration curve generated with dilutions of rHuPH20 assay working reference standard, and sample activity measurements were made relative to the calibration curve. The method was intended

for the analysis of rHuPH20 activity in solutions after dilution to a concentration of ~2 U/mL. The quantitative range was 0.3 to 3 U/mL, although for routine testing optimum performance was obtained in the range of 1 to 3 U/mL.

Enzyme Diluent was prepared fresh by dissolving 100 mg ± 10 mg gelatin hydrolysate (Sigma #G0262) in 75 mL of the Reaction Buffer Solution (140 mM NaCl, 50 mM PIPES (1,4 piperazine bis (2-ethanosulfonic acid)), pH 5.3) free acid (Mallinckrodt #V249) and 74.4 mL of Sterile Water for Irrigation (SWFI) and adding 0.6 mL 25 % Human Serum Albumin (HSA). A spectrophotometer blank was prepared by adding 1.0 mL Enzyme Diluent to a test tube and placing it in a heating block preheated to 37 °C. A Diluted Reference Standard was prepared by making a 1:25 dilution of the rHuPH20 Assay Working Reference Standard in triplicate by adding 120 µL of the Assay Working Reference Standard to 29.880 mL of Enzyme Diluent. Appropriate dilutions of each sample were prepared in triplicate to yield a ~2 U/mL solution.

The volumes of Enzyme Diluent were dispensed in triplicate into Standard test tubes according to Table 8. 500 µL of a solution of 1.0 mg/mL sodium hyaluronate (Lifecore, #81, with average molecular weight of 20-50 kDa) in SWFI was dispensed into all test tubes except the blank, and the tubes were placed in the 37 °C in the heating block for 5 minutes. The quantity of the Diluted Reference Standard indicated in Table 7 was added to the appropriate Standard test tubes, mixed and returned to the heating block. 500 µL of each sample to the appropriate tubes in triplicate. 30 minutes after the first Standard tube was started, 4.0 mL of Stop Solution (5.0 mg /mL cetylpyridinium chloride (Sigma, Cat # C-5460) dissolved in SWFI and passed through a 0.22 micron filter) to all tubes (including the Blank), which were then mixed and placed at room temperature.

The spectrophotometer was "blanked" at 640 nm fixed wavelength. After 30 minutes incubation at room temperature. Approximately 1 mL of Standard or Sample was transferred to a disposable cuvette and the absorbance read at 640 nm. The Reference Standard and Sample raw data values were analyzed employing GRAPHPAD PRISM® computer software (Hearne Scientific Software) using an exponential decay function constrained to 0 upon complete decay The best fit

standard curve was determined and used to calculate the corresponding Sample concentrations.

Table 8. Dilutions for Enzyme Standards			
Standard	**U/mL**	**Enzyme Diluent (µL)**	**Diluted Reference Standard (µL)**
1	0.0	500	0
2	0.6	400	100
3	1.2	300	200
4	1.8	200	300
5	2.4	100	400
6	3.0	0	500

Example 3

RP-HPLC

In this example, reverse phase-HPLC (RP-HPLC) was used to determine the apparent solubility of insulin and insulin analogs and the percent purity of rHuPH20. Apparent solubility was measured as the percent insulin recovery as compared to the initial formulations/conditions.

Reference Standards

For regular insulin, Humulin® (insulin regular, 100 U) was used as the standard. For insulin Lispro, one vial of USP Lispro was reconstituted with 1.72 mL 0.01 N HCl resulting in a 3.5 mg/mL USP Lispro (100 U/mL) reference standard. For insulin Aspart, one vial of EP Aspart was reconstituted with 1.00 mL 0.01 N HCl resulting in a 3.89 mg/mL EP Aspart reference standard. For rHuPH20, three reference standards were used, each containing 2.5 µg/mL, 5.0 µg/mL or 7.5 µg/mL rHuPH20, generated by diluting a 50 µg/mL rHuPH20 sample (Lot HUB0701EB) with an appropriate amount of sample diluent (20 mM Tris, 130 mM NaCl, 0.01 % Poloxamer 188, pH 7.3).

Preservative Standards

Three preservative reference standards were used, each containing 0.05 % metacresol/phenol, 0.10 % metacresol/phenol or 0.15 % metacresol/phenol.

Sample Preparation

Samples were diluted, if necessary, in sample diluent such that the insulin/insulin analog was present at 100 U/mL and the rHuPH20 was present at 5 µg/mL. Samples were prepared by withdrawing 100 µL from each vial at every time

point and centrifuging prior to loading. Samples were stored in an autosampler at 4 °C prior to use, and were considered stable for 3 days after preparation. All samples were tested in duplicate.

For insulin, 20 µL was injected for each HPLC run. For rHuPH20, 100 µL was injected for each HPLC run. The HPLC column and method parameters are set forth in Table 9 below. The HPLC gradient is set forth in Table 10 below.

Table 9. HPLC Column and Method Parameters	
Column	Phenomenex Jupiter C5 4.6 x 250mm, 5 µM, 300Å or Agilent Zorbax 300SB-C18 4.6 x 250 mm column (Cat No. 880995-902)
Injection Volume	20 µL for Insulin content and 100 µL for rHuPH20 content
Blank Injections	10 µL of Mobile Phase B
Column Temperature	40 °C ± 1 °C
Sample Thermostat	4 °C ± 3 °C
Detection λ	280 nm
Mobile Phase A	0.1 % Trifluoroacetic Acid (TFA) in water
Mobile Phase B	0.1 % TFA in acetonitrile
Run Time	39 minutes
Column Storage	100 % Acetonitrile

Table 10. HPLC Gradient			
Time	**Flow**	**% A**	**% B**
0	1.0	72	28
3	1.0	72	28
20	1.0	65	35
29	1.0	15	85
29.1	1.0	0	100
34	1.0	0	100
34.1	1.0	72	28
39	1.0	72	28

A. Insulin Solubility

To determine the apparent solubility, the peak area of insulin/insulin analog main peak and desamido peak were integrated and combined together to calculate the percentage relative to the standards. Solubility was expressed as relative percentage to the standard, with 100 % being 120 U/mL. The data were processed using Design-Expert® 7.0 (StatEase) and the "historical data" feature. Insulin percent purity was expressed as the percent of main insulin versus all insulin species.

B. rHuPH20 Percent Purity and Percent Recovery

rHuPH20 percent purity was expressed as the percent of rHuPH20 versus all rHuPH20 species. rHuPH20 percent recovery was expressed as relative percentage to the standard, with 100 % being 5 μg/mL. The target specification is 3-7 μg/mL (60-140 %).

Example 4

Size Exclusion Chromatography

In this example, the insulin/rHuPH20 formulations were analyzed by size exclusion high performance liquid chromatography (SEC-HPLC) to determine the relative amounts of high molecular weight protein (HMWP), *i.e.*, covalently bound aggregates, of insulin and rHuPH20 present in the sample. For denaturing SEC, the mobile phase was L-arginine and glacial acetic acid in acetonitrile. For non-denaturing SEC, which was used as described in Example 29, the mobile phase was phosphate buffered saline.

A. Denaturing SEC

1. Reference Standards

Humulin® R (insulin regular, Lilly, concentrated, NDC 0002-8501-01) or USP Human Insulin (USP Cat # I1F270) and rHuPH20 (lot HUA0703MA or HUB0701EB with their specific activities at 120,000 U/mg and 110,000 U.mg, respectively) were used as reference standards. Reference standards, containing both insulin and rHuPH20, were prepared at concentrations similar to the expected sample concentrations, *i.e.*, 100 U/mL insulin and 5 μg/mL rHuPH20. Liquid formulations were diluted in 25 mM Tris diluent, pH 7.3. Weighed amounts of USP Human Insulin were diluted in Tris diluent spiked with TFA (2 μL TFA/mL Tris). Reference samples were stored in HPLC vials at 10 °C prior to use.

2. Sample Preparation

If necessary, samples were diluted in Tris diluent such that insulin was present in the range of 1-100 U/mL and rHuPH20 was present in the range of 1-1000 μg/mL. Samples were stored in HPLC vials at 10 °C prior to use, for up to 3 days.

3. HPLC Preparation

The mobile phase was prepared by combining 650 mL 1.0 mg/mL L-arginine, 150 mL glacial acetic acid and 200 mL acetonitrile. The HPLC column and method

parameters are set forth in Table 11 below. The rHuPH20 peak occurs around 13.8 minutes and the insulin peak occurs around 20.2 minutes.

Table 11. HPLC Column and Method Parameters	
Column	Waters Insulin HMWP, 300 mm x 7.8 mm (Cat # 201549)
Flow Rate	0.6 mL/min
Injection Volume	100 µL
Blank Injections	100 µL of mobile phase, Tris, Tris TFA
Column Temperature	25 °C ± 1 °C
Sample Thermostat	10 °C ± 3 °C
Detection λ	280 nm
Run Time	30 minutes
Column Storage	20 % ethanol

B. Non-Denaturing SEC

1. Reference Standards

Humalog® 100 (insulin Lispro, Eli Lilly Cat # VL-1510), NovoLog® 100 (insulin Aspart, Novo Nordisk Cat # 750111) and rHuPH20 (lot T09RD02 [specific activity 122,000U/mg], HUA0703MA [specific activity 120,000 U/mg] or HUB0701EB [specific activity 122,000 U/mg]) were used as reference standards. Reference standards, containing both insulin and rHuPH20, were prepared at concentrations similar to the expected sample concentrations, *i.e.*, 100 U/mL insulin and 5 µg/mL rHuPH20. Reference samples were stored in HPLC vials at 10 °C prior to use.

2. Sample Preparation

If necessary, samples were diluted in sample diluent (20 mM Tris, 130 mM NaCl, 0.01 % Poloxamer 188, pH 7.3) such that insulin was present in the range of 1-100 U/mL and rHuPH20 was present in the range of 1-20 µg/mL. Samples were stored in HPLC vials at 10 °C prior to use, for up to 3 days.

3. HPLC Preparation

0.5 X Phosphate Buffered Saline (PBS) solution was prepared by diluting 10 X PBS with HPLC grade water. The HPLC column and method parameters are set forth in Table 12 below. The rHuPH20 peak occurs around 13 minutes and the insulin peak occurs around 18.6 minutes.

Table 12. HPLC Column and Method Parameters	
Column	Waters Insulin HMWP, 300 mm x 7.8 mm (Cat # 201549)

Flow Rate	0.5 mL/min
Injection Volume	30 µL
Blank Injections	30 µL of mobile phase, sample diluent, 0.01 N HCl
Column Temperature	25 °C ± 1 °C
Sample Thermostat	10 °C ± 3 °C
Detection λ	280 nm
Run Time	60 minutes
Column Storage	20 % ethanol

C. Insulin Percent Purity

Insulin percent purity was expressed as percentage of the insulin main peak versus the total insulin peaks. The target specification is less than 2 % high molecular weight protein, *i.e.*, insulin aggregates.

Example 5

Test Methods – Osmolality, Turbidity and pH

In this example, test methods used to determine appearance, osmolality, turbidity and pH of the insulin/insulin analog and rHuPH20 formulations are described. These test methods are used in subsequent Examples.

A. Appearance

1. Visual Analysis

Appearance of the insulin/rHuPH20 formulations was determined by qualitative visual analysis of the insulin/rHuPH20 solution in a type I glass vial. The evaluation of the coloration and clarity of the solution was determined by comparison to that of USP (or equivalent) sterile water for irrigation (SWFI). The aqueous solution was determined to be clear if its clarity was the same as that of the SWFI. The aqueous solution was determined to be colorless if it had the appearance of the SWFI. Vials were tested at room temperature and care was taken not to cause unnecessary turbulence and/or air bubbles in the solutions while swirling and/or inverting. If necessary, vials were wiped with non-shedding or lint-free wipes and 70 % ethanol prior to testing. The procedure was as follows: A directional light source with a 150 W or greater lamp was turned on. The samples were prepared in a hood. Samples to be tested were gently inverted to ensure homogeneity prior to transferring >0.5 mL using aseptic technique to a Type I glass vial for testing. A SWFI sample was prepared in the same way. The prepared test sample and SWFI vials were gently inverted to ensure homogeneity, taking care not to introduce any air bubbles. The test

sample and SWFI vials were visually compared for color against a white background with the light source shining at an angle through the bottom of the vials. The solution was considered colorless if it had the same appearance of the SWFI. The test sample and SWFI vials were visually compared for clarity against a black background with the light source shining at an angle through the bottom of the vials. The solution was considered clear if its clarity was the same as that of the SWFI. Color, clarity and the presence and extent of any visible particles and/or foreign matter were recorded. The acceptance criteria was a clear, colorless solution.

2. Illuminator Method

In this method, the degree of clarity and coloration of liquids were evaluated to ensure (visually) product quality against the applicable appearance specifications. Inspection was performed in a specifically designed inspection booth with high intensity light, against a white and black background. A liquid is clear if its opalescence is not more pronounced than that of Reference Suspension I. An aqueous solution is colorless if it has the appearance of water or is not more intensely colored than the specified reference solution (reference solution B9).

Preparation of Reference Solutions

Color reference solutions were prepared by mixing dilute HCl and EP Color Standard B (Brown Standard Solution, Ricca Chemical, #2880) as set forth in Table 13 below. Reference Solutions B_9, B_8, B_7, B_6 were stored in 2 mL vials sealed with a stopper and overseal. Reference solutions were prepared daily. 2 mL SWFI was place in a vial sealed with a stopper and overseal and stored for up to 1 year.

Clarity reference solutions were prepared as follows. A primary opalescent suspension was prepared by mixing 25 mL hexamethylenetetramine (2.5 g in 25 mL SWFI) and 25 mL hydrazine sulphate (1 g in 100 mL SWFI) and allowing the solution to stand for 24 hours. The suspension was stored for up to 2 months in a glass container. The Standard or Opalescence solution was prepared by diluting 45 µL of primary opalescent solution to 2955 µL of SWFI and mixing well. This solution was stable for 3 months. Reference suspension I and II were prepared by mixing Standard or Opalescence and SWFI as set forth in Table 13 below. Reference suspensions were stored in 2 mL vials sealed with a stopper and overseal and were prepared daily.

Table 13. Reference Solutions		
Color	**3.3 M HCl (µL)**	**EP Color Standard B (µL)**
Reference B_9	2970	30
Reference B_8	2955	45
Reference B_7	2925	75
Reference B_6	2850	150
Clarity	**SWFI (µL)**	**Standard of Opalescence (µL)**
Reference Suspension I	2850	150
Reference Suspension II	2700	300

Product samples were prepared by first mixing the product solution and then transferring 2 mL to a vial while working in a Class 100 hood. The vials were sealed with a stopper and overseal. A visual inspection booth was prepared and the intensity of the light source was checked to ensure a Lux greater than 1750. Product samples were compared to reference solutions and suspensions by visual inspection. Color was compared to SWFI and Reference solutions B_6, B_7, B_8 and B_9 as viewed horizontally against a white background. Clarity was compared to Reference suspensions I and II as viewed vertically against a black background. The degree of coloration and clarity and degree of opalescence were recorded as set forth in Table 14 below.

Table 14. Degree of Coloration, Clarity and Degree of Opalescence	
Degree of Coloration	**Description**
Colorless	Product sample is color of SWFI or is not more intensely colored than the Reference Solution B_9
Less colored than B_X and more colored than B_{X+1}; X = 6, 7 or 8	Product sample is less colored than B_X but more colored than $B_{(X+1)}$
As colored as B_X; X = 6, 7 or 8	Product sample is as colored as B_X, with X = 6, 7 or 8
More colored than B_6	Product sample is more colored than Reference Solution B_6
Clarity and Degree of Opalescence	**Description**
Clear	Opalescence of product sample is not more pronounced than that of Reference Suspension I
Less clear than Reference Suspension I and clearer than Reference Suspension II	Clarity of the product sample falls between the clarity of the two Reference Suspensions (I and II).
Less clear than Reference Suspension II	Clarity of the product sample is less than that of Reference Suspension II

B. Osmotic concentration

Osmotic concentration (osmolality) was determined by freezing point depression measurement. The test method is intended for the analysis of an aqueous

solution with an osmotic concentration between 100 and 500 mOsm/kg water. Freezing point depression osmometry involves pipetting a sample of the solution to be tested into a tube and placing the tube in the cooling chamber of an osmometer. The sample is supercooled (cooled below the freezing point) and then seeded (crystallization initiated) by one of a number of methods (*i.e.*, mechanical vibration, ultrasonic vibration, thermal shock or by the addition of solid seed particles). The sample temperature rises due to the heat of fusion released during the freezing process until equilibrium; at this point only a small fraction of the water is frozen, after which more water freezes and the temperature begins to decrease again, resulting in a flat region, or plateau, in the cooling curve. The temperature at the plateau is the freezing point of the sample and can be converted to units of osmolality (osmotic concentration) by observing that 1.0 Osmole depresses the freezing point of water by 1.858 °C, where 1.0 Osmole = 1.0 mole of osmotically active particles = $\Phi(n)(C)$, where:

Φ = osmotic coefficient;

n = number of particles resulting from dissociation of each molecule in solution; and

C = concentration of each molecule in mol/kg water.

A calibration check (0-2000 mOsm/kg water) was performed prior to each use of the osmometer (MicroOsmette Freeze Point Osmometer, Model #5004, Precision Systems Inc.) with 500 mOsm/kg and 200 mOsm/kg standards. After the initial turn-on, 10-15 minutes of warm up time was allowed to completely equilibrate the temperature. Samples, the probe and the seed wire were wiped with a KimWipe prior to each use. Samples for testing were prepared by pipetting 50 µL of the insulin/rHuPH20 solution into a clean, dry sample tube. The tube was placed in the refrigerator well and the osmolality was measured according to standard operating procedure. This procedure was repeated two more times for a total of three independent results per insulin/rHuPH20 sample. The raw data for each reading was recorded and a reading was considered valid if all three readings were within ± 5 mOsm/kg. The average value for each sample was reported. Osmolality varies based on formulation, and the acceptance criteria are listed below in each individual example.

C. Turbidity

Turbidity was determined by measuring the absorbance of an insulin/rHuPH20 solution at 350 nm. When light passes through a solution, the intensity is attenuated by the absorbance and light scattering effect from the solution. To measure the light scattering effect due to a protein in a solution, the wavelength of 350 nm was selected to avoid the effect of absorbance from the protein. The amount of light scattered is significantly affected by the concentration and size of the molecule/particles. The OD350 of a blank solution containing all components except the protein is subtracted to obtain a final reading. 200 μL samples of each insulin/rHuPH20 formulation to be tested were transferred to three adjacent wells of a 96-well UV flat bottom microtiter plate. 200 μL of each sample's respective excipient mixture (minus the protein) was added to three adjacent wells of the microtiter plate for use as a sample blank. SWFI (200 μL) was used as a plate blank. The OD350 of each sample was read in a UV-Vis Spectramax 384plus (Molecule Devices) plate reader. Each reading was recorded, the triplicate readings were averaged and the average absorbance of the respective excipient blank was subtracted resulting blank adjusted turbidity was recorded.

D. pH

pH was measured as described in U.S. Pharmacopeia Compendial <791> (pharmacopeia.cn/v29240/usp29nf24s0_c791.html). Acceptance criteria varied per formulation and is listed below in each individual example. In general, tight pH control (*i.e.*, ±0.2) was necessary to ensure insulin solubility and PH20 stability. However, in non-preserved insulin, insulin solubility was not affected by pH therefore the acceptance criteria is large (*i.e.*, pH 7.0 to 7.8).

Example 6

Stability of rHuPH20 in commercial formulations of Insulin and Insulin Analogs

In this example, rHuPH20 stability in commercial insulin and insulin analog formulations was determined by measuring rHuPH20 enzymatic activity after storage at 5° C and 25° C. In short, about 1500 U/mL of rHuPH20 was prepared in a buffer containing 10 mM Hepes and 130 mM NaCl, pH 7.0. Subsequently, 0.4 mL of commercial Humulin® (each mL contains 100 U insulin, 2.5 mg/mL metacresol and 16 mL glycerin (Eli Lilly)) or Humalog® (each mL contains 100 U insulin, 3.15

mg/mL metacresol, 16 mg glycerin, 1.88 mg Na_2HPO_4, 0.0197 mg zinc ion (zinc oxide), and trace amounts of phenol (Eli Lilly)) were added to 3.6 mL of rHuPH20 solution to form an insulin-rHuPH20 or insulin-analog rHuPH20 mixture (10x dilution for insulin products, final rHuPH20 concentration of 1350 U/mL). These solutions were stored at 5° C and 25° C for up to 1 week. rHuPH20 enzymatic activity was measured on days 0, 1, 2, 3, and 7 as described in Example 2B above. The results are set forth in Table 15 below. As shown in Table 15, 50 % of rHuPH20 activity was lost for the Humalog®-rHuPH20 (insulin Lispro-rHuPH20) combination when it was incubated for 2 days or longer at 25° C. No significant loss was observed for Humulin®-rHuPH20 stored at 25° C, presumably due to lower level of preservative in that specific product and the dilution factor.

Table 15. rHuPH20 Enzymatic Activity in presence of Humulin® or Humalog®

Sample	rHuPH20 Enzymatic Activity (U/mL)				
	0 day	1 day	2 days	3 days	7 days
Humulin®-PH20, 5° C	1339	1383	1329	1430	1332
Humulin®-PH20, 25° C	1318	1401	1325	1387	1308
Humalog®-PH20, 5° C	1264	1202	1172	1239	1140
Humalog®-PH20, 25° C	1305	966	740	763	248

Example 7

Effects of preservatives on rHuPH20

In this example, various common insulin and insulin analog preservatives were evaluated for their effects on the enzymatic activity and stability of rHuPH20. rHuPH20 enzymatic activity was measured as described in Example 2 above. Where applicable, rHuPH20 stability was determined by size exclusion chromatography (SEC) as described in Example 4 above.

A. rHuPH20 enzymatic activity

In this example, common insulin and insulin analog preservatives phenol, m-cresol and methylparaben were evaluated for their effects on the enzymatic activity of rHuPH20 at varying concentrations, temperatures and time. Each preservative was added (for a final concentration indicated in Table 16 below), to insulin/rHuPH20 formulations containing 3.7 mg/mL insulin (Organon Insulin API [Recombinant Human insulin SIHR 143] powder was used to prepare the stock solution as detailed in Example 1), and 20 μg/mL rHuPH20 in 20 mM Tris/HCl, pH 7.1 and 140 mM

NaCl and the samples were incubated at the indicated temperature for the predetermined amount of time.

The results are shown in Table 16 below, which sets forth the preservative and its concentration, the incubation time and temperature, and the rHuPH20 enzymatic activity. The results are temperature dependent. The enzymatic activity of rHuPH20 was significantly reduced after one week of incubation at 35 °C when the overall preservative level is relative high (>0.2 %). In contrast, at room temperature (25 °C) and lower preservative concentrations, rHuPH20 maintains its relative activity for at least one month. Generally, as the level of preservatives increases, rHuPH20 enzymatic activity decreases. Additionally, it appears that among the three preservatives, m-cresol is the most detrimental to rHuPH20 followed by phenol and methylparaben.

Table 16. Effect of preservative species and concentration on rHuPH20 activity at elevated temperatures

Preservative	Concentration (%)	Enzymatic Activity (U/mL)		
		1 Month, 25 °C	1 Week, 35 °C	2Weeks, 35 °C
Phenol	0.05	2103	2128	1996
Phenol	0.1	1980	2094	1997
Phenol	0.2	2128	1995	1822
Phenol	0.4	1910	835	447
m-cresol	0.05	2103	2019	1955
m-cresol	0.1	2188	2147	2069
m-cresol	0.2	2013	466	185
m-cresol	0.4	<LOD	<LOD	<LOD
methylparaben	0.05	2061	1058[2]	1018[2]
methylparaben	0.1	1919	2085	1968
methylparaben	0.2	2196	1927	1590
methylparaben	0.3[1]	2049	730	447
No preservative	0.0	2006	1984	1994

LOD, level of detection; [1] Methylparaben is not soluble at 0.4 %; [2] These numbers were unexpectedly low and were treated as "outliers".

B. Apparent melting temperature (Tm) of rHuPH20

In this example, the melting temperature (Tm) of rHuPH20 in the presence and absence of m-cresol, propyl paraben or phenoxyethanol was determined by measuring the hydrodynamic radius of particles using dynamic light scattering. Particle size increase is presumably due to denaturation and subsequent aggregation

of rHuPH20. As temperature increases, proteins will unfold with will lead to aggregate formation.

In brief, rHuPH20 (Lot HuB, 10 mg/mL stock) was diluted to 1 mg/mL in 25 mM Tris-HCl, pH 7.5. Indicated preservatives were spiked into the PH20 samples from a 100 % stock solution. Z-average particle size was measured by dynamic light scattering using a Malvern Zeta sizer Nano-ZS as a function of increasing temperature. A total of 3 measurements were made at each temperature in a low volume quartz cuvette (Helma, 3.00 mm). The temperature started at 20 °C, with a ramp of 2 °C, to a final temperature of 66 °C, with a 5 minute equilibration period at each temperature. Light scattering intensity was measured with a 173° backscatter detector equipped with the instrument and the cumulative Z-Average particle data was calculated with the DTS (dispersion technology software) software using a refractive index of 1.45 for the protein samples, and using a refractive index of 1.33 for water as dispersant. The inflection point on the temperature axis at which there is a significant increase in the particle size is considered to be the apparent Tm (melting temperature) where the protein is denatured and begins to aggregate.

The results are shown in Table 17 below, which sets forth the average particle size at various temperatures for 5 different PH20 formulations, as shown in Table 17 below. The data in Table 17 is an average of 3 measurements per point, at 2 ° temperature increments, with a 5 minute equilibration point. The results show that the Tm of rHuPH20 dropped from above 40 °C without any preservative down to 26 °C in the presence of 0.25 % m-cresol. A similar trend was observed using differential scanning calorimetry (DSC) to measure the Tm of rHuPH20 with and without m-cresol, however the Tm reduced only about 2 °C in the presence of 0.25 % m-cresol.

Table 17. Average particle size of rHuPH20, with and without various preservatives, as measured by Dynamic Light Scattering

Temp. (°C)	rHuPH20	rHuPH20 + 0.25 % m-cresol	rHuPH20 + 0.2 % propyl paraben	rHuPH20 + 1.0 % phenoxy-ethanol	rHuPH20 + 0.5 % phenoxy-ethanol
20	9.48	8.86	9.66	10.51	8.95
22	9.19	11.30	11.40	10.89	9.48
24	9.89	12.49	13.58	10.72	9.95
26	9.96	23.29	28.10	11.54	9.41
28	9.27	113.50	124.57	12.63	9.57

30	8.94	150.93	188.03	13.28	9.40
32	8.96	159.37	237.27	12.97	9.52
34	9.42	158.57	242.13	12.56	9.68
36	9.28	163.27	260.77	14.47	10.13
38	8.91	---	266.43	29.75	11.03
40	9.39	---	277.43	40.18	12.71
42	9.72	---	---	52.28	24.31
44	11.36	---	---	93.99	28.43
46	13.08	---	---	159.13	35.87
48	21.35	---	---	238.33	45.08
50	22.74	---	---	314.00	63.67
52	28.50	---	---	653.13	94.19
54	32.95	---	---	834.30	139.47
56	37.01	---	---	1060.73	---
Estimated Tm	44°C	26°C	26°C	38°C	42°C

C. Binding affinity for rHuPH20

Titration fluorescence spectroscopy was used to measure the apparent binding affinity of the preservatives to rHuPH20 in order to further understand how preservatives interact with rHuPH20. This study was carried out at Legacy BioDesign, LLC (Johnstown, CO).

Insulin analogs Lispro (USP reference standard, Cat. #1342321) and Aspart (EU reference standard, Cat. #Y0000349) were received in powder form and reconstituted with 857 µL of 18 MΩ deionized water and 23 µL of 1 M HCl. 120 µL of 250 mM Tris (pH 10.65) was added to each standard solution bringing the pH to 7.4 and the volume to 1 mL. 2.5 mL of 1 mg/mL solutions of each standard and the two insulin solutions were prepared by dilution with 30 mM Tris pH 7.4. All solutions were clear and colorless at the time of dilution, except the USP insulin lispro standard, which was hazy upon dilution, but became clear within an hour of preparation. Two samples each were prepared for insulin Aspart and insulin Lispro, with the reference standard and the manufactured material. Fluorescence emission spectra of 1 mg/mL insulin solutions were collected using an Aviv Model ATF 105 Spectrofluorometer and Aviv 105 software version 1.3. A quartz fluorescence cell was loaded with 2.5 mL of solution for each measurement. Excitation was performed at 275 nm with a bandwidth of 4 nm. Emission spectra were collected between 340 nm and 280 nm with a resolution of 1 nm. PMT voltage was set to 850 V, while the reference (QC) PMT voltage was set at 250 V.

The data showed that although there was some interaction between the preservatives and rHuPH20, the three tested preservatives (m-cresol, phenol, and benzyl alcohol) did not alter the structure of rHuPH20. The apparent dissociation constants (K_D) for the interactions of rHuPH20 with the preservatives, ranged from 40 to 3000 μM, with most data typically in the 50 to 100 μM range. The data also indicated that although rHuPH20 is sensitive to phenolic compounds, it appears that this is a non-specific interaction and is highly dependent on the environmental temperature. The conclusion of non-specific interaction was further supported by the fact that the addition of a structurally similar compound, phenylalanine, did not protect rHuPH20 against degradation caused by m-cresol or phenol (see section E below).

D. Other common preservatives

In order to determine whether rHuPH20 is sensitive to all preservatives, or just to those typically used in insulin products, various other commercial preservatives were tested for their effect on rHuPH20 enzymatic activity and stability. Additionally, phenylalanine was added as a potential stabilizer to evaluate whether the detrimental effect of preservatives on rHuPH20 activity is mediated solely by the phenolic ring present on several of the deleterious preservatives. It was hypothesized that phenylalanine might be able to compete with phenolic preservatives and provide a stabilizing effect.

In these studies, a total of 100 μg/mL of rHuPH20 was added to base formulations which contained 25 mM Tris/HCl, pH 7.3, 140 mM NaCl, 0.01 % polysorbate 80 and the selected preservatives at the specified level. The concentration chosen for each of the preservatives was based mainly on literature data (see, *e.g.*, Kibbe, A. H., (2000) Handbook of Pharmaceutical Excipients, 3rd edition, Pharmaceutical Press; Powell *et al.*, (1998) PDA Journal of Pharmaceutical Science and Technology) and the levels known to be present in existing commercial products.

The results are set forth in Tables 18-20 below. The results show that chlorhexidine salts and thimerosal do not affect rHuPH20 enzymatic activity. In contrast, the addition of either benzalkonium chloride or 4-chloro-1-butanol caused a significant reduction in rHuPH20 enzymatic activity after just 24 hours at 25 °C. The addition of either phenoxyethanol or m-cresol caused a reduction in rHuPH20 activity

in a temperature dependent manner. At 4 °C, the activity of rHuPH20 was about the same as the control sample that did not contain a preservative, whereas at 35 °C rHuPH20 activity was abolished in as little as 48 hours (see Table 20). Methylparaben generally had little effect on rHuPH20 enzymatic activity at 25 °C or for short time periods, *e.g.*, 24 hours, at 35 °C, but all enzymatic activity was lost after incubation at 35 °C for 6 days. Additionally, methylparaben is a less effective preservative as compared to m-cresol or phenol. The addition of phenylalanine at either low concentration (5 mM) or high concentration (50 mM) did not affect the loss or rHuPH20 activity in the presence of phenolic preservatives m-cresol and methylparaben.

Table 18. rHuPH20 activity with various preservatives at different storage temperatures

Formulation	t = 0	24 hours, 25 °C	5 days, 25 °C
Benzalkonium Chloride	11,740	10750	2710
4-Chloro-1-butanol	11,350	3500	1440
Chlorhexidine dihydrochloride	7,720	8130	8750
Chlorhexidine digluconate	9,590	9520	12310
Thimerosal	10,290	8990	8960
L-Phenylalanine	13,420	12100	11460
L-Phenylalanine / m-cresol	9,990	8390	13470
L-Phenylalanine / methylparaben	10,780	10620	9390
No preservative control	12,200	12410	10620

Table 19. rHuPH20 activity with various preservatives at different storage temperatures

Formulation	t = 0	6 days, 35 °C
Benzalkonium Chloride	11,740	<LOQ
4-Chloro-1-butanol	11,350	<LOQ
Chlorhexidine dihydrochloride	7,720	11890
Chlorhexidine digluconate	9,590	12270
Thimerosal	10,290	10070
L-Phenylalanine	13,420	10660
L-Phenylalanine / m-cresol	9,990	<LOQ
L-Phenylalanine / methylparaben	10,780	<LOQ
No preservative control	12,200	11870

Table 20. rHuPH20 activity with various preservatives at different storage temperatures

Formulation	48 hours,	48 hours,

	4 °C	35 °C
Phenoxyethanol	14,700	<LOQ
Chlorhexidine digluconate	12,090	13,110
L-Phenylalanine	12,540	13,130
L-Phenylalanine / m-cresol	12,250	<LOQ
L-Phenylalanine / methylparaben	10,480	10,690
m-cresol	10,950	<LOQ
methylparaben	10,380	12,660
No Preservative control	12,520	13,200

The loss of enzymatic activity caused by the addition of preservatives and elevated temperature was mainly attributed to the formation of rHuPH20 aggregates. As shown in Tables 21-22 below, at 35 °C, a loss of the main peak as measured by size exclusion chromatography was concomitant with the loss or rHuPH20 enzymatic activity (see Tables 21-22). Additionally, a significant aggregate peak was observed for m-cresol containing samples when stored at 35 °C. Common preservatives cause loss of rHuPH20 activity at elevated temperatures and over time. More compatible preservatives, *e.g.*, thimerosal and chlorhexidine salts, were identified.

Table 21. Effect of preservatives on rHuPH20 stability as measured by SEC

Preservatives and additives	Concentrations/ Percentages	% Main rHuPH20 peak by non-denaturing SEC	
		4 °C, 1 day	35 °C, 1 day
Benzalkonium Chloride	0.01 %	99.64	<LOD
4-Chloro-1-butanol	0.5 %	97.89	<LOD
Chlorhexidine dihydrochloride	0.002 %	98.76	98.12
Chlorhexidine digluconate	0.002 %	98.35	98.23
Thimerosal	0.01 %	98.59	98.31
L-Phenylalanine	5 mM	98.27	98.86
L-Phenylalanine / m-cresol	5 mM / 0.25 %	97.42	31.29
L-Phenylalanine / methylparaben	5 mM / 0.2 %	98.36	39.94
No preservative control	0	98.33	98.85

LOD: Limit of detection

Table 22. Effect of preservatives on rHuPH20 stability as measured by SEC

Preservatives and additives	Concentrations/ Percentage	% Main rHuPH20 peak by non-denaturing SEC	
		4°C	35°C,
Phenoxyethanol	1 %	99.81	8.51
Chlorhexidine digluconate	0.002 %	99.77	98.30
L-Phenylalanine	50 mM	100.00	99.80
L-Phenylalanine / m-cresol	50 mM / 0.25 %	95.10	3.287
L-Phenylalanine / methylparaben	50 mM / 0.2 %	96.75	95.07
m-cresol	0.25	100.00	0.834

methylparaben	0.2	100.00	96.91
No Preservative control	0	99.63	99.75

E. Preservative levels and antimicrobial effectiveness

Currently different regulatory agencies have different pharmacopeial criteria for antimicrobial effectiveness for pharmaceutical products designed for multiple dosing. Table 23 shows the criteria for injectable drugs to meet USP and EP criteria. Thus, it was necessary to determine minimum preservative concentrations needed to meet the various criteria in order to further evaluate the effects of the preservatives on rHuPH20.

Table 23. USP and EP requirements for antimicrobial effectiveness testing

Requirement		United States	Europe	
	Timepoint	USP	EPB (Minimum)	EPA (Preferred)
Bacterial Log Reduction*	6 h			2
	24 h		1	3
	7 d	1.0	3	---
	14 d	3.0	---	---
	28 d	No increase	No increase	No recovery
Fungal Log Reduction*	7 d	No increase		2
	14 d	No increase	1	---
	28 d	No increase	No increase	No increase

* Log_{10} unit reduction from initial measured inoculum; No increase: not more than 0.5 log_{10} unit increase than previously measured value.

Several batches of formulations containing different levels of preservatives with targeted amounts of insulin and rHuPH20 were prepared for microbial effectiveness testing. The tests were performed according to the guidance of EP and USP by a contract analytical laboratory (Quadrants Scientific, Inc., San Diego, CA). The insulin/rHuPH20 formulations contained: 100 U/mL insulin (Organon Insulin API, Recombinant Human insulin SIHR 143), 5 μg/mL rHuPH20, 20 mM Tris/HCl, pH 7.2, 150 mM NaCl and 0.02% poloxamer 188. The insulin was prepared as described in Example 1 above. The various preservative containing formulations were tested for anti-microbial effectiveness against *Aspergillus niger*, *Pseudomonas aeruginosa*, *E. coli*, *Staphylococcus aureus* and *Candida albicans*. Tests were conducted by 1) adding an initial inoculum (at least 10^5 CFU/mL) of each bacteria to the sample and 2) measuring the CFU/mL of each bacteria at 6 hours, 1 day and 7 days. Raw data (CFU/mL) was converted to a log 10 unit reduction from the

measured inoculum. The formulations were tested at a temperature of 37 °C and each organism was incubated separately with each formulation.

The results are shown in Table 24, which sets forth the percentages of the preservatives and whether the combination passed or failed the antimicrobial effectiveness criteria for EPA, EPB and USP.

Table 24. Antimicrobial effectiveness tests of different preservative levels and combinations

#	m-Cresol (%)	Methyl-paraben (%)	Phenol (%)	Antimicrobial Effectiveness Criteria		
				EPA	EPB	USP
1	---	---	0.15	Fail	Fail	Pass
2	---	---	0.3	Fail	Fail	Pass
3	0.1	---	---	Fail	Fail	Fail
4	0.15	---	---	Fail	Fail	Pass
5	0.1	0.15	---	Fail	Fail	Pass
6	0.1	0.1	---	Fail	Fail	Pass
7	0.1	0.15	---	Fail	Fail	Pass
8	0.15	0.1	---	Fail	Fail	Pass
9	0.15	0.15	---	Fail	Pass	Pass
10	0.1	---	0.1	Fail	Fail	Pass
11	0.1	---	0.15	Fail	Pass	Pass
12	0.15	---	0.1	Fail	Pass	Pass
13	0.15		0.15	Fail	Pass	Pass
14	0.1	0.15	---	Fail	Fail	Pass
15	---	0.15	0.15	Fail	Fail	Pass
16	---	0.15	0.2	Fail	Fail	Pass
17	---	0.2	0.15	Fail	Fail	Pass
18	---	0.2	0.2	Fail	Fail	Pass
Humalog ®	0.315	---	---	Pass*	Pass*	Pass*
Novo Log®	0.172	---	0.15	Fail*	Pass*	Pass*

*Result, based on 7 day value.

In order to determine suitable preservative levels that meet the USP antimicrobial effectiveness criteria, a further study of combinations of benzyl alcohol, phenol and m-cresol was undertaken. The basic formulation contains: 3.75 mg/mL insulin (Organon Insulin API, Recombinant Human insulin SIHR 143 and the stock solution was prepared in the say way as detailed in Example 1), 5 µg/mL rHuPH20, 20 mM tris/HCl, pH 7.4, 140 mM NaCl and 0.02% poloxamer 188. The results are shown in Table 25 below, which sets forth the percentages of the preservatives and

whether the combination passed or failed the antimicrobial effectiveness criteria for USP. With the exception of formulation # 2, all combinations passed the USP microbial effectiveness criteria.

Table 25. USP Antimicrobial effectiveness tests of different preservative levels and combinations

#	Benzyl Alcohol (%)	Phenol (%)	m-Cresol (%)	USP Antimicrobial Effectiveness Criteria*		
				7 days	14 days	28 days
1	0.25	0.1	---	Pass	Pass	Pass
2	0.1	0.15	---	Fail	Pass	Pass
3	0.1	0.1	---	Pass	Pass	Pass
4	0.05	0.2	---	Pass	Pass	Pass
5	0.05	0.15	---	Pass	Pass	Pass
6	0.05	0.1	---	Pass	Pass	Pass
7	0.1	---	0.1	Pass	Pass	Pass
8	0.05	---	0.1	Pass	Pass	Pass
9	0.05	---	0.15	Pass	Pass	Pass
10	0.5	---	---	Pass	Pass	Pass
11	---	0.1	0.08	Pass	Pass	Pass
12	---	0.1	0.06	Pass	Pass	Pass
13	---	0.08	0.08	Pass	Pass	Pass
14	---	0.06	0.1	Pass	Pass	Pass
15	---	0.08	0.1	Pass	Pass	Pass

Similar experiments were conducted with other percentage combinations of phenol and m-cresol. The results are depicted in Table 26.

Table 26. Minimum preservative levels necessary for USP and EPB guidelines

criteria		log reduction										standard	
		24 hours			7 days					14 days			
		bacteria			bacteria			fungi		fungi			
phenol %	m-cresol %	PA	EC	SA	PA	EC	SA	AN	CA	AN	CA	UPS	EPB
0.10	0.15	>4.5	1.9	0.9	>4.5	>48.	>4.8	2.9	2.0	>4.2	>4.4	pass	pass
0.15	0.10	>4.1	0.7	0.3	>4.6	>4.7	>4.9	2.0	1.6	>3.8	>4.3	pass	fail
0.175	0.10	>4.6	1.0	0.3	>4.6	>4.7	>4.8	2.5	1.8	>4.4	>4.3	pass	fail
0.20	0.10	>4.5	2.0	0.7	>4.5	>4.7	>4.9	3.0	2.5	>4.4	>4.2	pass	pass
0.125	0.125	>4.6	1.1	0.4	>4.6	>4.8	>4.9	2.5	2.1	>4.3	>4.2	pass	fail
0.15	0.125	>4.5	2.1	0.7	>4.5	>4.8	>4.8	2.9	2.8	>4.4	>4.4	pass	pass
0.175	0.125	>4.6	3.6	1.0	>4.6	>4.8	>4.6	3.5	2.6	>4.3	>4.4	pass	pass
0.125	0.15	>4.5	4.1	1.0	>4.5	>4.7	>4.7	3.8	4.0	>4.3	>4.6	pass	pass

PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; SA: *Staphylococcus aureus*; AN: *Aspergillus niger*; CA: *Candida albicans*.

Example 8

Effect of combinations of preservatives and NaCl and pH on the solubility of insulin and the enzymatic activity of rHuPH20

A. Effect of combinations of preservatives on rHuPH20 enzymatic activity

In this example, combinations of common insulin and insulin analog preservatives were tested for their effects on the enzymatic activity of rHuPH20 at varying concentrations at a temperature of 30 °C for 1 month. Each preservative was added (for final percentages indicated in Table 26 below), to insulin/rHuPH20 formulations containing 100 U/mL insulin, 5 µg/mL (600 U/mL) rHuPH20, 20 mM Tris/HCl, pH 7.2, 150 mM NaCl and 0.02 % poloxamer 188 and the samples were tested at T=0 and after 1 month incubation at 30 °C. Insulin was prepared as described in Example 1 above and rHuPh20 was prepared as described in Example 2.

The results are set forth in Tables 27 below, which sets forth the preservatives and their percentages, and the rHuPH20 enzymatic activity at time T=0 and after 1 month incubation at 30 °C. The results show that incubation of insulin/rHuPH20 for 1 month at 30 °C in the presence of one or a combination of phenolic preservatives causes a reduction in the enzymatic activity of rHuPH20.

Table 27. Effect of phenolic preservative combinations on rHuPH20 activity

Formulation				PH20 activity (U/mL)	
#	m-Cresol (%)	Methylparaben (%)	Phenol (%)	T=0	1 Month, 30 °C
1	---	---	0.15	629	564
2	---	---	0.3	579	490
3	0.1	---	---	621	549
4	0.15	---	---	601	538
5	0.1	0.15	---	569	474
6	0.1	0.1	---	351	404
7	0.1	0.15	---	557	481
8	0.15	0.1	---	545	418
9	0.15	0.15	---	530	256
10	0.1	---	0.1	585	526
11	0.1	---	0.15	578	487
12	0.15	---	0.1	550	390
13	0.15	---	0.15	553	262
14	0.1	0.15	---	544	465
15	---	0.15	0.15	546	484
16	---	0.15	0.2	545	392
17	---	0.2	0.15	533	407
18	---	0.2	0.2	513	215

In this example, combinations of phenol and m-cresol were tested for their effects on the enzymatic activity of rHuPH20 at varying concentrations, temperatures and incubation times. Each preservative was added (for final percentages indicated in Table 27 below), to insulin/rHuPH20 formulations containing 100 U/mL insulin, 5 µg/mL (600 U/mL) rHuPH20, 20 mM Tris/HCl, pH 7.4, 50 mM NaCl, 50 mL glycerin, 50 mM methionine, and 0.01 % poloxamer 188.

The results are set forth in Tables 28 below, which sets forth the preservatives and their percentages, and the rHuPH20 enzymatic activity at various incubation times and temperatures. As was observed above, storage temperature has a significant effect on enzymatic activity. Regardless of preservative, samples that were incubated at 30 °C or 37 °C had significantly reduced enzymatic activity as compared to those incubated at 25 °C. Additionally, m-cresol is more deleterious on the activity of rHuPH20 than phenol. As the percentage of m-cresol was increased, the enzymatic activity of rHuPH20 decreased.

Table 28. Effect of phenol and m-cresol combinations on rHuPH20 activity

Formulation	Phenol %	m-cresol %	rHuPH20 activity, U/mL				
			25 °C, 1 week	25 °C, 2 weeks	30 °C, 1 week	30 °C, 2 weeks	37 °C, 3 days
F1	0.35	---	603	569	178	72	5
F2	0.2	0.1	574	493	114	29	13
F3	0.25	0.1	475	356	23	0.3	8
F4	0.175	0.15	462	342	10	0	26
F5	0.2	0.15	385	297	0	ND	10
F6	0.15	0.175	421	318	3	ND	22
F7	0.2	0.175	265	141	0	ND	15
F8	---	0.25	401	332	3	ND	18

ND, Not determined.

B. Effect of NaCl and pH on insulin Lispro solubility and rHuPH20 enzymatic activity

In this example, a full factorial study design was employed to determine the effects of NaCl and pH on the solubility of insulin Lispro and the enzymatic activity of rHuPH20 in the presence of 0.15 % meta-cresol (m-cresol) and 0.15 % phenol. Four different pH values and 4 concentrations of NaCl were evaluated, generating a total of 16 samples.

All components, except pH and NaCl were kept constant at 120 U/mL insulin Lispro, 5 µg/mL rHuPH20, 20 mM Tris/HCl (Trizma, Sigma, Cat No. T6066), 0.15 %

m-cresol, 0.15 % phenol, 0.01 % Poloxamer 188 (Poloxamer 188, Spectrum, Cat No. P1169), and 0.1 mM added $ZnCl_2$ (EMD, Cat No. ZX0065-1). The pH values tested were 7.0, 7.2, 7.4 and 7.6. The NaCl concentrations tested were 50, 80, 110 and 140 mM. The preservatives were phenol (Riedel-dh Haen, 16017, multiple compendia) and m-cresol (Fluka, Cat No. 65996). Percent insulin Lispro recovery was determined for each sample, after storage at 2-8 °C for 0, 0.25, 0.5, 1, 3, 5, 9 and 12 months. rHuPH20 enzymatic activity was measured for each sample, after storage at 5° C for 2 weeks, 1, 5, 9 and 12 months, 25° C for 1 week, 2 weeks and 1 month, 30° C for 1 week and 2 weeks, and 35° C for 1 week.

To prepare the 16 samples, 4 stock solutions, each with a different NaCl concentration, were prepared. Insulin Lispro was prepared as described in Example 1 above, with the final insulin pH set at 7.6. All of the other common components were added to their final concentrations. The pH of each stock solution was then titrated with 1 N or 0.1 N NaOH from 7.6 down to 7.0, sequentially. The precision of pH was controlled at ± 0.02. Each time the designated pH was reached, 1 mL of the solution was removed and filled into a 2 mL type-1 glass vial. Once all the samples were prepared, they were stored at 2-8 °C until the tests were performed. Reverse Phase-HPLC (RP-HPLC) was performed as described in Example 3 above with the following modifications. The mobile phase started with 75 % 0.1 % trifluoroacetic acid (TFA) in water (A) and 25 % 0.1 % TFA in acetonitrile (B) with a linear gradient to 68 % A + 32 % B over 16 minutes, with a hold for 4 minutes, followed by a linear increase to 100 % B for 5 minutes.

Table 29 below, sets forth the solubility, expressed as % remaining of the original concentration (120 U/mL), after storage at 2-8 °C for 12 months. Tables 30-31 below, set forth the rHuPH20 enzymatic activity after various time points at different temperatures. Table 32 below sets forth the rHuPH20 percent recovery after various time points at different temperatures. The results of insulin Lispro solubility and rHuPH20 activity are summarized below.

Insulin Lispro Solubility

As is shown in Table 29 below, insulin Lispro is stable at low salt concentrations and high pH. For example, at pH 7.6, insulin Lispro was stable for at least 12 months at 50 and 80 mM NaCl, whereas it was stable for only 2 weeks at 140

mM NaCl. In contrast, at pH 7.0, insulin Lispro was only stable at 50 mM NaCl for 5 months. Solubility dropped below 65 % when after just 1 week at 80, 110 or 140 mM NaCl.

rHuPH20 Enzymatic Activity

As is shown in Tables 29-30 below, rHuPH20 is stable at high salt concentrations and low pH. rHuPH20 is stable at 5 °C for 12 months, albeit with a lower rHuPH20 activity at 50 mM NaCl then at 140 mM NaCl. At 25 °C, rHuPH20 enzymatic activity was greatly reduced at low salt concentrations after just one week at pH 7.6 and after 2 weeks at lower pH. At 30 °C , rHuPH20 retained useful enzymatic activity only at a concentration of 140 mM NaCl and a pH of 7.0. After one week at 35 °C, no meaning rHuPH20 enzymatic activity remained.

Table 29. Solubility of Insulin Lispro at 2-8 °C

Form #	pH	NaCl	% Insulin Recovery (months)							
			0	0.25	0.5	1	3	5	9	12
F16	7.0	50	96.41	95.91	95.12	93.26	81.51	88.84	72.51	58.91
F12	7.0	80	96.36	64.78	40.76	35.90	33.47	32.72	29.34	26.22
F8	7.0	110	96.35	31.18	19.52	18.50	15.05	14.72	14.23	13.63
F4	7.0	140	95.78	21.70	15.04	13.80	11.11	11.14	11.13	10.08
F15	7.2	50	96.68	95.95	95.80	95.42	95.64	96.28	98.59	91.50
F11	7.2	80	96.50	96.06	95.81	86.34	60.90	61.48	50.74	47.02
F7	7.2	110	96.45	66.09	39.64	32.94	58.68	28.02	23.68	21.88
F3	7.2	140	95.82	37.37	25.12	23.62	19.03	17.15	15.99	15.90
F14	7.4	50	96.74	96.44	96.36	95.89	95.90	96.58	98.78	95.82
F10	7.4	80	96.34	96.51	96.49	96.64	96.05	96.25	98.62	95.61
F6	7.4	110	96.69	96.86	93.50	74.63	63.68	68.64	43.83	38.80
F2	7.4	140	95.73	83.13	50.31	39.27	37.45	34.80	27.30	24.01
F13	7.6	50	95.66	96.72	97.02	96.38	96.21	96.71	98.74	96.11
F9	7.6	80	95.48	95.96	96.22	96.34	95.43	96.17	96.91	92.06
F5	7.6	110	95.47	96.74	96.40	96.35	95.89	95.50	84.82	91.38
F1	7.6	140	95.73	95.53	94.46	87.46	64.62	67.99	46.20	41.61

Table 30. Effect of salt and pH on rHuPH20 Enzymatic Activity at 35° C, 30° C and 25° C

Form #	NaCl (mM)	pH	t = 0	Enzyme Activity (U/mL)					
				35° C	30° C		25° C		
				1W	1W	2W	1W	2W	1M
F1	140	7.6	588	-*	244	133	545	528	461
F2	140	7.4	593	15	322	238	557	494	496
F3	140	7.2	583	31	370	300	585	530	514

F4	140	7.0	576	84	418	387	579	507	513
F5	110	7.6	577	-	163	73	525	496	412
F6	110	7.4	574	-	256	159	541	489	449
F7	110	7.2	581	14	327	257	565	501	505
F8	110	7.0	580	49	376	304	576	512	512
F9	80	7.6	599	9	152	82	498	430	385
F10	80	7.4	574	-	91	17	451	401	290
F11	80	7.2	544	-	230	133	522	444	416
F12	80	7.0	549	-	283	199	518	448	435
F13	50	7.6	526	-	38	-	361	296	178
F14	50	7.4	535	-	47	5	426	329	265
F15	50	7.2	529	14	115	51	481	371	324
F16	50	7.0	522	-	172	87	507	405	339

W = week, M = month * Below limit of detection.

Table 31. Effect of salt and pH on rHuPH20 Enzymatic Activity at 5° C

Form #	NaCl (mM)	pH	t = 0	Enzyme Activity (U/mL)				
				2W	1M	5M	9M	12M
F1	140	7.6	588	613	597	595	524	539
F2	140	7.4	593	572	594	586	542	551
F3	140	7.2	583	563	592	586	537	546
F4	140	7.0	576	569	586	582	555	559
F5	110	7.6	577	569	582	582	532	552
F6	110	7.4	574	655	592	618	560	580
F7	110	7.2	581	574	581	593	553	553
F8	110	7.0	580	564	588	615	551	576
F9	80	7.6	599	540	584	546	515	534
F10	80	7.4	574	613	557	565	510	488
F11	80	7.2	544	536	548	566	518	536
F12	80	7.0	549	536	548	575	514	520
F13	50	7.6	526	518	514	508	465	456
F14	50	7.4	535	521	525	527	466	473
F15	50	7.2	529	518	544	482	452	456
F16	50	7.0	522	574	543	494	437	438

W = week, M = month

Table 32. Effect of salt and pH on rHuPH20 % Recovery

Form #	NaCl (mM)	pH	% rHuPH20 Recovery					
			35° C 1W	30° C 2W	25° C 1M	5° C 1M	5° C 9M	5° C 12M
F1	140	7.6	41.90	52.70	70.78	101.78	94.05	76.91
F2	140	7.4	42.91	67.13	83.24	98.45	93.36	80.46

F3	140	7.2	49.99	68.94	89.84	102.18	102.07	75.42
F4	140	7.0	49.55	70.64	91.10	97.59	101.54	78.66
F5	110	7.6	37.50	53.04	82.95	96.95	92.45	81.42
F6	110	7.4	45.20	58.19	88.46	107.24	97.80	79.46
F7	110	7.2	42.96	59.21	86.68	93.10	108.11	80.83
F8	110	7.0	45.31	64.58	89.49	102.01	92.18	85.45
F9	80	7.6	42.91	56.60	73.76	90.92	92.18	78.13
F10	80	7.4	34.26	53.21	73.82	88.45	98.33	75.11
F11	80	7.2	38.33	59.21	82.78	87.93	95.23	78.77
F12	80	7.0	37.83	56.55	78.93	97.18	101.16	74.05
F13	50	7.6	34.87	49.07	42.13	87.93	83.47	69.38
F14	50	7.4	40.29	52.53	60.91	92.41	90.85	71.76
F15	50	7.2	39.56	52.47	69.92	87.76	91.54	73.41
F16	50	7.0	38.50	50.55	72.96	97.30	93.52	74.10

W = week, M = month

Example 9

Effect of NaCl and pH on Insulin Stability and rHuPH20 enzymatic activity under different combinations of preservatives

In this example, the effects of NaCl and pH on insulin (regular insulin) and/or insulin analog (lispro or aspart) stability, and rHuPH20 enzymatic activity, were determined for various storage conditions, including short term and long term storage (7 days, 5 months or 9 months) at 2-8 °C and short term storage (one month or less) at elevated temperatures, including 35 °C, 30 °C and 25 °C.

The basic formulations are set forth in sections A-C below. For each individual study, pH and NaCl concentration were varied while the other components of the compositions remained the same. To prepare the samples for each of the insulin/insulin analogs at each of the predetermined preservative combinations, 4 stock solutions were prepared for each of the insulin and/or insulin analogs. Insulin and insulin analog stocks were prepared as described in Example 1 above, with the final insulin pH set at 7.6. Every stock solution contained the proper levels of preservatives and NaCl concentrations (50, 80, 110 or 140 mM), and all of the other common components which were added to their final concentrations. The pH of each stock solution was then titrated with 1 N or 0.1 N NaOH from 7.6 down to the final target pH, sequentially. The precision of pH was controlled at ± 0.02. Each time the designated pH was reached, 1 mL of the solution was removed, filtered through a 0.2

micron PES filter, and filled into a 2 mL type-1 glass vial. Once all the samples were prepared, they were stored at 2-8 °C until the tests were performed.

A. Full factorial study for effects of NaCl and pH on insulin/insulin analog solubility at 2-8 °C for 7 days

In this example, a full factorial study design was employed to determine the effects of NaCl and pH on the solubility of regular insulin and analogs lispro or aspart in the presence of different combinations of preservatives. Other formulation components, except pH, NaCl and preservatives, were kept constant at: 120 U/mL insulin/insulin analog, 5 μg/mL rHuPH20 (600 U/mL), 20 mM Tris/HCl (Trizma, Sigma, Cat No. T6066), 0.02 % Pluronic® F68 (Poloxamer 188, Spectrum, Cat No. P1169), and 0.1 mM $ZnCl_2$ (EMD, Cat No. ZX0065-1). Three different combinations of preservative levels were utilized: 1) 0.15 % m-cresol (Fluka, Cat No. 65996) and 0.2 % phenol (Riedel-dh Haen, 16017, multiple compendia); 2) 0.15 % m-cresol and 0.15 % phenol; and 3) 0.15 % m-cresol and 0.2 % methylparaben (Fluka, Cat No. 85265). With each preservative combination, a full combination of 6 levels of pH and 4 levels of NaCl concentrations (total of 24 samples) were generated. The pH values tested were 6.6, 6.8, 7.0, 7.2, 7.4 and 7.6. The NaCl concentrations tested were 50, 80, 110 and 140 mM.

Reverse Phase-HPLC (RP-HPLC) was performed as described in Example 3 above with the following modifications. The mobile phase started with 75 % 0.1 % trifluoroacetic acid (TFA) in water (A) and 25 % 0.1 % TFA in acetonitrile (B) with a linear gradient to 68 % A + 32 % B over 16 minutes, with a hold for 4 minutes, followed by a linear increase to 100 % B for 5 minutes. The results are shown in Tables 33-35 below, which set forth the solubility, expressed as % remaining of the original concentration (120 U/mL), after storage at 2-8 °C for 7 days. Table 33 sets forth the results for Lispro. Table 34 sets forth the results for regular insulin. Table 35 sets forth the results for Insulin Aspart.

All three insulin/insulin analog molecules responded similarly to pH and NaCl concentration. At low pH and high NaCl concentrations, the insulin/insulin analogs formed crystals and precipitates, as indicated by a decrease in the percentage of remaining insulin/insulin analog. These results were verified by visual inspection. All three insulin/insulin analogs were soluble in all preservative combinations for pH

≥ 7.2 at 50 mM NaCl, pH ≥ 7.4 at 80 mM NaCl and at pH ≥ 7.6 at 110 mM NaCl. Similarly, all three insulin/insulin analogs were not adequately soluble (observed concentration < 90 U/mL or 75 %) for pH ≤ 6.8 at 140 mM NaCl and pH 6.6 at both 80 and 110 mM NaCl. The exact trends for solubility varied among the three insulin/insulin analogs. Under the tested conditions, insulin Aspart was the most soluble followed by insulin Lispro and regular insulin, which was the least soluble. There were also differences in preservative compatibility, which insulin Aspart and regular insulin most soluble in 0.15 % phenol and 0.15 % m-cresol and insulin Lispro most soluble in 0.15 % m-cresol and 0.2 % methylparaben. Methylparaben appears to be a better preservative when the salt concentration is low, however, at higher salt concentrations, no difference was observed between samples containing phenol or methylparaben.

Regular insulin did not completely dissolve at higher (> 110 mM) NaCl concentrations, even at high pH. Table 34 confirms insulin's low solubility, with more than 50 % of the test conditions having insulin concentrations below the 90 % of the original values after only 7 days at 2-8 °C. Reducing the phenol concentration and/or replacing the phenol with methylparaben increases the solubility slightly. Under these experiments, the loss in solubility accompanying a 30 mM increase in NaCl concentration was comparable to a 0.2 pH unit reduction. This data demonstrates that high NaCl concentrations will affect insulin solubility during 5 °C storage and thus regular insulin formulations will likely require reduced NaCl concentration and/or increased pH relative to the insulin analogs.

Table 33. Solubility of Lispro after 7 days at 2-8 °C

0.2 % phenol + 0.15 % m-cresol formulation				
pH	**NaCl concentration (mM)**			
	50	**80**	**110**	**140**
7.6	97.5	97.7	97.3	96.8
7.4	97.7	97.6	97.0	96.9
7.2	97.6	97.4	95.2	90.8
7.0	97.4	95.5	75.2	65.3
6.8	91.4	65.1	44.9	40.4
6.6	52.8	32.8	26.0	N/A
0.15 % phenol + 0.15 % m-cresol formulation				
pH	**NaCl concentration (mM)**			
	50	**80**	**110**	**140**
7.6	100.9	102.1	101.6	101.3

7.4	100.8	101.8	100.7	98.4
7.2	100.5	99.2	97.3	93.1
7.0	98.7	96.4	77.0	61.2
6.8	93.6	60.8	44.9	35.9
6.6	60.3	43.3	24.2	27.7
0.2 % methylparaben + 0.15 % m-cresol formulation				
pH	**NaCl concentration (mM)**			
	50	**80**	**110**	**140**
7.6	95.7	98.3	100.2	98.8
7.4	95.6	98.2	99.9	98.5
7.2	95.7	97.9	99.6	98.0
7.0	95.3	98.1	94.1	65.6
6.8	82.3	62.6	47.8	42.0
6.6	42.0	28.2	24.1	25.9

Table 34. Solubility of regular Insulin after 7 days at 2-8 °C

0.2 % phenol + 0.15 % m-cresol formulation				
pH	**NaCl concentration (mM)**			
	50	**80**	**110**	**140**
7.6	102.1	99.2	98.5	82.2
7.4	101.8	99.2	87.8	40.6
7.2	101.8	92.2	37.4	18.9
7.0	100.3	35.7	17.7	11.6
6.8	38.4	17.1	10.2	6.5
6.6	26.8	8.7	6.5	5.8
0.15 % phenol + 0.15 % m-cresol formulation				
pH	**NaCl concentration (mM)**			
	50	**80**	**110**	**140**
7.6	97.2	97.1	97.1	91.7
7.4	96.7	97.3	94.2	57.9
7.2	97.0	96.4	58.7	33.1
7.0	96.7	80.8	32.9	17.1
6.8	66.6	24.9	17.0	10.2
6.6	31.2	18.6	14.6	12.9
0.2 % methylparaben + 0.15 % m-cresol formulation				
pH	**NaCl concentration (mM)**			
	50	**80**	**110**	**140**
7.6	98.1	97.9	97.0	95.4
7.4	98.1	97.7	94.6	59.1
7.2	98.0	90.6	47.1	32.9
7.0	86.9	46.6	23.0	15.9
6.8	34.1	19.0	11.4	10.2
6.6	15.3	9.0	7.4	5.8

Table 35. Solubility of Insulin Aspart after 7 days at 2-8 °C

0.2 % phenol + 0.15 % m-cresol formulation

pH	NaCl concentration (mM)			
	50	80	110	140
7.6	99.2	100.8	100.2	99.8
7.4	99.2	100.6	100.0	99.7
7.2	99.1	100.5	100.0	99.7
7.0	99.1	100.6	99.9	88.9
6.8	98.8	91.8	72.8	50.9
6.6	91.8	59.2	58.0	39.3
0.15 % phenol + 0.15 % m-cresol formulation				
pH	**NaCl concentration (mM)**			
	50	**80**	**110**	**140**
7.6	100.1	99.7	99.4	101.1
7.4	100.0	99.6	99.5	101.1
7.2	99.1	99.2	99.2	101.1
7.0	99.7	99.3	99.3	100.2
6.8	99.8	97.9	97.5	60.5
6.6	97.9	86.3	54.4	38.3
0.2 % methylparaben + 0.15 % m-cresol formulation				
pH	**NaCl concentration (mM)**			
	50	**80**	**110**	**140**
7.6	99.3	100.6	99.8	99.8
7.4	99.1	100.5	99.6	99.6
7.2	99.0	100.2	99.5	101.6
7.0	98.7	100.2	96.3	67.9
6.8	97.2	84.1	54.2	32.2
6.6	57.7	32.1	21.1	14.7

B. Follow-up study with reduced m-cresol level

Insulin solubility was further evaluated in a simplified follow-up study with a reduced m-cresol level. Four combinations of preservative levels were utilized: 1) 0.1 % m-cresol and 0.15 % phenol; 2) 0.1 % m-cresol and 0.2 % phenol; 3) 0.1 % m-cresol and 0.15 % methylparaben; and 4) 0.1 % m-cresol and 0.2 % methylparaben. Two pH levels (7.3 and 7.1) and two NaCl concentrations (120 and 100 mM) were evaluated. The remaining formulation components were kept constant at: 120 U/mL regular insulin, 5 µg/mL rHuPH20 (600 U/mL), 20 mM Tris/HCl (Trizma, Sigma, Cat No. T6066), 0.02 % Poloxamer 188 (Poloxamer 188, Spectrum, Cat No. P1169), and 0.1 mM $ZnCl_2$ (EMD, Cat No. ZX0065-1). The formulations were prepared and tested as described above.

The results are set forth in Table 36 below. The overall solubility of regular insulin increases slightly in the presence of lower amounts of m-cresol, and additionally, when phenol is replaced with methylparaben.

Table 36. Solubility of regular insulin with reduced levels of m-cresol at 2-8 °C							
Formulation #	pH	NaCl (mM)	m-cresol (%)	phenol (%)	methylparaben (%)	Insulin Recovery	
						1 day	3 days
1	7.3	120	0.1	0.15	---	84.15	52.13
2	7.3	120	0.1	0.2	---	72.75	40.78
3	7.3	120	0.1	---	0.15	92.88	92.68
4	7.3	120	0.1	---	0.2	94.76	94.80
5	7.1	100	0.1	0.15	---	72.60	38.87
6	7.1	100	0.1	0.2	---	64.86	32.69
7	7.1	100	0.1	---	0.15	93.87	91.99
8	7.1	100	0.1	---	0.2	92.32	90.59

C. Long-term Effect of pH and NaCl on rHuPH20 and Insulin Stability

In this example, a full factorial study design was employed to determine the effects of pH and NaCl on regular insulin and rHuPH20 solubility in order to identify a condition that maximizes insulin solubility at 2-8 °C and maximizes rHuPH20 stability at room temperature or higher at a high preservative level. The preservative level was set to meet EP-A criteria. Four levels of NaCl concentrations and 4 levels of pH were evaluated generating a total of 16 samples. The samples were evaluated for stability under both short-term accelerated conditions (high temperature) and long-term storage at 2-8 °C.

Regular insulin (100 U/mL, prepared as described in Example 1 above, with a final pH of 7.0) and 5 μg/mL rHuPH20 were formulated in a common buffer containing 20 mM Tris/HCl, 0.1 mM $ZnCl_2$, 0.01 % Poloxamer 188, 0.15 % m-cresol and 0.2 % phenol. Solubility was determined by RP-HPLC as described in Example 3 above. Solubility was expressed as relative percent compared to the standard, with 100 % being 100 U/mL. rHuPH20 enzymatic activity was assessed as described in Example 2 above. RP-HPLC was used to monitor the total content and purity of rHuPH20 (see Example 3 above). Data was processed by Design Expert 7.0 (StatEase). ANOVA and correlation analyses were performed by JMP 8.0 software.

The results are set forth in Tables 37-42 below. Tables 37-38 set forth the rHuPH20 enzymatic activity and Table 39 sets forth the rHuPH20 percent recovery. Under short-term accelerated conditions, rHuPH20 enzymatic activity was effected by pH, NaCl and storage temperatures, as indicated in Table 37 below. After 1 week storage at 35 °C, there was no meaningful rHuPH20 activity remaining. The

enzymatic activity after storage at 30 °C was improved compared to 35 °C, but the only formulation that retained >375 U/mL had a high NaCl concentration (140 mM) and low pH (7.0). In general, the higher the pH and the lower the salt concentration, the lower the enzymatic activity. At a storage temperature of 25 °C the effect on rHuPH20 enzymatic activity was greatly reduced, although the trends observed for 30 °C remained, especially for formulations having low salt and high pH. A majority of the formulations maintained enzymatic activity above the set criteria of 375 U/mL at the 4 week time point. At 5 °C there was essentially no loss in rHuPH20 enzymatic activity, even after storage for 1 month. This trend continued over 9 months storage at 2-8 °C, as seen in Table 38 below. In summary, rHuPH20 maintains enzymatic activity when stored at 25 °C or lower temperature, especially when the NaCl concentration is kept above 80 mM. Stability quickly diminishes at temperatures higher than 25 °C. RP-HPLC was used to monitor the total content of rHuPH20 and its purity. As shown in Table 39 below, loss of enzymatic activity is correlated with loss of rHuPH20 content (statistical analysis, $p<0.001$). An analysis of rHuPH20 purity revealed no significant trends or differences in the relative peak area purity values for the rHuPH20 main peak area (data not shown), indicating that the purity is consistent and therefore the loss of activity is due to a loss of total content. Loss of content is likely due to protein unfolding at high preservative concentrations and temperatures, leading to aggregation and precipitation of the rHuPH20.

Tables 40-41 set forth the percent of insulin main peak and percent of insulin recovery after long term storage at 2-8 °C. In Table 38, the percent of insulin recovery was based on the sum of the insulin main peak and desamido peak. As is shown in Table 40, the insulin main peak percentage remained high (about 97 %) without significant changes, indicating that the loss was not due to insulin chemical or physical degradation, such as deamidation or aggregation. Visual inspection of the vials indicated mixtures of tiny shiny grits or clear crystalline or crystalline-like particles with an occasional cloudy solution. The data in Table 41 summarize insulin content remaining in solution in the formulation at each time point. Insulin recovery as compared to initial conditions is an indication of solubility. Insulin precipitation/crystallization varied depending on the ranges of pH and NaCl concentrations tested. Generally, when the pH was low and the salt concentration

high (conditions that favor rHuPH20 activity), the insulin formed crystals very quickly and reached equilibrium conditions in a couple of months. At low salt concentration and high pH, the crystallization was slow and most of the insulin molecules remained in solution at 9 months. The statistical analyses (see Table 42 below) of the insulin recovery data show that pH, NaCl, time, pH*NaCl and NaCl*time all significantly influence insulin solubility. These results indicated that insulin solubility for extended time periods is dependent on high pH (higher than 7.4) and low NaCl (less than 80 mM), in direct contrast to conditions that maintain rHuPH20 enzymatic activity.

Table 37. rHuPH20 enzymatic activity

#	NaCl, mM	pH	T=0	rHuPH20 activity (U/mL)							
				35 °C	30 °C		25 °C			5 °C	
				1W	1W	2W	1W	2W	1M	2W	1M
1	140	7.6	588	---	244	133	545	528	461	613	597
2	140	7.4	593	15	322	238	557	494	496	572	594
3	140	7.2	583	31	370	300	585	530	514	563	592
4	140	7.0	576	84	418	387	579	507	513	569	586
5	110	7.6	577	---	163	73	525	496	412	569	582
6	110	7.4	574	---	256	159	541	489	449	655	592
7	110	7.2	581	14	327	257	565	501	505	574	581
8	110	7.0	580	49	376	304	576	512	512	564	588
9	80	7.6	599	9	152	82	498	430	385	540	584
10	80	7.4	574	---	91	17	451	401	290	613	557
11	80	7.2	544	---	230	133	522	444	416	536	548
12	80	7.0	549	---	283	199	518	448	435	536	548
13	50	7.6	526	---	38	---	361	296	178	518	514
14	50	7.4	535	---	47	5	426	329	265	521	525
15	50	7.2	529	14	115	51	481	371	324	518	544
16	50	7.0	522	---	172	87	507	405	339	574	543

--- Below level of detection

Table 38. rHuPH20 enzymatic activity after long term storage at 2-8 °C

5 Months				
pH	NaCl concentration (mM)			
	50	80	110	140
7.6	508.0571	546.4209	581.6326	594.6197
7.4	526.6628	564.7769	618.3856	585.9813
7.2	481.6035	566.2521	593.2027	585.9948
7.0	494.4796	575.3274	615.3546	582.4274
9 Months				
pH	NaCl concentration (mM)			

	50	80	110	140
7.6	464.8964	515.3382	531.9202	523.5385
7.4	466.1871	509.9032	560.4847	542.4534
7.2	451.8324	518.0740	553.1847	536.7846
7.0	436.5115	513.9692	551.0218	554.6513

Table 39. Percent recovery rHuPH20

#	NaCl, mM	pH	% recovery rHuPH20*				
			35 °C, 1W	30 °C, 2W	25 °C, 4W	5 °C,1M	5 °C, 9M
1	140	7.6	41.90	52.70	70.78	101.78	94.05
2	140	7.4	42.91	67.13	83.24	98.45	93.36
3	140	7.2	49.99	68.94	89.84	102.18	102.07
4	140	7.0	49.55	70.64	91.10	97.59	101.54
5	110	7.6	37.50	53.04	82.95	96.95	92.45
6	110	7.4	45.20	58.19	88.46	107.24	97.80
7	110	7.2	42.96	59.21	86.68	93.10	108.11
8	110	7.0	45.31	64.58	89.49	102.01	92.18
9	80	7.6	42.91	56.60	73.76	90.92	92.18
10	80	7.4	34.26	53.21	73.82	88.45	98.33
11	80	7.2	38.33	59.21	82.78	87.93	95.23
12	80	7.0	37.83	56.55	78.93	97.18	101.16
13	50	7.6	34.87	49.07	42.13	87.93	83.47
14	50	7.4	40.29	52.53	60.91	92.41	90.85
15	50	7.2	39.56	52.47	69.92	87.76	91.54
16	50	7.0	38.50	50.55	72.96	97.30	93.52

* % recovery was based on total measure peak area in comparison to a known reference standard.

Table 40. Percent of insulin main peak after long term storage at 2-8 °C

140 mM NaCl							
pH	Time (months)						
	0	0.25	0.5	1	3	5	9
7.6	97.64	97.61	97.41	97.51	97.35	97.56	97.07
7.4	97.64	97.57	97.33	97.23	97.38	97.73	96.10
7.2	97.56	97.37	96.98	96.62	96.60	98.04	95.49
7.0	97.65	97.15	97.05	96.39	96.16	97.97	95.17
110 mM NaCl							
pH	Time (months)						
	0	0.25	0.5	1	3	5	9
7.6	97.58	97.57	97.60	97.52	97.42	97.61	97.31
7.4	97.65	97.58	97.39	97.36	97.36	97.79	96.91
7.2	97.62	97.46	97.35	97.03	97.49	97.61	95.91
7.0	97.64	97.31	97.57	96.64	96.10	97.79	95.03
80 mM NaCl							
pH	Time (months)						

	0	0.25	0.5	1	3	5	9
7.6	97.63	97.62	97.52	97.37	97.64	97.67	97.31
7.4	97.56	97.61	97.51	97.43	97.43	97.75	97.29
7.2	97.57	97.59	97.30	97.45	97.34	97.64	97.00
7.0	97.65	97.50	97.30	97.20	97.04	97.81	96.18
50 mM NaCl							
pH	Time (months)						
	0	0.25	0.5	1	3	5	9
7.6	97.65	97.58	97.33	97.44	97.35	97.66	97.34
7.4	97.58	97.54	97.48	97.47	97.45	97.74	97.27
7.2	97.64	97.59	97.56	97.46	97.47	97.75	97.27
7.0	97.64	97.59	97.50	97.44	97.24	97.73	97.20

Table 41. Percent of insulin recovery after long term storage at 2-8 °C

140 mM NaCl							
pH	Time (months)						
	0	0.25	0.5	1	3	5	9
7.6	95.73	95.53	94.46	87.46	64.62	67.99	46.20
7.4	95.73	83.13	50.31	39.27	37.45	34.80	27.30
7.2	95.82	37.37	25.12	23.62	19.03	17.15	15.99
7.0	95.78	21.70	15.04	13.80	11.11	11.14	11.13
110 mM NaCl							
pH	Time (months)						
	0	0.25	0.5	1	3	5	9
7.6	95.47	96.74	96.40	96.35	95.89	95.50	84.82
7.4	96.69	96.86	93.50	74.63	63.68	68.64	43.83
7.2	96.45	66.09	39.64	32.94	58.68	28.02	23.68
7.0	96.35	31.18	19.52	18.50	15.05	14.72	14.23
80 mM NaCl							
pH	Time (months)						
	0	0.25	0.5	1	3	5	9
7.6	95.48	95.96	96.22	96.34	95.43	96.17	96.91
7.4	96.34	96.51	96.49	96.64	96.05	96.25	98.62
7.2	96.50	96.06	95.81	86.34	60.90	61.48	50.74
7.0	96.36	64.78	40.76	35.90	33.47	32.72	29.34
50 mM NaCl							
pH	Time (months)						
	0	0.25	0.5	1	3	5	9
7.6	95.66	96.72	97.02	96.38	96.21	96.71	98.74
7.4	96.74	96.44	96.36	95.89	95.90	96.58	98.78
7.2	96.68	95.95	95.80	95.42	95.64	96.28	98.59
7.0	96.41	95.91	95.12	93.26	81.51	88.84	72.51

Table 42. Statistical Analyses

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	6	82776.03	13796.0	51.2972
Error	105	28239.00	268.9	Prob > F
C. Total	111	111015.02		<.0001*
Effect Tests				
Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
pH	1	1	30142.891	112.0792	<.0001*
NaCl	1	1	35065.510	130.3828	<.0001*
pH*NaCl	1	1	4786.627	17.7979	<.0001*
Time	1	1	9068.524	33.7192	<.0001*
pH*Time	1	1	752.462	2.7979	0.0974
NaCl*Time	1	1	2960.012	11.0061	0.0012*

*significant

Example 10

Insulin Analog Formulation Development: Stabilizer Screening for Insulin Formulated with rHuPH20

Preservatives protect against potential microbial contamination of insulin that is possible due to multiple dosing. Typical preservatives are m-cresol, phenol and parabens. These preservatives serve as antimicrobials but also serve to stabilize higher order structures of insulin. Phenolic preservatives have been shown to decrease the stability of rHuPH20 (see Example 6). In this example, various stabilizers were screened for their ability to prevent the degradation of rHuPH20 in the presence of phenolic preservatives while maintaining insulin/insulin analog stability. Stabilizers that were screened included commonly used pharmaceutical excipients, including amino acids and their derivatives, salts and buffer species, polyols and others compounds. Stability was determined by rHuPH20 enzymatic activity and insulin solubility. Specific stabilizing effects included prevention of adsorptive loss and/or oxidation of rHuPH20 and general stabilizing effects as measured by rHuPH20 enzymatic activity.

A. Effect of various surfactants on rHuPH20 enzymatic activity

Several common surfactants, namely polysorbate 80 (PS80), polysorbate 20 (PS20) and poloxamer 188 (Pluronic® F68) were screened for their ability to preserve rHuPH20 formulations. All formulations contained 100 µg/mL rHuPH20 (12,000 U) and 150 mM NaCl at pH 6.5. The formulations varied in the surfactant and surfactant concentration and the buffer (either histidine or phosphate). The formulations were

subjected to agitation at 35 °C for 10 days, with samples analyzed for rHuPH20 activity at days 3 and 10. rHuPH20 enzymatic activity was determined as described in Example 2 above. rHuPH20 stability was determined by measuring the oxidation peak of rHuPH20 by RP-HPLC and by size exclusion chromatography (SEC) (see Examples 3 and 4 above).

The formulations and results of rHuPH20 enzymatic activity are set forth in Table 43 below. An ANOVA analysis of rHuPH20 oxidation as measured by RP-HPLC shows no significant difference in enzymatic activity with respect to surfactant type, surfactant concentration, buffer or agitation time ($F = 0.6832$, $p = 0.6397$). Additionally, SEC results did not show detectable differences in the sizes of the main peaks (data not shown).

The results of rHuPH20 oxidation are set forth in Table 44 below. The results show that the oxidation peak area increased with increasing levels of surfactant and time of agitation. Polysorbate 20 is known to contain measurable amounts of peroxide activity (see, *e.g.*, Donbrow *et al.*, (1978) *J. Pharm Sci.* 67(12):1676-1681, or Kibbe, A.H., ed. (2000) Handbook of Pharmaceutical Excipients. 3rd Edition, American Pharmaceutical Association & Pharmaceutical Press: Washington, DC & London, UK). In this study, the polysorbate 20 used was an old lot which resulted in high oxidation of rHuPH20; in contrast, Poloxamer 188 caused only trace amounts of oxidation. The multivariate analysis of variance indicated that the agitation time as well as surfactant type and concentration effected the oxidation level of rHuPH20 (see Table 45 below). Also, significant were interaction terms including surfactant versus concentration, surfactant versus time and concentration versus time.

Based on these results, it is clear that the addition of surfactant in rHuPH20 formulations could effectively reduce the loss of rHuPH20, presumably due to preventing adsorptive loss and possible denaturation at the air-water interface. However, a potential drawback from the addition of surfactant is that it may increase oxidation for rHuPH20.

Table 43. Effect of PS80, PS20 and F68 on rHuPH20 enzymatic activity

#	Surfactant, conc (% w/v)	Buffer	Enzyme activity (U/mL)	
			Agitated 3 days	Agitated 10 days
1	PS80, 0.1 %	50 mM histidine	11,515	11,444
2	PS80, 0.01 %	50 mM histidine	10,370	10,358

3	PS80, 0.001 %	50 mM histidine	9,758	9,850
4	PS20, 0.1 %	50 mM histidine	9,993	7,990
5	PS20, 0.01 %	50 mM histidine	10,566	10,448
6	PS20, 0.001 %	50 mM histidine	8,644	8,488
7	F68, 0.1 %	50 mM histidine	10,460	9,580
8	F68, 0.01 %	50 mM histidine	10,537	10,064
9	F68, 0.001 %	50 mM histidine	9,148	8,811
10	PS80, 0.1 %	50 mM phosphate	10,473	7,459
11	PS80, 0.01 %	50 mM phosphate	10,590	10,919
12	PS80, 0.001 %	50 mM phosphate	9,233	9,858
13	PS20, 0.1 %	50 mM phosphate	9,839	9,004
14	PS20, 0.01 %	50 mM phosphate	10,659	11,241
15	PS20, 0.001 %	50 mM phosphate	9,161	9,770
16	F68, 0.1 %	50 mM phosphate	11,274	11,197
17	F68, 0.01 %	50 mM phosphate	10,669	10,459
18	F68, 0.001 %	50 mM phosphate	9,605	9,655

Table 44. Effect of PS80, PS20 and F68 on rHuPH20 oxidation

#	Surfactant, conc (% w/v)	Buffer	% Oxidation Peak	
			Agitated 3 days	Agitated 10 days
1	PS80, 0.1 %	50 mM histidine	6.89	8.5
2	PS80, 0.01 %	50 mM histidine	4.35	4.89
3	PS80, 0.001 %	50 mM histidine	3.82	3.94
4	PS20, 0.1 %	50 mM histidine	44.13	74.39
5	PS20, 0.01 %	50 mM histidine	10.01	14.21
6	PS20, 0.001 %	50 mM histidine	4.56	4.91
7	F68, 0.1 %	50 mM histidine	4.52	17.63
8	F68, 0.01 %	50 mM histidine	3.83	5.38
9	F68, 0.001 %	50 mM histidine	3.79	4.31
10	PS80, 0.1 %	50 mM phosphate	5.41	12.40
11	PS80, 0.01 %	50 mM phosphate	3.83	5.01
12	PS80, 0.001 %	50 mM phosphate	3.41	4.62
13	PS20, 0.1 %	50 mM phosphate	43.79	65.34
14	PS20, 0.01 %	50 mM phosphate	10.32	12.48
15	PS20, 0.001 %	50 mM phosphate	4.25	5.15
16	F68, 0.1 %	50 mM phosphate	6.11	6.29
17	F68, 0.01 %	50 mM phosphate	3.80	4.72
18	F68, 0.001 %	50 mM phosphate	3.61	4.21

Table 45. Statistical Analyses

Summary of Fit	
RSquare	0.975911
RSquare Adj	0.959852
Root Mean Square Error	3.423151
Mean of Response	11.91222

Observations (or Sum Wgts)		36		
Analysis of Variance				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	14	9969.264	712.090	60.7691
Error	21	246.077	11.718	**Prob > F**
C. Total	35	10215.341		<.0001*
Effect Tests				
Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
surfactant	2	2	909.4801	38.8071	<.0001*
buffer	1	1	0.1152	0.0098	0.9220
conc	1	1	741.1067	63.2454	<.0001*
time	1	1	214.5248	18.3074	0.0003*
surfactant*buffer	2	2	20.3784	0.8695	0.4337
surfactant*conc	2	2	3557.3392	151.7900	<.0001*
surfactant*time	2	2	114.0738	4.8675	0.0183*
buffer*conc	1	1	14.8438	1.2668	0.2731
buffer*time	1	1	10.4329	0.8903	0.3561
conc*time	1	1	248.4121	21.1993	0.0002*

* Significant

B. Methionine

1. Effect of methionine on preventing rHuPH20 oxidation

rHuPH20 has two potential oxidation sites: Met458 and Met35. The "ox-1" peak corresponds to Met458 and is the main oxidation peak when assayed by RP-HPLC. The "ox-2" peak contains both methionine oxidations. Methionine oxidation can be prevented by addition of free methionine as a scavenger to react with potential oxidative compounds.

a. rHuPH20 Formulations

In this study, the effect of the addition of free methionine on the oxidation of rHuPH20 in the presence of polysorbate 20, polysorbate 80 and/or Poloxamer 188 was evaluated. Each formulation contained 5 μg/mL rHuPH20, 0.02% designated surfactant, 50 mM phosphate, pH 6.5, 150 mM NaCl and methionine (from 0 to 50 mM). The samples were incubated at 30 °C for 72 hours and examined by RP-HPLC as set forth in Example 3 above. The results are set forth in Table 46 below. The statistical analyses are set forth in Table 47 below. The results indicated that methionine prevents the oxidation of rHuPH20 at a concentration of 2 mM.

Table 46. Effect of methionine on the oxidation of rHuPH20

Methionine (mM)	% of Oxidation Peak 1		
	Poloxamer 188	Polysorbate 20	Polysorbate 80
0	3.98	4.01	4.01
2	3.39	3.43	3.50
10	3.36	3.37	3.44
50	3.34	3.38	3.34

Table 47. Statistical Analyses

Summary of Fit

RSquare	0.993397
RSquare Adj	0.987894
Root Mean Square Error	0.030551
Mean of Response	3.545833
Observations (or Sum Wgts)	12

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	0.84249167	0.168498	180.5339
Error	6	0.00560000	0.000933	Prob > F
C. Total	11	0.84809167		<.0001*

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Surfactant	2	2	0.00606667	3.2500	0.1106
Met	3	3	0.83642500	298.7232	<.0001*

* Significant

b. rHuPH20/Insulin Formulations

Methionine was tested for its ability to prevent rHuPH20 oxidation in rHuPH20/insulin formulations. The formulations contained 100 U/mL insulin (Organon Insulin API, Recombinant Human insulin SIHR 143, the stock solution was prepared as described in Example 1), 5 µg/mL rHuPH20, 20 mM tris/HCl, pH 7.4, 80 mM NaCl, 0.03 % poloxamer 188, 0.1 % Phenol and 0.1 % m-cresol in the presence or absence of 40 mM methionine. The formulations were incubated at 30 °C for 5 weeks to evaluate the oxidation peak of rHuPH20. The results showed that the ox-1 peak was significantly smaller as measured by RP-HPLC in formulations that contained methionine as compared to formulations that did not contain methionine.

2. Methionine as a general stabilizer

Methionine was further evaluated for its ability to prevent loss of rHuPH20 enzymatic activity at higher temperature and preservative content. A designed of experiment (DOE) response surface methodology study (RSM) was conducted to

evaluate the effect of methionine on rHuPH20 enzymatic activity at different levels of NaCl concentration and pH. The basic formulations contained 100 U/mL insulin (Organon Insulin API, Recombinant Human insulin SIHR 143, the stock solution was prepared as described in Example 1), 5 µg/mL rHuPH20, 20 mM Tris/HCl, 0.1 % m-cresol, 0.1 % phenol and 0.01% poloxamer 188. The methionine concentration range varied between 40 and 80 mM, the NaCl concentration range varied between 70 and 110 mM and the pH range was between 7.2 and 7.6. The formulations were incubated at either 30 °C for 4 weeks or 35 °C for 5 days and rHuPH20 enzymatic activity was determined as set forth in Example 2 above.

The data is set forth in Table 48 below. The statistical analyses of the data are set forth in Tables 49-50 below. The data shows that the concentration of NaCl has a significant effect on the stability of rHuPH20 at both 30 °C and 35 °C. pH has a significant effect at 35 °C. Methionine did not show stabilizing effect on rHuPH20 between 40 and 80 mM. A follow up study indicated that the results were the same in the presence or absence of methionine. Formulations with a starting rHuPH20 enzymatic activity of 650 U/mL dropped to 525 and 522 U/mL for 1 and 20 mM methionine, respectively, after storage at 30 °C for one month. Thus, methionine acts as an anti-oxidant, but does not improve the overall stability of rHuPH20 against preservative and thermal stress.

Table 48. Effects of methionine, NaCl and pH on rHuPH20 activity in insulin-PH20 formulations

Met (mM)	NaCl (mM)	pH	rHuPH20 activity (U/mL)	
			30 °C, 4 W	35 °C, 5 D
40	110	7.4	559	462
60	110	7.2	558	518
60	90	7.4	551	432
40	90	7.2	539	459
60	90	7.4	530	418
80	110	7.4	556	475
40	70	7.4	456	303
40	90	7.6	508	349
60	110	7.6	552	430
60	70	7.6	444	258
60	90	7.4	522	415
60	90	7.4	525	426
60	70	7.2	455	414
80	90	7.6	467	326

80	90	7.2	503	428
80	70	7.4	452	341
60	90	7.4	500	401

Table 49. Response activity of HuPH20 measured after stored at 30 °C for 2 weeks

Summary of Fit	
RSquare	0.91826
RSquare Adj	0.813165
Root Mean Square Error	17.88843
Mean of Response	510.4541
Observations (or Sum Wgts)	17

Analysis of Variance

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	9	25163.516	2795.95	8.7374
Error	7	2239.972	320.00	**Prob > F**
C. Total	16	27403.488		0.0046*

Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Met	1	1	884.795	2.7650	0.1403
NaCl	1	1	21696.424	67.8022	<.0001*
pH	1	1	882.210	2.7569	0.1408
Met*Met	1	1	349.423	1.0920	0.3308
Met*NaCl	1	1	0.936	0.0029	0.9584
NaCl*NaCl	1	1	487.419	1.5232	0.2570
Met*pH	1	1	6.812	0.0213	0.8881
NaCl*pH	1	1	7.784	0.0243	0.8805
pH*pH	1	1	674.368	2.1074	0.1899

* Significant

Table 50. Response activity of HuPH20 measured after stored at 35 °C for 5 days

Summary of Fit	
RSquare	0.970969
RSquare Adj	0.933643
Root Mean Square Error	17.2642
Mean of Response	403.3303
Observations (or Sum Wgts)	17

Analysis of Variance

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	9	69779.522	7753.28	26.0131
Error	7	2086.367	298.05	**Prob > F**
C. Total	16	71865.889		0.0001*

Effect Tests					
Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Met	1	1	1.347	0.0045	0.9483
NaCl	1	1	40555.093	136.0670	<.0001*
pH	1	1	25833.100	86.6730	<.0001*
Met*Met	1	1	1473.541	4.9439	0.0616
Met*NaCl	1	1	146.858	0.4927	0.5054
NaCl*NaCl	1	1	84.069	0.2821	0.6118
Met*pH	1	1	14.175	0.0476	0.8336
NaCl*pH	1	1	1176.764	3.9482	0.0873
pH*pH	1	1	345.281	1.1585	0.3175

* Significant

C. Screening of potential stabilizers for rHuPH20/insulin formulations

Various compounds were screened for their ability to act as protein stabilizers in the rHuPH20/insulin formulations. The excipients included amino acids and their derivatives, amines, polyols, salts and buffers and other compounds (see Table 51 below). Each compound was screened, typically at two concentrations, for its effect on rHuPH20 enzymatic activity and insulin solubility. The basic formulation typically contained 100 U/mL insulin/insulin analog, 5 µg/mL rHuPH20, 20 mM Tris/HCl, 0.15 % m-cresol and 0.2 % phenol at a pH 7.3 ±0.1. Formulations that contained 100-140 mM NaCl were used as reference formulations and the potential stabilizers replaced all or part of the NaCl in the formulations. rHuPH20 enzymatic activity was measured as described in Example 2 above on samples incubated at 30 °C for about one week. Insulin solubility was evaluated by RP-HPLC (see Example 3 above) on samples stored at 5 °C.

The results are set forth in Table 51 below. The evaluation was semi-quantitative in comparison with NaCl. A majority of the compounds tested had no effect on insulin solubility with the remaining causing a decrease in insulin solubility. Previous data (see Examples 8-9) indicated that pH and salt, two conditions that can potentially influence the charge of the protein in solution, influence insulin solubility. In the current screen, molecules or compounds containing divalent cations (Mg^{2+} (magnesium chloride) and SO_4^{2-} (sodium sulfate) and Lys-Lys) caused severe precipitation of insulin. It appears a charge repulsing mechanism might be important for keeping insulin molecules from interacting with each other. Lys-Lys, magnesium chloride and hyaluronic acid oligomers (HA, 4-16mers) were identified as molecules

that stabilized rHuPH20 more than NaCl. Due to the fact that both Lys-Lys and magnesium chloride decreased insulin solubility, these molecules were not considered viable stabilizers at high concentrations. Reducing the concentration of Lys-Lys will reduce its impact on solubility of insulin.

Table 51. Summary of rHuPH20/insulin stabilizer screening

Category	Compound name	Effect on rHuPH20 enzymatic activity	Effect on insulin solubility
Amino acid, derivative and amines	L-arginine	Similar to NaCl	Significantly reduced solubility; cause severe precipitation
	Glutamine	No effect	No effect
	Glycine	No effect	No effect
	Lysine	No effect	No effect
	Methionine	No effect	No effect
	Proline	No effect	No effect
	Lys-Lys	Strong stabilizer	Reduced solubility
	Gly-Gly	No effect	No effect
	Trimethylamine oxide (TMAO)	No effect	No effect
	Betaine	No effect	No effect
Polyols	glycerol	No effect	No/slight effect; might slightly defer crystal formation
	sorbitol	No effect	No/slight effect; might slightly defer crystal formation
	Mannitol	No effect	No/slight effect; might slightly defer crystal formation
	Inositol	No effect	No effect
	Sucrose	No effect	No/slight effect; might slightly defer crystal formation
	Trehalose	No effect	No/slight effect; might slightly defer crystal formation
Salts and buffers	Magnesium chloride	Effective stabilizer	Precipitation
	Sodium sulfate	No effect	Increased precipitation compared to NaCl
	Tris (100 mM)	No effect	No effect
	Sodium Benzoate	No effect	Reduced precipitation compared to NaCl
Others	Hyaluronic acid (HA) oligomers	Effective stabilizer	No effect

	(4-16mers)		
	Human serum albumin	No effect	No effect
	Phenyl butyric acid	No effect	No effect
	Taurocholate	No effect – may be destabilizing	No effect
	PVP	Unclear	No effect

D. Effect of HA oligomers on rHuPH20/insulin formulations

HA oligomers were examined further for their ability to stabilize the rHuPH20/insulin formulations. HA oligomers are the substrate/product of the rHuPH20 enzymatic reaction with hyaluronan, and as such could possibly bind to the enzyme active site thereby causing the stabilizing effect. A designed of experiment (DOE) study was performed to investigate the effects of pH, NaCl concentration and HA oligomer concentration on overall rHuPH20/insulin stability.

The basic formulation contained 3.75 mg/mL insulin (Organon Insulin API, Recombinant Human insulin SIHR 143, the stock solution was prepared as described in Example 1), 5 μg/mL rHuPH20, 15 mM Tris/HCl, 0.01 % $ZnCl_2$, 0.01 % poloxamer 188, 0.15 % phenol and 0.15 % m-cresol. A total of 17 samples were evaluated, with three different pH levels (7.1, 7.3 and 7.5), three NaCl concentrations (50, 75 and 100 mM) and three HA oligomer concentrations (1, 5.5 and 10 mg/mL). rHuPH20 enzyme activity was measured on samples incubated at 30 °C for 1 week or on samples stored at 2-8 °C for 9 months. rHuPH20 oxidation was measured by RP-HPLC on samples stored at 5 °C for 9 months. Insulin content was measured by RP-HPLC for samples stored at 2-8 °C for 9 months.

The formulations and results are set forth in Table 52 below. Insulin content is expressed as % recovery to a USP reference standard. Percent (%) rHuPH20 oxidation peak area is a sum of the ox-1 (major) and ox-2 (minor) peaks. Statistical analyses are set forth in Table 51 below. As shown in Table 53, HA and NaCl concentration both had a significant effect on rHuPH20 enzymatic activity when measured after incubation at 30 °C for 1 week. As the concentrations of NaCl and HA increased, the rHuPH20 enzymatic activity increased as well. This was particularly true when both excipient concentrations were higher, indicating a significant interaction between the two factors (see Table 53, P = 0.0150).

Table 52. Effect of HA, pH and NaCl on rHuPH20 activity and insulin solubility

Run #	pH	HA, mg/mL	NaCl, mM	Enzyme activity (U/mL) 30 °C, 1 week	Insulin content	% rHuPH20 oxidation peak area	Enzyme Activity (U/mL) 2-8 °C, 9 months
1	7.5	1	75	254	92.08	27.09	574
2	7.1	1	75	341	52.38	26.43	576
3	7.3	10	50	267	91.58	75.03	515
4	7.3	1	50	173	92.08	29.00	562
5	7.3	1	100	136	45.33	30.64	531
6	7.1	5.5	100	407	21.99	55.72	521
7	7.3	5.5	75	273	89.07	58.19	523
8	7.3	5.5	75	296	85.73	55.63	506
9	7.3	5.5	75	257	87.99	58.29	504
10	7.1	5.5	50	363	82.41	55.54	524
11	7.5	10	75	389	91.16	70.57	495
12	7.3	5.5	75	357	87.63	58.61	505
13	7.3	10	100	537	43.57	72.43	521
14	7.1	10	75	510	38.06	72.09	494
15	7.3	5.5	75	391	86.17	56.13	510
16	7.5	5.5	50	313	91.68	52.36	489
17	7.5	5.5	100	424	89.91	53.63	478

Table 53. Statistical analyses of the DOE study on HA, pH, and NaCl effect on rHuPH20 enzymatic activity at 30 °C for 1 week

Summary of Fit

RSquare	0.869567
RSquare Adj	0.768119
Root Mean Square Error	51.19549
Mean of Response	334.5882
Observations (or Sum Wgts)	17

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	7	157261.32	22465.9	8.5716
Error	9	23588.80	2621.0	Prob > F
C. Total	16	180850.12		0.0023*

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
pH	1	1	7260.125	2.7700	0.1304
HA	1	1	79800.125	30.4467	0.0004*
NaCl	1	1	18818.000	7.1798	0.0252*
pH*pH	1	1	26012.463	9.9247	0.0117*
HA*HA	1	1	1667.411	0.6362	0.4456
HA*NaCl	1	1	23562.250	8.9899	0.0150*
NaCl*NaCl	1	1	1167.253	0.4454	0.5213

* Significant

It was noted that in almost all previous studies assessing insulin/rHuPH20 formulations in the absence HA, increasing pH had a significant and negative effect on rHuPH20 enzymatic activity, especially in the pH range of 6.0 to 8.0. In contrast, in this study in which HA was included in the formulations, the pH appeared to have little or no effect on rHuPH20 enzymatic activity. Thus, it appears that the presence of HA in the insulin/rHuPH20 formulation reduces or negates the negative effect that higher pH can have on the enzymatic activity of rHuPH20.

To further evaluate the usefulness of HA as a stabilizer, the insulin content was assessed by RP-HPLC on samples that had been stored at 2-8 °C for 9 months. The insulin content data and statistical analysis results are shown in Tables 54 and 55 below. The results clearly demonstrate the significance of pH and NaCl concentration on insulin solubility ($p<0.0001$ for both factors). As the pH increased, insulin solubility increased. As the NaCl concentration increased, insulin solubility decreased. The effect of HA on insulin solubility/content was not significant ($p = 0.2755$). Thus, HA oligomers can be an excipient/stabilizer for rHuPH20/insulin or rHuPH20/insulin analog formulations.

Long term storage of formulations containing HA oligomers showed a significant increase in the level of rHuPH20 oxidation as compared to formulations that did not include added HA. The level of oxidation appeared to correlate well with the HA concentration, but was independent of pH or NaCl concentration (see Tables 55 and 56 below). HA contains glucuronic acid, which may be a potential oxygen donor so an anti-oxidant or oxygen scavenger might be called for if HA is an excipient in the final rHuPH20-insulin formulations. In this study some of the formulations were severely oxidized, yet the enzymatic activity remained reasonably intact (see Table 55), which confirmed that oxidized rHuPH20 still maintains a significant enzymatic activity. The multivariate statistical analyses indicated that the rHuPH20 enzymatic activities of these samples from long-term low temperature storage were significantly affected by HA ($p = 0.004$, reduced enzymatic activity), but not pH or NaCl ($p=0.1715$ and 0.4766, respectively) (see Table 56 below).

Table 54. Effect of HA, pH , and NaCl on the insulin content for samples stored at 2-8 °C for 9 months

Summary of Fit	
RSquare	0.978698
RSquare Adj	0.951309
Root Mean Square Error	5.231467
Mean of Response	74.63647
Observations (or Sum Wgts)	17

Analysis of Variance				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	9	8801.6746	977.964	35.7335
Error	7	191.5778	27.368	**Prob > F**
C. Total	16	8993.2524		<.0001*

Parameter Estimates				
Term	**Estimate**	**Std Error**	**t Ratio**	**Prob>|t|**
Intercept	-626.7315	67.81624	-9.24	<.0001*
pH	106.24375	9.248015	11.49	<.0001*
HA	-0.486111	0.411023	-1.18	0.2755
NaCl	-0.78475	0.073984	-10.61	<.0001*
(pH-7.3)*(pH-7.3)	-194.2562	63.73755	-3.05	0.0186*
(pH-7.3)*(HA-5.5)	3.7222222	2.906371	1.28	0.2411
(HA-5.5)*(HA-5.5)	-0.549519	0.125901	-4.36	0.0033*
(pH-7.3)*(NaCl-75)	2.9325	0.523147	5.61	0.0008*
(HA-5.5)*(NaCl-75)	-0.0028	0.023251	-0.12	0.9075
(NaCl-75)*(NaCl-75)	-0.01288	0.004079	-3.16	0.0160*

* Significant

Table 55. Effect of HA, pH and NaCl on the oxidation of rHuPH20 stored at 2-8 °C for 9 months

Summary of Fit	
RSquare	0.997321
RSquare Adj	0.993876
Root Mean Square Error	1.253363
Mean of Response	53.37529
Observations (or Sum Wgts)	17

Analysis of Variance				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	9	4093.6132	454.846	289.5415
Error	7	10.9964	1.571	**Prob > F**
C. Total	16	4104.6096		<.0001*

Parameter Estimates				
Term	**Estimate**	**Std Error**	**t Ratio**	**Prob>|t|**
Intercept	58.118819	16.24751	3.58	0.0090*
pH	-3.83125	2.215653	-1.73	0.1274
HA	4.9155556	0.098473	49.92	<0.0001*
NaCl	0.00245	0.017725	0.14	0.8940

(pH-7.3)*(pH-7.3)	-72.34375	15.27033	-4.74	0.0021*
(pH-7.3)*(HA-5.5)	-0.605556	0.696313	-0.87	0.4133
(HA-5.5)*(HA-5.5)	-0.26821	0.030164	-8.89	<0.0001*
(pH-7.3)*(NaCl-75)	0.0545	0.125336	0.43	0.6768
(HA-5.5)*(NaCl-75)	-0.009422	0.005571	-1.69	0.1346
(NaCl-75)*(NaCl-75)	-0.000262	0.000977	-0.27	0.7964

* Significant

Table 56. Effect of HA, pH and NaCl on the enzymatic activity of rHuPH20 stored at 2-8 °C for 9 months

Summary of Fit	
RSquare	0.8159
RSquare Adj	0.5792
Root Mean Square Error	18.33595
Mean of Response	519.2941
Observations (or Sum Wgts)	17

Analysis of Variance				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	9	10430.079	1158.90	3.4470
Error	7	2353.450	336.21	**Prob > F**
C. Total	16	12783.529		0.0584

Parameter Estimates				
Term	**Estimate**	**Std Error**	**t Ratio**	**Prob>|t|**
Intercept	917.96806	237.6915	3.86	0.0062*
pH	-49.375	32.41369	-1.52	0.1715
HA	-6.055556	1.440608	-4.20	0.0040*
NaCl	-0.195	0.25931	-0.75	0.4766
(pH-7.3)*(pH-7.3)	-51.25	223.3959	-0.23	0.8251
(pH-7.3)*(HA-5.5)	0.8333333	10.18664	0.08	0.9371
(HA-5.5)*(HA-5.5)	1.3432099	0.441276	3.04	0.0187*
(pH-7.3)*(NaCl-75)	-0.4	1.833595	-0.22	0.8335
(HA-5.5)*(NaCl-75)	0.0822222	0.081493	1.01	0.3466
(NaCl-75)*(NaCl-75)	-0.00728	0.014297	-0.51	0.6263

* Significant

Example 11

Effect of salt concentration, pH and buffer concentration on rHuPH20 in the presence of methylparaben

In this example, a Box-Behnken experimental design was used to determine the optimal pH, salt concentration (NaCl) and buffer concentration (Hepes) for the stability of rHuPH20 at elevated temperatures in the presence of the phenolic preservative methylparaben. In this design of experiment (DOE) response surface

method (RSM) experiment, three factors, namely varying concentrations of buffer and salt, and pH, were examined for their effects on rHuPH20 activity under accelerated conditions, whereby the samples were subjected to stresses, including elevated temperature and agitation. Measured responses included enzymatic activity and purity/content, as measured by reverse phased-HPLC.

The study was based on the DOE software package Design-Expert® 7.1. (StatEase, Minneapolis, MN). The ranges and midpoints of the Hepes concentration, NaCl concentration and pH used in the experiment are listed in Table 57 below. To carry out the study, a total of 13 different formulations, with 5 repeated central points, were made based on a random sequence that the software generated. Each sample contained 100 µg/mL rHuPH20 (from a 10 mg/mL solution in histidine/HCl, pH 6.5, 130 mM NaCl), 0.20 % methylparaben (Fluka, Cat. No. 85265) and 0.025 % propylparaben (American Custom Chemicals, San Diego, Cat. No. CHEM-19713), with varying concentrations of Hepes (Calbiochem, Cat No. 391338) and NaCl (EMD, SX0418-1) and pH as specified in Table 58 below. pH was adjusted using 1.0 N NaOH or HCl. The solutions were aliquoted into 2 mL type 1 glass vials (Wheaton, Cat. No. 223683) with rubber stoppers (Wheaton, Cat. No. 224100-072) and sealed with alumina caps, with 2 vials per formulation. The samples were placed in an incubator with the temperature set at 35 °C. One set of vials was subjected to agitation using a Titer Plate shaker (LabLine) at 600 rpm for 3 days (35 + ag). The other set of vials was kept at 35 °C for 5 days without agitation. Samples were submitted for testing following the incubation period.

Table 57. Ranges and Mid-points for Hepes, NaCl and pH

Factor	Mid-point	Ranges
Hepes concentration	11 mM	2-20 mM
NaCl concentration	150 mM	120-180 mM
pH	7	6-8

Table 58. Box-Behnken design of RSM, including responses

STD	Run #	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4
		NaCl mM	Hepes mM	pH	Enz (35 + ag)	SEC (35 + ag)*	Enz (35)	SEC (35)*
5	1	120	11	6	2119	38.91	7,474	95.54
15	2	150	11	7	6944	78.50	8,260	95.82
10	3	150	20	6	5744	70.96	8,784	94.94

1	4	120	2	7	5228	63.06	6,958	84.71
16	5	150	11	7	7023	80.69	8,022	93.95
7	6	120	11	8	2138	34.15	2,829	62.12
6	7	180	11	6	5864	70.36	8,704	93.53
8	8	180	11	8	5297	63.89	6,052	59.80
12	9	150	20	8	4554	55.72	5,378	74.59
13	10	150	11	7	5709	74.53	8,184	91.14
11	11	150	2	8	4031	42.09	4,392	61.61
4	12	180	20	7	6192	69.69	8,708	94.85
9	13	150	2	6	8178	81.28	9,642	91.32
17	14	150	11	7	7464	80.22	8,651	93.58
3	15	120	20	7	7101	71.51	8,681	89.31
2	16	180	2	7	7194	77.18	8,755	93.34
14	17	150	11	7	6482	74.79	8,721	93.51

*: Size exclusion chromatography (SEC) main peak percentage as compared to a reference.

Enzymatic activity was measured as described in Example 2b above. Size exclusion chromatography (SEC) was used to evaluate purity by measuring the percentage of the main peak as compared to a reference sample (see Example 4 above). SEC was performed using the following conditions: 1X Phosphate Buffered Saline (PBS), a Toso BioScience G2000 SWXL column, and a flow rate set at 1 mL/min. Data were collected as described above and means were reported and entered into the design table following the sample sequence provided by the DOE software. The data are shown in Table 58 above.

For data analysis, the raw data were analyzed as follows. Briefly, the data were fit into a quadratic model as the starting point. The ANOVA were performed based on the quadratic model but the parameters typically needed to be reduced in order to make the model meet more closely to all of the following criteria: model significant level $P<0.05$, p value for lack of fit ($P>0.1$), adequate precision level >4, and predicted R-square within 0.2 of the adjusted R-square. Finally, residual analysis and diagnostics plots were checked to make sure the ANOVA assumptions were met. In some cases, the data needed to be transformed in order to meet those criteria; in those cases, the raw data was transformed by a suitable mathematic equation then followed the statistical analysis as described previously. The ANOVA test models for the model used for each response are set forth in Table 59 below, which indicates the response, the model equation, whether a transformation was used and the type of transformation, the model P value and the lack of fit P value.

Table 59. ANOVA Test for each model				
	Model*	**Transform**	**Model P-Value**	**Lack of fit P-Value**
Response 1, enzymatic activity (35 °C - AG)	Y=A+C+A2+C2	None	Significant P=0.009	Not significant P=0.091
Response 2, SEC main peak (35 °C - AG)	Y=A+C+A2+C2	Logit	Significant P=0.0007	Not significant P=0.109
Response 3, enzymatic activity (35 °C - 5D)	Y=A+B+C+AB+ BC+AC+A2+B2+ C2	None	Significant P=0.0002	Not significant P=0.076
Response 4, SEC main peak (35 °C - 5D)	Y=A+B+C+A2+ C2	Logit	Significant P<0.0001	Not significant P=0.147

* A - NaCl; B - Hepes buffer; C - pH

A. Effects of pH, NaCl and buffer concentrations on rHuPH20 at accelerated temperature with agitation

The ANOVA models show, that under excessive agitation at 35 °C, buffer concentration has No effect of enzymatic activity and content/purity of rHuPH20 (see Table 59 above, Response 1 and 2, wherein the model equation does not include buffer). rHuPH20 was predicted to be most stable between pH 6.5-7.3 with a NaCl concentration between 145-180 mM. Outside these ranges, the model predicted rHuPH20 activity would decline, in particular, when the pH is 8.0 and when the salt concentration is at the lowest concentration (120 mM). Under these accelerated conditions, the highest rHuPH20 activity measured was about 8100 U/mL (STD 9 in Table 56 above), which is about 65 % of the expected rHuPH20 activity (about 12,000 U/mL) based on 100 µg/mL enzyme. Similar results were observed for the purity/content of the rHuPH20 as determined by SEC, with the loss of main peak content for the most stable formulation about 20 % that of the expected.

The optimal ranges for salt concentration and pH for rHuPH20 enzymatic activity were slightly narrower than those for rHuPH20 content/purity. Thus, enzymatic activity of rHuPH20 is more sensitive to stressed conditions, therefore indicating enzymatic activity of rHuPH20 is a better method to indicate stability.

B. Effects of pH, NaCl and buffer concentrations on rHuPH20 at accelerated temperature without agitation

The data shows that under less stressful conditions (*i.e.*, no agitation), smaller decreases in enzymatic activity and less main peak loss were observed. For example, for the better samples, only a 20 % loss in activity was observed (compared to 35 % for samples incubated with agitation) and less than 5 % loss in main peak was observed (compared to 20 % for samples incubated with agitation). Additionally, the ANOVA models show that the salt concentration does not have a significant effect on enzyme activity. rHuPH20 remains enzymatically active at a pH less than 6.5, as long as the salt concentration is at least 130 mM. Additionally, optimal enzymatic activity is observed with a pH less than 7.

Example 12

Stability Study of Insulin Lispro/rHuPH20 or Insulin Aspart/RHuPH20 with Preservatives
Six Month Interim Data Tables

In this example, the stability of various insulin Lispro/rHuPH20 formulations was evaluated under three storage conditions: 5 °C, 25 °C and 30 °C over time. One formulation was also evaluated under two physical accelerated stress conditions: multiple freeze/thaw cycles and agitation at 25 °C. The formulations varied in pH and preservative levels. Stability was evaluated by measuring general appearance and characteristics, rHuPH20 enzymatic activity and rHuPH20 and insulin analog purity.

The insulin Lispro/rHuPH20 formulations are set forth in Table 60 below.

Table 60. Insulin Lispro/rHuPH20 Formulations or Insulin Aspart/rHuPH20 Formulations

#	pH	Buffer	Tonicity Modifier		Surfactant	Preservatives		API	
1	7.2	30 mM Tris/HCl	50 mM NaCl	100 mM Methionine	0.01 % Poloxamer 188	0.125 % phenol	0.075 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Lispro or Aspart
2	7.4	30 mM Tris/HCl	50 mM NaCl	100 mM Methionine	0.01 % Poloxamer 188	0.125 % phenol	0.075 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Lispro or Aspart
3	7.6	30 mM Tris/HCl	50 mM NaCl	100 mM Methionine	0.01 % Poloxamer 188	0.125 % phenol	0.075 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Lispro or Aspart
4	7.2	30 mM Tris/HCl	50 mM NaCl	100 mM Methionine	0.01 % Poloxamer 188	**0.10 % phenol**	0.075 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Lispro or Aspart
5	7.4	30 mM Tris/HCl	50 mM NaCl	100 mM Methionine	0.01 % Poloxamer	**0.10 % phenol**	0.075 % m-cresol	600 U/mL	3.5 mg/mL

Table 60. Insulin Lispro/rHuPH20 Formulations or Insulin Aspart/rHuPH20 Formulations									
#	pH	Buffer	Tonicity Modifier		Surfactant	Preservatives		API	
					188			rHuPH20	Lispro or Aspart
6	7.6	30 mM Tris/HCl	50 mM NaCl	100 mM Methionine	0.01 % Poloxamer 188	**0.10 % phenol**	0.075 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Lispro or Aspart
7	7.2	30 mM Tris/HCl	50 mM NaCl	100 mM Methionine	0.01 % Poloxamer 188	**0.14 % phenol**	**0.085 % m-cresol**	600 U/mL rHuPH20	3.5 mg/mL Lispro or Aspart
8	7.6	30 mM Tris/HCl	50 mM NaCl	100 mM Methionine	0.01 % Poloxamer 188	**0.14 % phenol**	**0.085 % m-cresol**	600 U/mL rHuPH20	3.5 mg/mL Lispro or Aspart

One (1) mL of each formulation was stored in USP Type 1 borosilicate 2mL glass vial with a chlorobutyl rubber stopper and an aluminum seal. Each formulation was incubated, individually, at 5±3 °C, 25±2 °C and 30±3 °C and stability measurements were recorded at 0, 0.5, 1, 2, 3 and 6 months. Formulation #2 was subjected to agitation (shaking at 650 rpm) at 25 °C for 24 hours. Formulation #2 was subjected to 5 cycles of freeze/thaw by alternating the storage condition between a -30 °C freezer and room temperature at the bench. The sample was left at each condition for at least 2 hours for each cycle (*i.e.*, 2 hours in -30 °C freezer, remove and place on bench top at room temperature for 2 hours was considered 1 cycle).

Stability was assessed by measuring pH, appearance, including osmolality, turbidity at 350 nm and qualitative observation, rHuPH20 enzymatic activity, the percent purity and percent recovery of rHuPH20 by RP-HPLC, the percent purity and percent recovery of insulin Lispro by RP-HPLC, and the percent purity of insulin Lispro by non-denaturing and denaturing SEC (see Examples 1-5). For osmolality, the stability acceptance criteria was 275 ± 30 mOsm/kg. For appearance, the stability acceptance criteria was that it was a clear, colorless solution. For rHuPH20 activity, the stability acceptance criteria was that the formulation exhibited > 375 U/mL (based on > 75 U/μg). For rHuPH20 percent recovery by RP-HPLC, the target acceptable specification was 3-7 μg/mL (60-140 %) by RP-HPLC. For insulin purity by RP-HPLC, the target acceptable specification was ≥ 90 % purity by RP-HPLC. For insulin recovery by RP-HPLC, the target acceptable specification was 90-100 U/mL (90-110 %) by RP-HPLC. For percent purity by non-denaturing SEC, the target

acceptable specification was ≤ 2 % High Molecular Weight (HMWt) Insulin species by peak area. For insulin purity by denaturing SEC, the target acceptable specification was ≤ 2 % High Molecular Weight (HMWt) Insulin species by peak area with denaturing SEC.

The results are summarized below. It was determined that in the insulin Lispro/rHuPH20 formulations set forth in Table 58, formulations # 1, # 2 and # 3 were formulated with low content of insulin lispro at T = 0 (91.22 %, 91.40% or 91.30%, respectively), whereas the other formulations contained approximately 100% protein content.

1. 3-Month Time Point

a. Insulin Lispro

All of the tested formulations were stable at 5±3 °C up to the 3 month time point for both rHuPH20 and insulin Lispro. All formulations were clear and colorless with no particles at all time points and temperatures. Insulin Lispro content was low (approximately 91 % recovery of target) for formulations #1-#3, but this appears to be a formulation preparation error as the percent recovery was constant, as expected at 5 °C.

At the 25±2 °C, rHuPH20 in all of the formulations was stable, with the exception of formulation #8 which fell below the 375 U/mL target specification for rHuPH20 enzymatic activity between 2 and 3 months. This was not unexpected as formulation #8 had a high pH and high preservative concentration and in general, rHuPH20 is more stable at lower pH and lower preservative concentration. Insulin Lispro was generally stable in all formulations at 25 °C out to the 3 month time point, but purity began to slightly decline as assessed by RP-HPLC after approximately 1 month.

At 30±3 °C only the lowest pH formulations (#1 and #4, both pH 7.2) were above the target specification for rHuPH20 enzymatic activity (>375 U/mL) after 1 month at 30 °C. Formulation # 2 was above the target specification after 2 weeks at 30 °C but was below 375 U/mL by 1 month. Insulin Lispro was generally stable within the target specification for 3 months in all formulations at 30 °C, but there was a slightly greater decrease in purity than was observed at 25 °C. The decrease in

purity was accompanied by a decrease in content at 30 °C that was not apparent at 25 °C. After the 3-month time point, the 30 °C condition was terminated.

b. Insulin Aspart

All formulations were clear and colorless with no particles at all time points and temperatures. All of the tested formulations were stable at 5±3 °C up to the 3 month time point for both rHuPH20 and insulin Aspart.

rHuPH20 met the target specifications for all of the formulations held at 25±2 °C out to 3 months with the exception of formulation #7 which fell below the 375 U/mL target specification for rHuPH20 activity between 2 and 3 months. This is slightly unexpected as formulations #7 and #8 are identical except for pH values (7.2 and 7.6, respectively), and in general, rHuPH20 has been more stable at a lower pH. This unexpected result is likely due to the fact that formulation #7 started at a lower activity (500 U/mL) and content (93.8 %) than targeted (600 U/mL and 100 %, respectively), and is further supported by the acceptable purity values for formulations #7 and #8. Insulin Aspart was stable in all formulations at 25 °C out to the 3 month time point but there is a slight downward trend in purity and % recovery. rHuPH20 was less stable in all of the formulations held at 30±3 °C as compared to those at 25 °C. The lowest pH formulations (#1 and #4, both pH 7.2) and the lowest total preservative formulations (#4 and #5, total preservative level = 0.175 %) were still above target specification for rHuPH20 enzymatic activity after 1 month at 30 °C. Formulation #2 was just below target specification after 1 month at 30 °C and formulation #6 was slightly over target specification for activity after 1 month but slightly under after 2 weeks. Formulations #1-#6 remained generally within target specification for rHuPH20 enzymatic activity after 2 weeks at 30 °C. Insulin Aspart was stable within target specification for 3 months in all formulations at 30 °C but there was a slightly greater downward trend in purity and % recovery than observed at 25 °C. The 30±3 °C portion of the study was terminated after the 3-month time point.

2. 6-Month Time Point

a. Insulin Lispro

All formulations were clear and colorless with no particles at all time points and temperatures. All of the tested formulations were stable at 5 °C at the 6-month time point for both rHuPH20 and insulin Lispro. The low insulin Lispro content

(approximately 91% recovery of target) for formulations #1-#3 as previously observed but the percent recovery was still flat at 5 °C.

At 25 °, rHuPH20 continued to also was stable (> 375 U/mL rHuPH20 activity) for formulation #1, #2, #4, and #5. These formulations were all at lower pH (7.2 or 7.4) with either the current USP preservative level (#1, #2: 0.125 % phenol, 0.075 % m-cresol) or a slightly lower preservative level (#4, #5: 0.1 % phenol, 0.075 % m-cresol). All 8 of the formulations remained within the target rHuPH20 percent recovery specification at 6 months but there was clear decrease with respect to both rHuPH20 purity and recovery by RP-HPLC. The declining purity by RP-HPLC, content by RP-HPLC, and purity by non-denaturing SEC observed for insulin Lispro noted after 3 months at 25 °C were more apparent by 6 months. The purity by denaturing SEC may be decreasing very slightly but all of the main peak values are still > 99.4 % so any loss is very small up to this point.

b. Insulin Aspart

All formulations were clear and colorless with no particles at all time points and temperatures. All of the tested formulations were stable at 5±3 °C at the 6-month time point for both rHuPH20 and insulin Aspart.

rHuPH20 continued to meet target activity specifications for enzymatic activity (> 375 U/mL) for 4 of the formulations held at 25±2 °C (#1, #2, #4, and #5) out to 6 months. These formulations were all at lower pH (7.2 or 7.4) with either the current USP preservative level (#1, #2: 0.125 % phenol, 0.075 % m-cresol) or a slightly lower preservative level (#4, #5: 0.1 % phenol, 0.075 % m-cresol). All 8 of the formulations remained within target rHuPH20 percent recovery specification at 6 months but there was a clear decrease observed with respect to both rHuPH20 purity and recovery by RP-HPLC. The downward trends for insulin Aspart noted after 3 months at 25 °C were more apparent by 6 months for purity by RP-HPLC, content by RP-HPLC, and purity by non-denaturing SEC. The purity by denaturing SEC may be trending very slightly downward but all of the main peak values are still > 99.5 % so any loss is very small up to this point.

3. Accelerated conditions – Agitation at 25 °C and freeze/thaw

a. Insulin Lispro

Formulation #2 was cloudy after agitation at 25 °C for 24 hours. Insulin Lispro was stable for both conditions, although percent recovery decreased to about 75 % after 5 freeze thaw cycles. rHuPH20 met target activity specifications (>375 U/mL) for the sample subjected to agitation at 25 °C for 24 hours. After 5 freeze/thaw cycles, rHuPH20 was enzymatically inactive.

b. Insulin Aspart

Similar results were obtained for the Insulin Aspart Formulation #2 as was obtained for the Insulin Lispro formulation under agitations or freeze/thaw conditions.

Example 13

Stability Study of Insulin Lispro/rHuPH20, Insulin Aspart/rHuPH20 or Insulin Glulisine/rHuPH20 with Varied Preservatives Two or Three Month Interim Data Tables

In this example, the stability of various insulin Lispro-rHuPH20, insulin Aspart-rHuPH20 or Insulin Glulisine-rHuPH20 formulations were evaluated under four different storage conditions: 5 °C, 15 °C, 25 °C and 30 °C over time. The formulations varied in pH, salt concentration and glycerin concentration. Stability was evaluated by measuring general appearance and characteristics, rHuPH20 enzymatic activity and rHuPH20 and insulin analog purity. This study was intended to provide supporting data for phase 3 clinical development at 3 preservative levels: the preservative levels contained in commercial insulin preparations (Novolog) or EP-B (European Pharmacopoeia) and USP (United States Pharmacopeia) preservative levels.

The insulin Lispro/rHuPH20 formulations are set forth in Table 61 below. The insulin Aspart/rHuPH20 formulations are set forth in Table 62. The insulin Glulisine/rHuPH20 formulations are set forth in Table 63. The base formulation contained 3.5 mg/mL insulin Lispro or Aspart, 600 U/mL rHuPH20, 30 mM Tris/HCl, 20 mM methionine and 0.01 % Poloxamer 188. The pH, NaCl concentrations and glycerin concentrations were varied within the three base preservative concentrations.

For the insulin Lispro/rHuPH20 and insulin Aspart/rHuPH20 formulations set forth in Table 61 and 62, formulations A1-A4 have the commercial Novolog® preservative level: 0.15 % phenol and 0.172 % m-cresol; formulations B1-B5 have an

EP-B preservative level: 0.1 % phenol and 0.15 % m-cresol and formulation U1 has an USP preservative level: 0.125 % phenol and 0.075 % m-cresol.

In addition, for the insulin Glulisine/rHuPH20 formulations set forth in Table 63, formulation 1 has the commercial Novolog® preservative level: 0.15 % phenol and 0.172 % m-cresol. Formulation 2 has an EP-B preservative level: 0.1 % phenol and 0.15 % m-cresol. Formulation 3 has an USP preservative level: 0.125 % phenol and 0.075 % m-cresol. Formulations 4-7 have the Novolog® commercial preservative level and vary one or more factors for comparison with formulation 1. The variations between formulations are indicated in boldface type. Formulation 4 has a higher concentration of NaCl and formulation 7 has a different surfactant. Formulations 5 and 6 vary from formulation 4 in the concentration of methionine and the pH.

Table 61. Insulin Lispro/rHuPH20 Formulations

#	pH	Buffer	Tonicity Modifier			Surfac-tant	Preservatives		API	
A1	7.6	30 mM Tris/ HCl	100 mM NaCl	20 mM Methionine	50 mM Glycerin	0.01 % Poloxamer 188	0.15 % phenol	0.172 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Lispro
A2	7.6	30 mM Tris/ HCl	80 mM NaCl	20 mM Methionine	50 mM Glycerin	0.01 % Poloxamer 188	0.15 % phenol	0.172 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Lispro
A3	7.4	30 mM Tris/ HCl	80 mM NaCl	20 mM Methionine	50 mM Glycerin	0.01 % Poloxamer 188	0.15 % phenol	0.172 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Lispro
A4	7.6	30 mM Tris/ HCl	50 mM NaCl	20 mM Methionine	70 mM Glycerin	0.01 % Poloxamer 188	0.15 % phenol	0.172 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Lispro
B1	7.6	30 mM Tris/ HCl	100 mM NaCl	20 mM Methionine	50 mM Glycerin	0.01 % Poloxamer 188	0.1 % phenol	0.15 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Lispro
B2	7.6	30 mM Tris/ HCl	80 mM NaCl	20 mM Methionine	50 mM Glycerin	0.01 % Poloxamer 188	0.1 % phenol	0.15 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Lispro
B3	7.4	30 mM Tris/ HCl	80 mM NaCl	20 mM Methionine	50 mM Glycerin	0.01 % Poloxamer 188	0.1 % phenol	0.15 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Lispro
B4	7.6	30 mM Tris/ HCl	50 mM NaCl	20 mM Methionine	70 mM Glycerin	0.01 % Poloxamer 188	0.1 % phenol	0.15 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Lispro
B5	7.2	30 mM Tris/ HCl	50 mM NaCl	20 mM Methionine	70 mM Glycerin	0.01 % Poloxamer 188	0.1 % phenol	0.15 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Lispro
U1	7.4	30 mM Tris/ HCl	80 mM NaCl	20 mM Methionine	50 mM Glycerin	0.01 % Poloxamer 188	0.125 % phenol	0.075 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Lispro

Table 62. Insulin Aspart/rHuPH20 Formulations

#	pH	Buffer	Tonicity Modifier			Surfac-tant	Preservatives		API	
A1	7.4	30 mM Tris/ HCl	100 mM NaCl	20 mM Methionine	50 mM Glycerin	0.01 % Poloxamer 188	0.15 % phenol	0.172 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Aspart
A2	7.4	30 mM Tris/ HCl	80 mM NaCl	20 mM Methionine	50 mM Glycerin	0.01 % Poloxamer 188	0.15 % phenol	0.172 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Aspart
A3	7.2	30 mM Tris/ HCl	80 mM NaCl	20 mM Methionine	50 mM Glycerin	0.01 % Poloxamer 188	0.15 % phenol	0.172 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Aspart
A4	7.6	30 mM Tris/ HCl	50 mM NaCl	20 mM Methionine	70 mM Glycerin	0.01 % Poloxamer 188	0.15 % phenol	0.172 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Aspart
B1	7.4	30 mM Tris/ HCl	100 mM NaCl	20 mM Methionine	50 mM Glycerin	0.01 % Poloxamer 188	0.1 % phenol	0.15 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Aspart
B2	7.4	30 mM Tris/ HCl	80 mM NaCl	20 mM Methionine	50 mM Glycerin	0.01 % Poloxamer 188	0.1 % phenol	0.15 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Aspart
B3	7.2	30 mM Tris/ HCl	80 mM NaCl	20 mM Methionine	50 mM Glycerin	0.01 % Poloxamer 188	0.1 % phenol	0.15 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Aspart
B4	7.6	30 mM Tris/ HCl	50 mM NaCl	20 mM Methionine	70 mM Glycerin	0.01 % Poloxamer 188	0.1 % phenol	0.15 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Aspart
B5	7.2	30 mM Tris/ HCl	50 mM NaCl	20 mM Methionine	70 mM Glycerin	0.01 % Poloxamer 188	0.1 % phenol	0.15 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Aspart
U1	7.2	30 mM Tris/ HCl	80 mM NaCl	20 mM Methionine	50 mM Glycerin	0.01 % Poloxamer 188	0.125 % phenol	0.075 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Aspart

Table 63. Insulin Glulisine/rHuPH20 Formulations

#	pH	Buffer	Tonicity Modifier		Surfactant	Preservatives		API	
1	7.3	20 mM Tris/HCl	100 mM NaCl	20 mM Methionine	0.001 % Polysorbate 20	0.15 % phenol	0.172 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Glulisine
2	7.3	20 mM Tris/HCl	100 mM NaCl	20 mM Methionine	0.001 % Polysorbate 20	0.10 % phenol	0.150 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Glulisine
3	7.3	20 mM Tris/HCl	100 mM NaCl	20 mM Methionine	0.001 % Polysorbate 20	0.125 % phenol	0.075 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Glulisine
4	7.3	20 mM Tris/HCl	**140 mM NaCl**	20 mM Methionine	0.001 % Polysorbate 20	0.15 % phenol	0.172 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Glulisine
5	7.3	20 mM Tris/HCl	**140 mM NaCl**	**0 mM Methionine**	0.001 % Polysorbate 20	0.15 % phenol	0.172 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Glulisine
6	**7.0**	20 mM Tris/HCl	**140 mM NaCl**	20 mM Methionine	0.001 % Polysorbate 20	0.15 % phenol	0.172 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Glulisine

Table 63. Insulin Glulisine/rHuPH20 Formulations									
#	pH	Buffer	Tonicity Modifier		Surfactant	Preservatives		API	
7	7.3	20 mM Tris/HCl	100 mM NaCl	20 mM Methionine	**0.01 % Poloxamer 188**	0.15 % phenol	0.172 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Glulisine

One (1) mL of each formulation was stored and incubated at various temperatures as described in Example 13 above in USP Type 1 borosilicate 2mL glass vial with a chlorobutyl rubber stopper and an aluminum seal. Stability measurements were recorded at various times: t = 0, 0.25, 0.5, 1, 2, 3, 6 or 9 months. Stability also was assessed by measuring pH, appearance, rHuPH20 enzymatic activity, percent purity and percent recovery of rHuPH20 by RP-HPLC, percent purity and percent recovery of insulin Lispro by RP-HPLC, and the percent purity of insulin Lispro by non-denaturing and denaturing SEC (see Examples 1-5 and Example 13).- The stability acceptance criteria or target specification for each parameter tested are the same as described in Example 13. Samples were only evaluated at a subsequent timepoint if the results indicated ≥30 % of the initial level.

The results are summarized below.

1. 1-Month Time Point

a. Insulin Lispro

All formulations were clear and colorless with no particles at all time points and temperatures. All of the tested formulations were stable at 5±3 °C up to the 1 month time point for both rHuPH20 and insulin lispro. The formulations at 15±2 °C were not evaluated at the one month time point.

The rHuPH20 was stable in most of the formulations held at 25±2 °C out to 1 month with the exception of formulations A1, A2, A4 and B4. Formulations A1, A2, and B4 fell below the 375 U/mL target specification for rHuPH20 enzymatic activity between 2 weeks and 1 month and formulation A4 fell below target specification between 1 and 2 weeks after storage at 25 °C. These formulations had commercial Novolog or EP-B levels of preservative with a high pH (pH 7.6), which disfavors rHuPH20 stability. The single EP-B formulation (B4) that had < 375 U/mL hyaluronidase activity after 1 month at 25 °C had a pH 7.6 and low sodium chloride concentration (50 mM) which combined were disfavorable for rHuPH20. The other EP-B formulations at pH 7.6 with higher sodium chloride concentrations (80 mM or

100 mM) both remained well above the target level of 375 U/mL rHuPH20 enzymatic activity after 1 month at 25 °C. All other formulations were well above the target specification after 1 month of storage at 25 °C. Insulin lispro was stable in all formulations at 25 °C out to the 1 month time point but the percent purity by RP-HPLC began to decrease slightly.

The rHuPH20 was less stable in all of the formulations held at 30±2 °C as compared to those at 25±2 °C. The USP formulation maintained acceptable rHuPH20 activity out to 1 month at 30 °C but none of the commercial preservative (A1-A4) or EP-B (B1-B5) formulations remained above the target specification after 1 week storage at 30 °C. Insulin lispro was stable within target specification for 1 month in all formulations at 30 °C but the percent purity by RP-HPLC is decreasing slightly.

b. Insulin Aspart

All formulations were clear and colorless with no particles at all time points and temperatures. All formulations were stable at 5±3 °C up to the 1 month time point for both rHuPH20 and insulin Aspart. The formulations at 15±2 °C were not evaluated at the one month time point.

rHuPH20 was stable in all of the formulations held at 25±2 °C out to 1 month with the exception of formulations A4 and B4 which fell below the 375 U/mL target specification between 1-2 weeks and 2 weeks-1 month, respectively. These formulations had commercial Novolog® or EP-B levels of preservative with low salt (50 mM) and high pH (7.6), which are each conditions that disfavor rHuPH20 stability. All other formulations were above the target specification for rHuPH20 enzymatic activity after 1 month of storage at 25 °C. Insulin Aspart was stable in all formulations at 25 °C out to the 1 month time point. Insulin Aspart total content was lower than expected but values are consistent from time=0.

rHuPH20 was less stable in all of the formulations held at 30±2 °C as compared to those at 25±2 °C. The USP formulation (U1) maintained acceptable rHuPH20 activity out to 1 month at 30 °C. Several of the EP-B formulations maintained acceptable rHuPH20 activity out to 1 week but none of the Novolog® preservative formulations maintained acceptable activity after 1 week of storage at 30 °C. Insulin Aspart was stable within target specification for 1 month for all

formulations at 30 °C but there is a slight downward trend noticeable for percent purity by RP-HPLC.

c. Insulin Glulisine

All formulations were clear and colorless with no particles at all time points and temperatures. All formulations were stable at 5±3 °C up to the 1 month time point for both rHuPH20 and insulin Glulisine. The formulations at 15±2 °C were not evaluated at the one month time point.

rHuPH20 was stable in all of the formulations held at 25±2 °C out to 1 month. There was some slight downward trending for some of the formulations with respect to rHuPH20 activity and rHuPH20 purity by RP-HPLC. Insulin Glulisine was stable in all formulations at 25 °C out to the 1 month time point but also showed a slight downward trend with respect to purity and percent recovery.

The rHuPH20 was less stable in all of the formulations held at 30±2 °C as compared to those at 25±2 °C. Four (4) of the 7 test articles failed to meet the target rHuPH20 activity specification of > 375 U/mL after 1 week at 30 °C. Formulation 6 showed acceptable activity for only 1 week, formulation 2 demonstrated acceptable activity for 2 weeks, and the USP formulation (#3) maintained acceptable rHuPH20 activity (532 U/mL) out to 1 month at 30 °C. Insulin Glulisine was stable within target specification for 1 month in all formulations at 30 °C, but there was a clear downward trend noticeable for percent purity and percent recovery by RP-HPLC.

2. 2-Month Time Point

a. Insulin Lispro

All formulations were clear and colorless with no particles at all time points and temperatures. All of the tested formulations were stable at 5±3 °C up to the 2 month time point for both rHuPH20 and insulin lispro.

All test articles were also stable at 15±2 °C up to the 2 month time point for both rHuPH20 and insulin lispro. There may be some very slight trending downward for rHuPH20 enzymatic activity but no other obvious downward trending in stability.

The rHuPH20 was not stable in the commercial preservative formulations (A1-A4) at 25±2 °C out to 2 months, but formulation A3 had good activity at 1 month (436 U/mL) and was only slightly below the intended > 375 U/mL specification at 2 months (361 U/mL). Four (4) of the 5 EP-B formulations (B1, B2, B3, and B5) were

above the 375 U/mL target specification after 2 months at 25 °C and had good rHuPH20 purity and recovery. The USP formulation (U1) also was above the rHuPH20 activity target specification after 2 months at 25 °C with a value of 510 U/mL and correspondingly high purity and recovery values. Insulin lispro was stable in all formulations at 25 °C out to the 2 month time point but the downward trends identified at 1 month continued.

As identified at 1 month, the rHuPH20 was less stable in all of the formulations held at 30±2 °C as compared to those at 25±2 °C and all A (commercial preservative) and B (EP-B preservative) formulations were well below the target rHuPH20 activity specification by 2 months at 30 °C. The USP formulation just maintained acceptable rHuPH20 activity (375 U/mL) out to 2 months at 30 °C, but dipped below specification at the 1 month timepoint (334 U/mL). This may be due to inherent variability in the rHuPH20 activity assay or sample variation, but still demonstrates the general downward trend in stability at 30 °C. Insulin lispro was stable within target specification for 2 months in all formulations at 30 °C, but there is a definite downward trend noticeable for percent purity and recovery by RP-HPLC as well as percent purity by non-denaturing SEC.

b. Insulin Aspart

All formulations were clear and colorless with no particles at all time points and temperatures. All formulations were stable at 5±3 °C up to the 2 month time point for both rHuPH20 and insulin Aspart. All test articles were also stable at 15±2 °C up to the 2 month time point for both rHuPH20 and insulin Aspart. A slight decrease was observed for rHuPH20 enzymatic activity, purity, and recovery.

The rHuPH20 was not stable in 3 of the 4 commercial preservative formulations (A2, A3 and A4) at 25±2 °C out to 2 months, but formulation A1 had acceptable activity at 2 months (385 U/mL) and test article A3 was only slightly below the intended > 375 U/mL specification at 2 months (357 U/mL). Three (3) of the 5 EP-B formulations (B1, B2 and B3) were still well above the 375 U/mL target specification for rHuPH20 enzymatic activity after 2 months at 25 °C and had acceptable but uncharacteristically lower than expected rHuPH20 purity and recovery based on experience with similar formulations. The USP formulation (U1) was still well above the rHuPH20 activity target specification after 2 months at 25 °C with a

value of 444 U/mL with acceptable purity and recoveries. Insulin Aspart was stable in all formulations at 25 °C out to the 2 month time point but there were slight downward trends in purity by RP-HPLC and non-denaturing SEC.

As identified at 1 month, rHuPH20 was less stable in all of the formulations held at 30±2 °C as compared to those at 25±2 °C, and all A and B formulations were well below the target rHuPH20 enzymatic activity specification by 2 months at 30 °C. The USP formulation (U1) had acceptable rHuPH20 activity (382 U/mL) out to 2 months at 30 °C. rHuPH20 percent purity and recovery for the USP formulation were slightly lower than expected based on experience with similar formulations as described above but were within acceptable limits. Insulin Aspart was stable within target specification for 2 months in all formulations at 30 °C but there was a downward trend noticeable for percent purity by RP-HPLC as well as percent purity by denaturing and non-denaturing SEC. The low percent recoveries observed for previous time points were no longer evident and values for percent recovery of insulin Aspart by RP-HPLC have increased slightly between 1 and 2 months for all temperatures (most evident at 30 °C).

c. Insulin Glulisine

All formulations were clear and colorless with no particles at all time points and temperatures. All of the formulations tested were stable within target specifications at 5±3 °C up to the 2 month time point for both rHuPH20 and insulin Glulisine.

All test articles were stable within target specifications at 15±2 °C at the 2 month time point for both rHuPH20 and insulin Glulisine. The 2 month data were generally slightly lower for rHuPH20 activity and recovery but percent purity remained the same, except in formulation #5 (no added methionine) which had a significantly lower percent purity at 2 months. Insulin glulisine was also stable within target specifications at 15 °C up to the 2 month time point, showing only slightly lower values after 2 months at 15 °C for percent purity and recovery.

The rHuPH20 activity was well above the preliminary specifications (< 375 U/mL) for 3 of the 7 formulations (#2, #3 and #6) held at 25±2 °C out to 2 months and just above specifications for formulation #4. In general these formulations either contained the lower levels of preservative (#2 and #3) or higher salt and lower pH

(#6). Formulation #4, which was just above the rHuPH20 activity specification, had the highest level of preservative and pH in the study but also contained the a higher concentration of NaCl. There was downward trending for all of the formulations with respect to rHuPH20 activity, purity, and content. Insulin Glulisine was stable in all formulations at 25 °C out to the 2 month time point but the slight downward trend with respect to purity and percent recovery identified at 1 month continued.

The rHuPH20 was less stable in all of the formulations held at 30±2 °C as compared to those at 25±2 °C. Only the USP preservative level formulation (#3) met the target activity specification of > 375 U/mL after 2 months at 30 °C. Formulation #3 also showed acceptable rHuPH20 purity and recovery at the 2 month time point. Insulin Glulisine was stable within target specifications for 2 months in all formulations at 30 °C, but there was a clear downward trend noticeable for percent purity and percent recovery by RP-HPLC.

3. 3-Month Time Point

a. Insulin Lispro

All formulations were stable at 5±3 °C up to the 3 month time point for both rHuPH20 and insulin lispro. The rHuPH20 purity by RP-HPLC had somewhat lower values than expected, but this appears to be due to normal assay variation. All formulations were clear and colorless with no particles at all time points and temperatures, except formulation A3 that was identified to contain "2 red foreign slivers".

All formulations met all target specifications at 15±2 °C up to the 3 month time point for both rHuPH20 and insulin lispro. The rHuPH20 purity by RP-HPLC also had somewhat lower values than expected, which as mentioned previously b appears to be due to normal assay variation. There is some slight trending downward for rHuPH20 activity and insulin Lispro purity by non-denaturing SEC but no other obvious downward trending.

rHuPH20 was not stable in the commercial preservative formulations (A1-A4) at 25±2 °C out to 3 months. Four (4) of the 5 EP-B formulations (B1, B2, B3, and B5) were still above the 375 U/mL target specification for rHuPH20 enzymatic activity after 3 months at 25 °C, and have downward trending but acceptable rHuPH20 purity and recovery. The USP formulation (U1) was still well above the

rHuPH20 activity target specification after 3 months at 25 °C with a value of 522 U/mL and correspondingly high purity and recovery values. Insulin lispro was stable in all formulations at 25 °C out to the 3-month time, but downward trends for insulin lispro purity by RP-HPLC and non-denaturing SEC were apparent.

As identified previously, the rHuPH20 was less stable in all of the formulations held at 30±2 °C as compared to those at 25±2 °C and all formulations were below the target rHuPH20 activity specification after 3 months at 30°C. Insulin lispro was stable within the target specification for 3 months in all formulations at 30 °C, but there are definite downward trends for percent purity by RP-HPLC as well as percent purity by non-denaturing SEC.

b. Insulin Aspart

All formulations were clear and colorless with no particles at all time points and temperatures. All of the tested formulations were stable at 5±3 °C up to the 3 month time point for both rHuPH20 and insulin Aspart. All formulations met all target specifications at 15±2 °C up to the 3 month time point for both rHuPH20 and insulin Aspart. There was some trending downward for rHuPH20 activity, purity, and recovery. Insulin Aspart purity by non-denaturing SEC was somewhat lower than at the 2-month time point, but it is not clear if this is a downward trend or normal assay variability.

rHuPH20 was not stable in the commercial preservative formulations but formulation A1 was only slightly below the target rHuPH20 activity specification (< 375 U/mL) at 3 months (369 U/mL). Three (3) of the 5 EP-B formulations (B1, B2 and B3) were still well above the 375 U/mL target specification after 3 months at 25±2 °C and had acceptable but unexpectedly lower rHuPH20 purity and recovery as mentioned in the 2 month summary. The USP formulation (U1) was still well above the rHuPH20 activity target specification after 3 months at 25 °C with a value of 492 U/mL with acceptable purity and recoveries. Insulin Aspart was stable in all formulations at 25 °C out to the 3 month time point but there were clear downward trends in purity by RP-HPLC and non-denaturing SEC.

As previously discussed, rHuPH20 was less stable in all of the formulations held at 30±2 °C as compared to those at 25±2 °C. All A and B formulations were below the target rHuPH20 activity specification by 1 month at 30 °C. The USP

formulation (U1) was only slightly below the intended > 375 U/mL specification at 3 months (371 U/mL) but also had low rHuPH20 percent purity and percent recovery by RP-HPLC. Insulin aspart was stable within target specification for 3 months in all formulations at 30 °C by all techniques except non-denaturing SEC which showed a very slight decline to approximately 97% main peak for all formulations.

4. 6-Month Time Point

a. Insulin Lispro

All formulations were clear and colorless with no particles at all time points and temperatures. Formulations at 30±2 °C were not evaluated at the 6 month time point.

All formulations were stable at 5±3 °C and 15±2 °C up to the 6 month time point for both rHuPH20 and insulin lispro. There was some slight trending downward for rHuPH20 activity and rHuPH20 percent recovery for all formulations. Formulation A4 was the least stable at both temperatures.

Only formulations B3 and U1 met the stability requirements at 25±2 °C. Both of these formulations had a pH of 7.4 and 80 mM salt. The remaining formulations had rHuPH20 enzymatic activity less than 375 U/mL. The loss in activity coincided with a loss in rHuPH20 percent recovery. Non-denaturing SEC showed a slight downward trending in insulin lispro percent purity.

b. Insulin Aspart

All formulations were clear and colorless with no particles at all time points and temperatures. Formulations at 30±2 °C were not evaluated at the 6 month time point.

All formulations were stable at 5±3 °C and 15±2 °C up to the 6 month time point for both rHuPH20 and insulin aspart. There was some slight trending downward for rHuPH20 activity and rHuPH20 percent recovery for all formulations. Formulation A4 was the least stable at both temperatures.

Only formulation U1 met the stability requirements at 25±2 °C. The remaining formulations had less rHuPH20 enzymatic activity less than 375 U/mL. The loss in activity coincided with a loss in rHuPH20 percent recovery. Non-denaturing SEC showed a slight downward trending in insulin aspart percent purity.

5. 9-Month Time Point

a. Insulin Lispro

All formulations were clear and colorless with no particles at all time points and temperatures. Formulations at 25±2 °C and 30±2 °C were not evaluated at the 9 month time point.

All formulations were stable at 5±3 °C and 15±2 °C up to the 9 month time point for both rHuPH20 and insulin lispro. There is some slight trending downward for rHuPH20 activity and rHuPH20 percent recovery for all formulations. As was seen at 6 months, Formulation A4 is the least stable at both temperatures.

b. Insulin Aspart

All formulations were clear and colorless with no particles at all time points and temperatures. Formulations at 25±2 °C and 30±2 °C were not evaluated at the 9 month time point.

All formulations were stable at 5±3 °C and 15±2 °C up to the 9 month time point for both rHuPH20 and insulin aspart. There was some slight trending downward for rHuPH20 activity and rHuPH20 percent recovery for all formulations. As was seen at 6 months, Formulation A4 was the least stable at both temperatures.

Example 14

Accelerated Stability Study of Aspart-rHuPH20 and Lispro-rHuPH20: Freeze/Thaw, Agitation and High Storage Temperature

In this example, the stability of various insulin Aspart-rHuPH20 and insulin Lispro-rHuPH20 formulations was evaluated under three physical accelerated stress conditions: multiple freeze/thaw cycles; agitation at 25 °C, and thermal stress at 37 °C. Stability was evaluated by measuring general appearance and characteristics, rHuPH20 enzymatic activity and rHuPH20 and insulin analog purity.

The insulin analog/rHuPH20 formulations are set forth in Table 64 below. A Humalog® sample (insulin Lispro, Formulation 4) and NovoLog® sample (insulin Aspart, Formulation 8) were used as controls. The base formulations contained 3.47 mg/mL insulin Lispro or 3.5 mg/mL insulin Aspart, 600 U/mL rHuPH20, 30 mM Tris/HCl, and 0.01 % Poloxamer 188. Formulations 1 and 5 additionally contained 50 mM NaCl, 100 mM methionine, 0.13 % phenol and 0.075 % m-cresol, with a pH of 7.4. Formulations 2 and 6 (EP-B formulations) additionally contained 80 mM NaCl, 20 mM methionine and 50 mM glycerin with an EP-B preservative level: 0.15 %

phenol and 0.175 % m-cresol. Formulations 3 and 7 (USP formulations) additionally contained 80 mM NaCl, 20 mM methionine and 50 mM glycerin with an USP preservative level: 0.13 % phenol and 0.075 % m-cresol.

Table 64. Insulin Formulations

#	pH	Buffer	Tonicity Modifier			Surfact-ant	Preservatives		API	
1	7.4	30 mM Tris/ HCl	50 mM NaCl	100 mM Methionine	---	0.01 % Poloxamer 188	0.13 % phenol	0.075 % m-cresol	600 U/mL rHuPH20	3.47 mg/mL Lispro
2	7.4	30 mM Tris/ HCl	80 mM NaCl	20 mM Methionine	50 mM Glycerin	0.01 % Poloxamer 188	0.15 % phenol	0.175 % m-cresol	600 U/mL rHuPH20	3.47 mg/mL Lispro
3	7.4	30 mM Tris/ HCl	80 mM NaCl	20 mM Methionine	50 mM Glycerin	0.01 % Poloxamer 188	0.13 % phenol	0.075 % m-cresol	600 U/mL rHuPH20	3.47 mg/mL Lispro
4	7.0-7.8	---	---	---	---	---	---	0.315 % m-cresol	Humalog®	
5	7.4	30 mM Tris/ HCl	50 mM NaCl	100 mM Methionine	---	0.01 % Poloxamer 188	0.13 % phenol	0.075 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Aspart
6	7.2	30 mM Tris/ HCl	80 mM NaCl	20 mM Methionine	50 mM Glycerin	0.01 % Poloxamer 188	0.15 % phenol	0.175 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Aspart
7	7.2	30 mM Tris/ HCl	80 mM NaCl	20 mM Methionine	50 mM Glycerin	0.01 % Poloxamer 188	0.13 % phenol	0.075 % m-cresol	600 U/mL rHuPH20	3.5 mg/mL Aspart
8	7.2-7.6	---	---	---	---	---	0.15 % phenol	0.172 % m-cresol	Novolog®	

Each formulation was exposed to the following accelerated degradation conditions:

(1) Agitation at 25 °C: Each formulation was kept in a 25 °C incubator equipped with a shaker. The vials stood upright with shaking at 650 rpm. Samples were withdrawn for analysis after shaking for 6, 12 and 24 hours. All samples were stored at 2-8 °C prior to analysis.

(2) Multi-Cycle Freeze/Thaw: Each formulation was subjected to 5 cycles of freeze/thaw by alternating the storage condition between a -30 °C freezer and room temperature at the bench. The samples were left at each condition for at least 2 hours for each cycle (*i.e.*, 2 hours in -30 °C freezer, remove and place on bench top at room temperature for 2 hours was considered 1 cycle). Samples were withdrawn after 1, 3 and 5 cycles and stored at 2-8 °C prior to analysis.

(3) Thermal Stress: Each formulation was kept in a 37 °C incubator. Samples were withdrawn after 8, 24 and 48 hours and samples were stored at 2-8 °C prior to analysis.

Analysis was performed before and after the stress conditions were applied. All formulations were characterized by a complete set of analytical tests, including appearance, osmolality, pH, turbidity, hyaluronidase enzymatic activity, hyaluronidase purity, and insulin purity, HWM purity and content (see Examples 1-5 and 13 above for testing procedures). The stability acceptance criteria or target specification for each parameter tested are the same as described in Example 13. The results are summarized below.

1. Agitation at 25 °C

The EP-B formulations (#2 and #6) and the control formulations (#4 and #8) were clear and colorless with no particles at all time points. The UPS formulations (#3 and #7) were clear and colorless after 6 hours of agitation but precipitate had formed by the 12 hour time point. Formulations #1 and #5 had precipitate at the 6 hour time point.

rHuPH20 was stable in all formulations at all time points having >375 U/mL enzymatic activity. rHuPH20 percent recovery decreased slightly at all successive time points, but all formulations maintained an acceptable recovery level. Insulin Aspart and Insulin Lispro were stable at all time points. NovoLog® (insulin Aspart) and Humalog® (insulin Lispro) controls were stable at all time points.

2. Freeze/Thaw

All formulations were clear and colorless with no particles at all time points. After 1 freeze/thaw cycle, rHuPH20 enzymatic activity was severely diminished (highest formulation, #3, had an activity of 168 U/mL), although the percent purity remained >95 % for all formulations and time points. rHuPH20 percent recovery was below the acceptable levels for most of the formulations. Insulin Aspart and Insulin Lispro were stable at all time points. NovoLog® (insulin Aspart) and Humalog® (insulin Lispro) were stable at all time points.

3. Thermal Stress at 37 °C

All formulations were clear and colorless with no particles at all time points. Formulations #1, #3, #5 and #7 had acceptable rHuPH20 enzymatic activity (>375

U/mL) after 8 hours at 37 °C. rHuPH20 stability decreased at all successive time points for all formulations, as evidenced by a decrease in rHuPH20 activity (below 375 U/mL) and a decrease in percent recovery. Insulin Aspart and Insulin Lispro were stable at all time points. NovoLog® (insulin Aspart) and Humalog® (insulin Lispro) controls were stable at all time points.

Example 15

Effect of HA oligomers on insulin/rHuPH20 formulation stability

In this example, the effect of the addition of HA oligomers on the stability of various insulin/rHuPH20 formulations was evaluated. First, tests were completed to evaluate the stability under four storage conditions: 5 °C, 25 °C, 30 °C and 35 °C over time. Second, the effect of HA oligomers on rHuPH20 stability in the presence of different preservatives was tested. Third, the stabilizing effect of different sizes of HA oligomers was evaluated for insulin aspart-rHuPH20 formulations.

A. Effect of HA oligomers on Storage Stability

The formulations contained various amounts of HA (10, 15 or 20 mg/mL). Additional formulations contained 15 mg/mL HA with varying pH, glycerin and salt concentrations.

The insulin/rHuPH20 formulations are set forth in Table 65 below. The basic formulations contained 3.5 mg/mL insulin, 600 U/mL (5 µg/mL) rHuPH20, 30 mM Tris/HCl, 80 mM NaCl, 20 mM methionine and 0.01 % Poloxamer 188 (Poloxamer 188) with 0.1 % phenol and 0.15 % m-cresol at pH 7.4. Formulations #2-#4 additionally contained 10, 15 and 20 mg/mL HA oligomers, respectively. Formulations #1 and #6 additionally contained 50 mM glycerin, with formulation #6 containing 15 mg/mL HA oligomers. Formulations #5 and #7 varied from formulation #3 in their pH and NaCl concentrations. Three additional formulations (#8-#10) were evaluated containing no methionine, and each containing 3.5 mg/mL insulin, 600 U/mL rHuPH20, 15 mg/mL HA oligomers, 30 mM Tris/HCl, 80 mM NaCl and 0.01 % Poloxamer 188 with 0.1 % phenol and 0.15 % m-cresol at pH 7.4. Formulation #8 additionally contained 20 mM methionine and formulation #10 additionally contained 50 mM glycerin.

Table 65. Formulations containing 600 U/mL rHuPH20 and 3.5 mg/mL Insulin

#	pH	Buffer	Tonicity Modifier		Stabilizer			Preservatives	
1	7.4	30 mM Tris/HCl	80 mM NaCl	50 mM Glycerin		20 mM Methionine	0.01 % F68	0.1 % phenol	0.15 % m-cresol
2	7.4	30 mM Tris/HCl	80 mM NaCl		10 mg/mL HA	20 mM Methionine	0.01 % F68	0.1 % phenol	0.15 % m-cresol
3	7.4	30 mM Tris/HCl	80 mM NaCl		15 mg/mL HA	20 mM Methionine	0.01 % F68	0.1 % phenol	0.15 % m-cresol
4	7.4	30 mM Tris/HCl	80 mM NaCl		20 mg/mL HA	20 mM Methionine	0.01 % F68	0.1 % phenol	0.15 % m-cresol
5	7.2	30 mM Tris/HCl	80 mM NaCl		15 mg/mL HA	20 mM Methionine	0.01 % F68	0.1 % phenol	0.15 % m-cresol
6	7.4	30 mM Tris/HCl	80 mM NaCl	50 mM Glycerin	15 mg/mL HA	20 mM Methionine	0.01 % F68	0.1 % phenol	0.15 % m-cresol
7	7.4	30 mM Tris/HCl	**100 mM NaCl**		15 mg/mL HA	20 mM Methionine	0.01 % F68	0.1 % phenol	0.15 % m-cresol
8	7.4	30 mM Tris/HCl	80 mM NaCl		15 mg/mL HA	20 mM Methionine	0.01 % F68	0.1 % phenol	0.15 % m-cresol
9	7.4	30 mM Tris/HCl	80 mM NaCl		15 mg/mL HA		0.01 % F68	0.1 % phenol	0.15 % m-cresol
10	7.4	30 mM Tris/HCl	80 mM NaCl	50 mM Glycerin	15 mg/mL HA		0.01 % F68	0.1 % phenol	0.15 % m-cresol

One (1) mL of each formulation was stored in USP Type 1 borosilicate 2mL glass vial with a chlorobutyl rubber stopper and an aluminum seal. Each formulation was incubated, individually, at 5 ± 3 °C, 25 ± 2 °C, 30 ± 2 °C and 35 ± 2 °C and stability measurements were recorded at time t = 0, 2, 5, 7 and 9 days, 1 and 2 weeks, 1 month or 2 months. Stability was assessed by measuring pH, appearance, osmolality, rHuPH20 enzymatic activity, content of rHuPH20 by RP-HPLC, content of insulin by RP-HPLC, and the percent purity of insulin by non-denaturing SEC (see Examples 2-5 and Example 13). The stability acceptance criteria or target specification for each parameter tested are the same as described in Example 13.

1. **5 °C**

All formulations containing at least 15 mg/mL HA oligomers contained visible particles after only 1 week at 5 °C. The formulations containing only 10 mg/mL HA oligomers or no HA oligomers were clear, colorless and contained no particles after 2 weeks at 5 °C, but particles were observed after 1 month. All of the tested formulations were stable at 5 °C up to the 2 month time point for rHuPH20. Insulin was unstable in formulations containing 20 mg/mL HA oligomers (#4), having lower pH (#5) and containing higher salt (#7), after only 1 month at 5 °C.

2. **25 °C**

Formulations #5 (low pH) and #7 (high salt) contained particles after only 1 week at 25 °C. The remaining formulations were all clear and colorless and contained no particles. All of the tested formulations were stable at 25 °C up to the 2 month time point for both rHuPH20 and insulin.

3. 30 °C

Formulations #5 (low pH) and #7 (high salt) contained particles after only 1 week at 30 °C. The remaining formulations were all clear and colorless and contained no particles. All formulations containing HA were stable for 1 month at 30 °C for rHuPH20. By the 2 month time point, rHuPH20 enzymatic activity had decreased below acceptable levels of 375 U/mL. Insulin was stable in all formulations at the 1 month time point, with a clear decrease in stability observed after 2 months at 25 °C. The additional of HA oligomers had a clear positive effect on rHuPH20 stability at 25 °C without affecting the stability of insulin.

Formulations #8-#10 were tested for rHuPH20 oxidation at time zero and after 3 days at 25 °C. Formulation #10 containing 50 mM glycerin exhibited increased oxidation after only 3 days at 25 °C.

4. 35 °C

Formulations containing HA oligomers were stable for 2 days at 35 °C for rHuPH20. At 5 days, only formulations #3 and #5, containing 15 mg/mL HA oligomers, with varying pH, were stable for rHuPH20. rHuPH20 content was low in all formulations after only 2 days at 35 °C. The addition of HA oligomers had a clear positive effect on rHuPH20 stability at 35 °C without affecting the stability of insulin.

B. Preservative Effects

1. USP Preservatives

In this example, the ability of HA oligomers to stabilize insulin aspart-rHuPH20 or insulin lispro-rHuPH20 formulations containing USP levels of preservatives was evaluated as determined by rHuPH20 enzymatic activity. Formulations F1-F7 were tested. The basic formulations contained 3.5 mg/mL insulin aspart or insulin lispro, 600 U/mL rHuPH20, 30 mM Tris/HCl, pH 7.4, 5 mM methionine, 0.01 % Poloxamer 188, 0.125 % phenol and 0.075 % m-cresol. Formulation F1 did not contain Insulin Aspart. rHuPH20 enzymatic activity was measured as described in Example 2B above. The results are set forth in Tables 66-67

below. All formulations were stable at 5° C for 3 or 6 days. All formulations containing HA, regardless of their pH and NaCl concentrations, had higher enzymatic activities than the same formulations that did not contain HA. As shown in Table 67 below, formulation F4 containing 10 mg/mL HA had higher rHuPH20 enzymatic activity over time at 37° C as compared to formulations F2 and F3, which did not contain HA, either at the same or lower pH. For formulation F5 (see Table 67 below), which had a reduced concentration of NaCl, the addition of 10 mg/mL in the formulation limited loss of the rHuPH20 activity due to the lower NaCl concentration, which is destabilizing for rHuPH20 (see, *e.g.*, F3).

Table 66. Enzymatic Activities for Insulin Aspart-PH20 Formulations

Form #	pH	NaCl mM	HA mg/mL	rHuPH20 Enzymatic Activity (U/mL)					
				5° C	37° C				
				6d	2d	4d	6d	8d	12d
F1	7.0	200	0	679	575	553	513	481	487
F2	7.0	140	0	674	512	454	394	400	346
F3	7.2	140	0	671	481	424	363	352	302
F4	7.2	140	10	667	542	491	442	431	371
F5	7.2	100	10	658	517	432	392	385	319

Table 67. Enzymatic Activities for Insulin Lispro-PH20 Formulations

Form #	pH	NaCl mM	HA mg/mL	Activity (U/mL)			
				5° C	37° C		
				3d	3d	5d	7d
F1	7.2	140	0	641	248	141	98
F2	7.2	140	10	642	426	323	284
F3	7.2	120	10	650	431	363	288
F4	7.2	100	10	643	420	350	253
F5	7.2	80	10	659	419	349	229
F6	7.4	120	10	642	353	284	175
F7	7.4	100	10	643	345	264	158

2. EP-B Level Preservative

In this example, the ability of HA oligomers to stabilize insulin-rHuPH20 formulations with EP-B levels of preservatives was evaluated as determined by rHuPH20 enzymatic activity. The basic formulations contained 3.5 mg/mL insulin, 600 U/mL rHuPH20, 30 mM Tris/HCl, pH 7.4, 20 mM methionine, 0.01 % Poloxamer 188, 0.10 % phenol and 0.15% m-cresol. rHuPH20 enzymatic activity was measured as described in Example 2B above. The results are set forth in Table 68 below. At 35° C, the formulations that contained HA (F2-F6) all had higher

rHuPH20 enzymatic activities than formulation F1, which did not contain HA. At day 9, only about 10 % of enzymatic activity was left for F1, whereas formulations F2-F6, depending on their pH, HA and NaCl concentrations, still had about 30 % to 60 % rHuPH20 enzymatic activity. Similar results were observed for these formulations incubated at 30° C for a longer term stability study. As shown in Table 66 below, at the end of 3 months storage at 30° C, about 30 % of rHuPH20 enzymatic activity remained for formulation F1 which did not contain HA; for all other formulations that contained HA, on average, slightly above 50 % of rHuPH20 enzymatic activity remained.

At lower temperature, the rHuPH20 experienced less thermal stress which might explains the smaller difference with respect to relative enzymatic activity changes between formulations with and without HA. It is noted that under the same pH and NaCl conditions, rHuPH20 activity does not correlate with the HA concentration tested in this study. Due to the fact that HA is a substrate for rHuPH20, its stabilizing mechanism most likely links to specific enzyme-substrate binding effect. This interaction certainly is affected by the molar ratio between the two molecules. At certain substrate:enzyme ratio, the maximal stabilizing effect is reached and the addition of more HA does not further improve the activity. In this example, about 10 mg/mL of HA is necessary to stabilize 5 μg/mL rHuPH20.

Table 68. Enzymatic Activities for Insulin-PH20 Formulations

Form #	pH	NaCl mM	HA mg/mL	Activity (U/mL)						
				5° C	35° C			30° C		
				0d	2d	5d	9d	1 m	2m	3m
F1	7.4	80	0	592	254	170	64	358	239	194
F2	7.4	80	10	604	434	365	209	465	342	296
F3	7.4	80	15	559	444	380	269	463	348	288
F4	7.4	80	20	556	438	364	287	459	323	286
F5	7.2	80	15	559	471	397	326	504	365	312
F6	7.4	100	15	556	457	312	278	461	365	299

C. Effect of molecular weight of HA on rHuPH20 stabilization

In this example, the stabilizing effect of different sizes of HA oligomers was evaluated for insulin aspart-rHuPH20 formulations as determined by measuring rHuPH20 enzymatic activity. Three different molecular weight sodium hyaluronates were acquired from Lifecore Biomedical (Minnesota, USA): 6.4 kDa (Lot: GSP252-5-7), 74 kDa (Lot:GSP252-60-2) and 234.4 kDa (Lot:002799). Ten (10) mg of each

HA was added to 1 mL of an insulin aspart-PH20 formulation that contained 100 U/mL insulin aspart, 5 μg/mL rHuPH20, 30 mM Tris/HCl, pH 7.4, 80 mM NaCl, 50 mM glycerol, 20 mM methionine, 0.01% Poloxamer 188, 0.10% phenol, and 0.15% m-cresol. A formulation that contained no HA also was included in the study as a control. These solutions were incubated at 5° C and 37° C for up to 9 days to assess the stabilizing effect of different sizes of HA on rHuPH20 enzymatic activity. rHuPH20 enzymatic activity was measured as described in Example 2B above.

The results are shown in Table 69 below. The insulin aspart-rHuPH20 formulation that did not contain HA (F1) was enzymatically inactive after 3 days incubation at 37° C. In contrast, formulations F2-F4 retained about 30-40 % rHuPH20 enzymatic activity. In general, there was a trend that the smaller HA polymers seemed to provide a better protection than the bigger ones. This could possibly be due to HA size alone or to HA to rHuPH20 molar ratio.

Table 69. Effect of Molecular Weight of HA on rHuPH20 Enzymatic Activity

Formulation	MW of HA, kDa	Activity (U/mL)				
		5° C	37° C			
		9d	3d	5d	7d	9d
F1	NA	600	34	2	-15	-16
F2	6.4	645	279	145	59	27
F3	74.0	585	211	94	30	10
F4	234.4	575	161	52	5	-6

Example 16

Stability Study of Insulin Aspart/rHuPH20 formulations

In this example, various insulin Aspart/rHuPH20 formulations were evaluated for stability under two storage conditions, 30 °C and 37 °C, and under accelerated conditions (agitation at 25 °C for 9 days). The tested insulin/rHuPH20 formulations are set forth in Table 70 below. The reference formulation (#7) contained 3.5 mg/mL insulin Aspart, 600 U/mL (5 μg/mL) rHuPH20, 30 mM Tris/HCl, 80 mM NaCl, 50 mM glycerin, 20 mM methionine and 0.01 % Poloxamer 188 (Poloxamer 188) with 0.1 % phenol and 0.15 % m-cresol at pH 7.4. Formulations #1-#3 varied in tested different preservative levels (0.13 % phenol and 0.12 % m-cresol) with 3 different levels of stabilizer Poloxamer 188. Formulations #4-#6 varied in tested different preservative levels (0.15 % phenol and 0.12 % m-cresol) with 3 different levels of

stabilizer Poloxamer 188. Formulations #8 and #9 additionally contained 10 mg/mL HA oligomers with formulation #8 having a lower level of glycerin (20 mM). Formulations #10 and #11 contained a lower level of methionine (5 mM) and #11 additionally contained 10 mg/mL HA oligomers.

Table 70. Formulations containing 600 U/mL rHuPH20 and 3.5 mg/mL Insulin Aspart

#	pH	Buffer	Tonicity Modifier		Stabilizer			Preservatives	
1	7.4	30 mM Tris/ HCl	80 mM NaCl	50 mM Glycerin		20 mM Methionine	0.01 % F68	0.13 % phenol	0.12 % m-cresol
2	7.4	30 mM Tris/ HCl	80 mM NaCl	50 mM Glycerin		20 mM Methionine	0.05 % F68	0.13 % phenol	0.12 % m-cresol
3	7.4	30 mM Tris/ HCl	80 mM NaCl	50 mM Glycerin		20 mM Methionine	0.1 % F68	0.13 % phenol	0.12 % m-cresol
4	7.4	30 mM Tris/ HCl	80 mM NaCl	50 mM Glycerin		20 mM Methionine	0.01 % F68	0.15 % phenol	0.12 % m-cresol
5	7.4	30 mM Tris/ HCl	80 mM NaCl	50 mM Glycerin		20 mM Methionine	0.05 % F68	0.15 % phenol	0.12 % m-cresol
6	7.4	30 mM Tris/ HCl	80 mM NaCl	50 mM Glycerin		20 mM Methionine	0.1 % F68	0.15 % phenol	0.12 % m-cresol
7	7.4	30 mM Tris/ HCl	80 mM NaCl	50 mM Glycerin		20 mM Methionine	0.01 % F68	0.1 % phenol	0.15 % m-cresol
8	7.4	30 mM Tris/ HCl	80 mM NaCl	20 mM Glycerin	10 mg/mL HA	20 mM Methionine	0.01 % F68	0.1 % phenol	0.15 % m-cresol
9	7.4	30 mM Tris/ HCl	80 mM NaCl	50 mM Glycerin	10 mg/mL HA	20 mM Methionine	0.01 % F68	0.1 % phenol	0.15 % m-cresol
10	7.4	30 mM Tris/ HCl	80 mM NaCl	50 mM Glycerin		5 mM Methionine	0.01 % F68	0.1 % phenol	0.15 % m-cresol
11	7.4	30 mM Tris/ HCl	80 mM NaCl	50 mM Glycerin	10 mg/mL HA	5 mM Methionine	0.01 % F68	0.1 % phenol	0.15 % m-cresol

One (1) mL of each formulation was stored in USP Type 1 borosilicate 2mL glass vial with a chlorobutyl rubber stopper and an aluminum seal. Each formulation was incubated, individually, at 30 ± 2 °C and 37 ± 2 °C and stability measurements were recorded at time t = 0, 2, 4, 7 and 9 days, 2 weeks, 1 month or 1.5 months. For each formulation, one set of vials was subjected to agitation using a Titer Plate shaker

(LabLine) at 600 rpm for 9 days at 25 °C. NovoLog® (insulin Aspart) was also subjected to the same agitation conditions as a control.

Stability was assessed by measuring pH, appearance, osmolality, rHuPH20 enzymatic activity, content of rHuPH20 by RP-HPLC and content of insulin by RP-HPLC (see Examples 1-5 and 13). The acceptable criteria or target specification for the stability parameters was the same as set forth in Example 13.

1. 30 °C

All formulations were all clear and colorless and contained no particles after 2 weeks at 30 °C. All of the tested formulations were stable at 30 °C up to the 2 week time point for both rHuPH20 and insulin Aspart, with a slight downward trend in stability observed for all formulations over time. A stabilizing effect on rHuPH20 activity was observed for formulations containing HA oligomers (#8, #9, #11) as evidenced by higher enzymatic activity and content as compared to the formulations that did not contain HA oligomers. Additionally, a decrease in either the glycerin and methionine concentrations did not result in a visible effect on the stability of these formulations (#8, #9, and #11).

A comparison of preservative concentrations indicated that decreasing the amount of metacresol and a modest increase in the amount of phenol is beneficial for rHuPH20 stability, but the benefit is eliminated when the concentration of phenol is high. In contrast, changes in preservative concentrations had little effect on insulin Aspart stability, as a modest decrease in insulin Aspart content was observed for all formulations. For example, rHuPH20 was more stable in formulations containing 0.13 % phenol (#1-#3) than those containing 0.15 % phenol (#4-#6). Formulations containing increased phenol concentrations and decreased metacresol concentrations (#1-#3) were more stable than the reference formulation (#7) that contained a lower amount of phenol and a higher amount of metacresol. In contrast, formulations #4-#6 were as stable as reference formulation #7.

2. 37 °C

All formulations were all clear and colorless and contained no particles after 9 days at 37 °C. A stabilizing effect on rHuPH20 activity was observed for formulations containing HA oligomers (#8, #9, and #11) as evidenced by higher enzymatic activity and content after 2 days at 37 °C as compared to the formulations

that did not contain HA oligomers, although none of the formulations had an acceptable level of rHuPH20 enzymatic activity. Loss of rHuPH20 enzymatic activity was associated with loss of rHuPH20 content. In contrast, insulin Aspart content showed a downward trend for formulations containing HA oligomers indicating insulin Aspart is not as stable at 37 °C in the presence of HA oligomers.

Similar to the effects seen at 25 °C, a comparison of preservative concentrations indicated that decreasing the amount of metacresol and a modest increase in the amount of phenol is beneficial for rHuPH20 stability, but the benefit is eliminated when the concentration of phenol is high. In contrast, changes in preservative concentrations had little effect on insulin Aspart stability, as a modest decrease in insulin content was observed for all formulations.

3. Agitation at 25 °C

rHuPH20 and insulin Aspart were stable in all formulations after 24 or 48 hours at 25 °C with agitation, although formulations containing HA oligomers (#8, #9, and #11) were cloudy after 24 hours. Novolog® was stable after 24 hours at 25 °C with agitation. Changes in preservative concentrations had no effect on rHuPH20 or insulin Aspart stability.

Example 17

Formulations Containing HA oligomers and divalent metal ion (Mg^{2+})

In this example, the effect of EP-B levels of preservatives on rHuPH20 enzymatic activity was evaluated under two storage conditions: 5 °C at 5 days and 37 °C for 3-5 days. The formulations contained differing amounts of HA oligomers and divalent metal ion (Mg^{2+}).

The rHuPH20 formulations are set forth in Table 71 below. The basic formulations contained 600 U/mL rHuPH20, 30 mM Tris/HCl, 200 mM NaCl, 5 mM methionine, 0.01 % Poloxamer 188 (Poloxamer 188) with 0.100 % phenol and 0.150 % m-cresol at pH 6.8. Formulations 2 and 4 additionally contained 10 mg/mL HA oligomers and formulations 3 and 5 contained 20 mg/mL HA oligomers. Formulations 2 and 3 additionally contained 3 mM Mg^{2+} and formulations 4 and 5 contained 10 mM Mg^{2+}.

Table 71. Formulations containing 600 U/mL rHuPH20

#	pH	Buffer	Tonicity Modifier	Stabilizer			Metal Ion	Preservatives	
1	6.8	30 mM Tris/HCl	200 mM NaCl	---	5 mM Methionine	0.01 % F68	---	0.100 % phenol	0.150 % m-cresol
2	6.8	30 mM Tris/HCl	200 mM NaCl	10 mg/mL HA	5 mM Methionine	0.01 % F68	3 mM Mg^{++}	0.100 % phenol	0.150 % m-cresol
3	6.8	30 mM Tris/HCl	200 mM NaCl	20 mg/mL HA	5 mM Methionine	0.01 % F68	3 mM Mg^{++}	0.100 % phenol	0.150 % m-cresol
4	6.8	30 mM Tris/HCl	200 mM NaCl	10 mg/mL HA	5 mM Methionine	0.01 % F68	10 mM Mg^{++}	0.100 % phenol	0.150 % m-cresol
5	6.8	30 mM Tris/HCl	200 mM NaCl	20 mg/mL HA	5 mM Methionine	0.01 % F68	10 mM Mg^{++}	0.100 % phenol	0.150 % m-cresol

One (1) mL of each formulation was stored in USP Type 1 borosilicate 2 mL glass vial with a chlorobutyl rubber stopper and an aluminum seal. Each formulation was incubated, individually, at 5 and 37 °C and stability measurements were recorded at time t = 3, 4 and/or 5 days. Stability was assessed by measuring pH, appearance, osmolality, rHuPH20 enzymatic activity and content of rHuPH20 by RP-HPLC (see Examples 2-3 and 5 and Example 13). The acceptance criteria or target specification for each of the above parameters is the same as set forth in Example 13.

The results show that all formulations were clear and colorless with no particles. Osmolarity tended to increase with the addition of Mg^{2+}. rHuPH20 enzymatic activity and content was stable at 5 °C for all formulations. At 37 °C, rHuPH20 enzymatic activity was decreased at 3 days and after 4 days, was at the accepted target specification of 375 U/mL. After 5 days, approximately half of the initial rHuPH20 enzymatic activity remained. Drop in activity correlated with drop in rHuPH20 content.

Example 18

Study of USP Preservative Formulations at 5 °C or 37 °C

In this example, the effect of USP levels of preservatives on rHuPH20 enzymatic activity was evaluated under two storage conditions: 5 °C at 4 days and 37 °C for 2, 4, 6, 8 and 12 days. The formulations contained differing amounts of HA oligomers and salt and varying pH. The rHuPH20 formulations are set forth in Table 72 below. The basic formulations contained 600 U/mL rHuPH20, 3.5 mg/mL insulin

aspart, insulin lispro or regular insulin, 30 mM Tris/HCl, 5 mM methionine, 0.01 % Poloxamer 188 (Poloxamer 188) with 0.125 % phenol and 0.075 % m-cresol. Formulation 1 did not contain insulin. Formulations 2-5 contained insulin aspart, formulations 6-9 contained regular insulin and formulations 10-13 contained insulin lispro. Four basic formulations were prepared for each insulin analog. Formulations 2, 6 and 10 were the same as 3, 7 and 11, respectively, with varying pH (7.0 versus 7.2). Formulations 3, 8 and 12 were the same as 4, 9 and 13, respectively, with varying NaCl. These formulations additionally contained 10 mg/mL HA oligomers.

Table 72. Formulations

#	pH	Buffer	Tonicity Modifier	Stabilizer			Preservatives		rHuPH20	insulin
1	7.0	30 mM Tris/HCl	200 mM NaCl	---	5 mM Methionine	0.01 % F68	0.125 % phenol	0.075 % m-cresol	600 U/mL	---
2	7.0	30 mM Tris/HCl	140 mM NaCl	---	5 mM Methionine	0.01 % F68	0.125 % phenol	0.075 % m-cresol	600 U/mL	3.5 mg/mL Aspart
3	7.2	30 mM Tris/HCl	140 mM NaCl	---	5 mM Methionine	0.01 % F68	0.125 % phenol	0.075 % m-cresol	600 U/mL	3.5 mg/mL Aspart
4	7.2	30 mM Tris/HCl	140 mM NaCl	10 mg/mL HA	5 mM Methionine	0.01 % F68	0.125 % phenol	0.075 % m-cresol	600 U/mL	3.5 mg/mL Aspart
5	7.2	30 mM Tris/HCl	100 mM NaCl	10 mg/mL HA	5 mM Methionine	0.01 % F68	0.125 % phenol	0.075 % m-cresol	600 U/mL	3.5 mg/mL Aspart
6	7.0	30 mM Tris/HCl	140 mM NaCl	---	5 mM Methionine	0.01 % F68	0.125 % phenol	0.075 % m-cresol	600 U/mL	3.5 mg/mL Insulin
7	7.2	30 mM Tris/HCl	140 mM NaCl	---	5 mM Methionine	0.01 % F68	0.125 % phenol	0.075 % m-cresol	600 U/mL	3.5 mg/mL Insulin
8	7.2	30 mM Tris/HCl	140 mM NaCl	10 mg/mL HA	5 mM Methionine	0.01 % F68	0.125 % phenol	0.075 % m-cresol	600 U/mL	3.5 mg/mL Insulin
9	7.2	30 mM Tris/HCl	100 mM NaCl	10 mg/mL HA	5 mM Methionine	0.01 % F68	0.125 % phenol	0.075 % m-cresol	600 U/mL	3.5 mg/mL Insulin
10	7.0	30 mM Tris/HCl	140 mM NaCl	---	5 mM Methionine	0.01 % F68	0.125 % phenol	0.075 % m-cresol	600 U/mL	3.5 mg/mL Lispro
11	7.2	30 mM Tris/HCl	140 mM NaCl	---	5 mM Methionine	0.01 % F68	0.125 % phenol	0.075 % m-cresol	600 U/mL	3.5 mg/mL Lispro
12	7.2	30 mM Tris/HCl	140 mM NaCl	10 mg/mL HA	5 mM Methionine	0.01 % F68	0.125 % phenol	0.075 % m-cresol	600 U/mL	3.5 mg/mL Lispro
13	7.2	30 mM Tris/HCl	100 mM NaCl	10 mg/mL	5 mM Methionine	0.01 %	0.125 %	0.075 % m-	600 U/mL	3.5 mg/mL

				HA		F68	phenol	cresol		Lispro

One (1) mL of each formulation was stored in USP Type 1 borosilicate 2 mL glass vial with a chlorobutyl rubber stopper and an aluminum seal. Each formulation was incubated, individually, at 5 and 37 °C and stability measurements were recorded at time t = 2, 4, 6, 8 and/or 12 days. Stability was assessed by measuring pH, appearance, osmolality, rHuPH20 enzymatic activity, content of rHuPH20 by RP-HPLC and content of insulin by RP-HPLC (see Examples 2-3, 5 and 13). The acceptable criteria and target specification for stability in each of the parameters tested was the same as set forth in Example 13.

The results show that formulation 1 and all of the insulin aspart formulations (#2-5) were clear and colorless with no particles after 12 days at either 5 or 37 °C. Crystals were observed in formulations 6 and 8-9 and 11-13 after 6 days at 5 °C and crystals were observed in formulation 10 after just 2 days at 5 °C.

All of the formulations were stable after 4 days at 5 °C. The insulin lispro formulations (#10-13) were stable for 8 days at 37 °C whereas the insulin formulations (#6-9) were stable for only 2 days (with the exception of #7) at 37 °C. The insulin aspart formulations (#2-5) were stable for 4-6 days at 37 °C. rHuPH20 content decreased over time for all formulations at 37 °C. Insulin content decreased over time for all formulations.

Example 19

Antimicrobial effectiveness tests of different preservative levels

In this example, several batches of formulations containing different levels of preservatives with targeted amounts of insulin and rHuPH20 were prepared for microbial effectiveness testing. The tests were performed according to the guidance of EP and USP by a contract analytical laboratory (Quadrants Scientific, Inc., San Diego, CA and Lancaster Laboratories, Lancaster, PA). The various preservative containing formulations were tested for anti-microbial effectiveness against bacteria *Pseudomonas aeruginosa*, *E. coli* and *Staphylococcus aureus* and fungi *Aspergillus niger* and *Candida albicans*. Tests were conducted by 1) adding an initial inoculum (at least 10^5 CFU/mL) of each bacteria to the sample and 2) measuring the CFU/mL of each bacteria or fungi at 24 hours, 7 days and 14 days. Raw data (CFU/mL) was

converted to a log 10 unit reduction from the measured inoculum. The formulations were tested at a temperature of 37 °C.

All formulations contained 100 U/mL insulin or insulin analog and 5 μg/mL rHuPH20. The remaining components varied among the formulations such that the effect of various components could be compared. The results are summarized in Table 73, which summarizes the effects of the various formulation components on antimicrobial effectiveness. A neutral effect indicates the indicated component and concentrations have no effect on antimicrobial effectiveness. For example, insulin analog has a neutral effect on antimicrobial effectiveness, when comparing two formulations that vary only in the insulin analog, *i.e.*, insulin aspart or insulin lispro. NaCl concentration had either a neutral or negative effect on antimicrobial effectiveness depending on the formulations.

Table 73. Summary of antimicrobial effectiveness testing

	Concentration	**Effect**
Insulin vs analog		Insufficient data
Aspart vs Lispro		Neutral
pH	(7.2 - 7.4)	Neutral
Methionine	(50 - 100 mM)	Neutral
	(20 - 50 mM)	Insufficient data
NaCl	(50 - 100 mM)	Negative
	(50 - 100 mM)	Neutral
	(80 - 100 mM)	Neutral
F68	(0.01 - 0.03 %)	Neutral
	(0.01 - 0.03 %)	Slightly negative
Glycerol	(0 - 50 mM)	Insufficient data
Company	(Quadrant vs Lancaster)	Neutral

Example 20

Generation of a soluble rHuPH20-expressing cell line

The HZ24 plasmid (set forth in SEQ ID NO:52) was used to transfect Chinese Hamster Ovary (CHO cells) (see *e.g.* U.S. Patent Nos. 7,76,429 and 7,781,607 and U.S. Publication No. 2006-0104968). The HZ24 plasmid vector for expression of soluble rHuPH20 contains a pCI vector backbone (Promega), DNA encoding amino acids 1-482 of human PH20 hyaluronidase (SEQ ID NO:49), an internal ribosomal entry site (IRES) from the ECMV virus (Clontech), and the mouse dihydrofolate reductase (DHFR) gene. The pCI vector backbone also includes DNA encoding the

Beta-lactamase resistance gene (AmpR), an f1 origin of replication, a Cytomegalovirus immediate-early enhancer/promoter region (CMV), a chimeric intron, and an SV40 late polyadenylation signal (SV40). The DNA encoding the soluble rHuPH20 construct contains an NheI site and a Kozak consensus sequence prior to the DNA encoding the methionine at amino acid position 1 of the native 35 amino acid signal sequence of human PH20, and a stop codon following the DNA encoding the tyrosine corresponding to amino acid position 482 of the human PH20 hyaluronidase set forth in SEQ ID NO:1, followed by a BamHI restriction site. The construct pCI-PH20-IRES-DHFR-SV40pa (HZ24), therefore, results in a single mRNA species driven by the CMV promoter that encodes amino acids 1-482 of human PH20 (set forth in SEQ ID NO:3) and amino acids 1-186 of mouse dihydrofolate reductase (set forth in SEQ ID NO:53), separated by the internal ribosomal entry site (IRES).

Non-transfected DG44 CHO cells growing in GIBCO Modified CD-CHO media for DHFR(-) cells, supplemented with 4 mM Glutamine and 18 ml/L Plurionic F68/L (Gibco), were seeded at 0.5 x 10^6 cells/ml in a shaker flask in preparation for transfection. Cells were grown at 37 °C in 5 % CO_2 in a humidified incubator, shaking at 120 rpm. Exponentially growing non-transfected DG44 CHO cells were tested for viability prior to transfection.

Sixty million viable cells of the non-transfected DG44 CHO cell culture were pelleted and resuspended to a density of 2 $\times 10^7$ cells in 0.7 mL of 2x transfection buffer (2x HeBS: 40 mM Hepes, pH 7.0, 274 mM NaCl, 10 mM KCl, 1.4 mM Na_2HPO_4, 12 mM dextrose). To each aliquot of resuspended cells, 0.09 mL (250 µg) of the linear HZ24 plasmid (linearized by overnight digestion with Cla I (New England Biolabs) was added, and the cell/DNA solutions were transferred into 0.4 cm gap BTX (Gentronics) electroporation cuvettes at room temperature. A negative control electroporation was performed with no plasmid DNA mixed with the cells. The cell/plasmid mixes were electroporated with a capacitor discharge of 330 V and 960 µF or at 350 V and 960 µF.

The cells were removed from the cuvettes after electroporation and transferred into 5 mL of Modified CD-CHO media for DHFR(-) cells, supplemented with 4 mM Glutamine and 18 ml/L Plurionic F68/L (Gibco), and allowed to grow in a well of a 6-

well tissue culture plate without selection for 2 days at 37 °C in 5 % CO_2 in a humidified incubator.

Two days post-electroporation, 0.5 mL of tissue culture media was removed from each well and tested for the presence of hyaluronidase activity, using the microturbidity assay described in Example 2.

Table 74: Initial Hyaluronidase Activity of HZ24 Transfected DG44 CHO cells at 40 hours post-transfection

	Dilution	Activity (Units/ml)
Transfection 1 330V	1 to 10	0.25
Transfection 2 350V	1 to 10	0.52
Negative Control	1 to 10	0.015

Cells from Transfection 2 (350V) were collected from the tissue culture well, counted and diluted to 1 $\times 10^4$ to 2 $\times 10^4$ viable cells per mL. A 0.1 mL aliquot of the cell suspension was transferred to each well of five, 96 well round bottom tissue culture plates. One hundred microliters of CD-CHO media (GIBCO) containing 4 mM GlutaMAX™-1 supplement (GIBCO™, Invitrogen Corporation) and without hypoxanthine and thymidine supplements were added to the wells containing cells (final volume 0.2 mL).

Ten clones were identified from the 5 plates grown without methotrexate.

Table 75. Hyaluronidase activity of identified clones

Plate/Well ID	Relative Hyaluronidase
1C3	261
2C2	261
3D3	261
3E5	243
3C6	174
2G8	103
1B9	304
2D9	273
4D10	302

Six HZ24 clones were expanded in culture and transferred into shaker flasks as single cell suspensions. Clones 3D3, 3E5, 2G8, 2D9, 1E11, and 4D10 were plated into 96-well round bottom tissue culture plates using a two-dimensional infinite dilution strategy in which cells were diluted 1:2 down the plate, and 1:3 across the plate, starting at 5000 cells in the top left hand well. Diluted clones were grown in a background of 500 non-transfected DG44 CHO cells per well, to provide necessary

growth factors for the initial days in culture. Ten plates were made per subclone, with 5 plates containing 50 nM methotrexate and 5 plates without methotrexate.

Clone 3D3 produced 24 visual subclones (13 from the no methotrexate treatment, and 11 from the 50 nM methotrexate treatment. Significant hyaluronidase activity was measured in the supernatants from 8 of the 24 subclones (>50 Units/mL), and these 8 subclones were expanded into T-25 tissue culture flasks. Clones isolated from the methotrexate treatment protocol were expanded in the presence of 50 nM methotrexate. Clone 3D35M was further expanded in 500 nM methotrexate giving rise to clones producing in excess of 1,000 Units/ml in shaker flasks (clone 3D35M; or Gen1 3D35M). A master cell bank (MCB) of the 3D35M cells was then prepared.

Example 21

Production Gen2 Cells Containing Soluble human PH20 (rHuPH20)

The Gen1 3D35M cell line described in Example 20 was adapted to higher methotrexate levels to produce generation 2 (Gen2) clones. 3D35M cells were seeded from established methotrexate-containing cultures into CD CHO medium containing 4 mM GlutaMAX-1™ and 1.0 μM methotrexate. The cells were adapted to a higher methotrexate level by growing and passaging them 9 times over a period of 46 days in a 37 °C, 7 % CO_2 humidified incubator. The amplified population of cells was cloned out by limiting dilution in 96-well tissue culture plates containing medium with 2.0 μM methotrexate. After approximately 4 weeks, clones were identified and clone 3E10B was selected for expansion. 3E10B cells were grown in CD CHO medium containing 4 mM GlutaMAX-1™ and 2.0 μM methotrexate for 20 passages. A master cell bank (MCB) of the 3E10B cell line was created and frozen and used for subsequent studies.

Amplification of the cell line continued by culturing 3E10B cells in CD CHO medium containing 4 mM GlutaMAX-1™ and 4.0 μM methotrexate. After the 12th passage, cells were frozen in vials as a research cell bank (RCB). One vial of the RCB was thawed and cultured in medium containing 8.0 μM methotrexate. After 5 days, the methotrexate concentration in the medium was increased to 16.0 μM, then 20.0 μM 18 days later. Cells from the 8th passage in medium containing 20.0 μM methotrexate were cloned out by limiting dilution in 96-well tissue culture plates

containing CD CHO medium containing 4 mM GlutaMAX-1™ and 20.0 μM methotrexate. Clones were identified 5-6 weeks later and clone 2B2 was selected for expansion in medium containing 20.0 μM methotrexate. After the 11th passage, 2B2 cells were frozen in vials as a research cell bank (RCB).

The resultant 2B2 cells are dihydrofolate reductase deficient (dhfr-) DG44 CHO cells that express soluble recombinant human PH20 (rHuPH20). The soluble PH20 is present in 2B2 cells at a copy number of approximately 206 copies/cell. Southern blot analysis of Spe I-, Xba I- and BamH I/Hind III-digested genomic 2B2 cell DNA using a rHuPH20-specific probe revealed the following restriction digest profile: one major hybridizing band of ~7.7 kb and four minor hybridizing bands (~13.9, ~6.6, ~5.7 and ~4.6 kb) with DNA digested with Spe I; one major hybridizing band of ~5.0 kb and two minor hybridizing bands (~13.9 and ~6.5 kb) with DNA digested with Xba I; and one single hybridizing band of ~1.4 kb observed using 2B2 DNA digested with BamH I/Hind III. Sequence analysis of the mRNA transcript indicated that the derived cDNA (SEQ ID NO:56) was identical to the reference sequence (SEQ ID NO:49) except for one base pair difference at position 1131, which was observed to be a thymidine (T) instead of the expected cytosine (C). This is a silent mutation, with no effect on the amino acid sequence.

Example 22

A. Production of Gen2 soluble rHuPH20 in 300 L Bioreactor Cell Culture

A vial of HZ24-2B2 was thawed and expanded from shaker flasks through 36L spinner flasks in CD-CHO media (Invitrogen, Carlsbad, CA) supplemented with 20 μM methotrexate and GlutaMAX-1™ (Invitrogen). Briefly, the a vial of cells was thawed in a 37°C water bath, media was added and the cells were centrifuged. The cells were re-suspended in a 125 mL shake flask with 20 mL of fresh media and placed in a 37 °C, 7 % CO_2 incubator. The cells were expanded up to 40 mL in the 125 mL shake flask. When the cell density reached greater than 1.5 x 10^6 cells/mL, the culture was expanded into a 125 mL spinner flask in a 100 mL culture volume. The flask was incubated at 37 °C, 7 % CO_2. When the cell density reached greater than 1.5 x 10^6 cells/mL, the culture was expanded into a 250 mL spinner flask in 200 mL culture volume, and the flask was incubated at 37 °C, 7 % CO_2. When the cell

density reached greater than 1.5 x 10^6 cells/mL, the culture was expanded into a 1 L spinner flask in 800 mL culture volume and incubated at 37 °C, 7 % CO_2. When the cell density reached greater than 1.5 x 10^6 cells/mL the culture was expanded into a 6 L spinner flask in 5000 mL culture volume and incubated at 37 °C, 7 % CO_2. When the cell density reached greater than 1.5 x 106 cells/mL the culture was expanded into a 36 L spinner flask in 32 L culture volume and incubated at 37 °C, 7 % CO_2.

A 400 L reactor was sterilized and 230 mL of CD-CHO media was added. Before use, the reactor was checked for contamination. Approximately 30 L cells were transferred from the 36L spinner flasks to the 400 L bioreactor (Braun) at an inoculation density of 4.0 × 10^5 viable cells per ml and a total volume of 260L. Parameters were temperature set point, 37°C; Impeller Speed 40-55 RPM; Vessel Pressure: 3 psi; Air Sparge 0.5- 1.5 L/Min.; Air Overlay: 3 L/ min. The reactor was sampled daily for cell counts, pH verification, media analysis, protein production and retention. Also, during the run nutrient feeds were added. At 120 hrs (day 5), 10.4L of Feed #1 Medium (4× CD-CHO + 33 g/L Glucose + 160 mL/L Glutamax-1™ + 83 mL/L Yeastolate + 33 mg/L rHuInsulin) was added. At 168 hours (day 7), 10.8 L of Feed #2 (2× CD-CHO + 33 g/L Glucose + 80 mL/L Glutamax-1™ + 167 mL/L Yeastolate + 0.92 g/L Sodium Butyrate) was added, and culture temperature was changed to 36.5°C. At 216 hours (day 9), 10.8 L of Feed #3 (1× CD-CHO + 50 g/L Glucose + 50 mL/L Glutamax-1™ + 250 mL/L Yeastolate + 1.80 g/L Sodium Butyrate) was added, and culture temperature was changed to 36 °C. At 264 hours (day 11), 10.8 L of Feed #4 (1× CD-CHO + 33 g/L Glucose + 33 mL/L Glutamax-1™ + 250 mL/L Yeastolate + 0.92 g/L Sodium Butyrate) was added, and culture temperature was changed to 35.5° C. The addition of the feed media was observed to dramatically enhance the production of soluble rHuPH20 in the final stages of production. The reactor was harvested at 14 or 15 days or when the viability of the cells dropped below 40 %. The process resulted in a final productivity of 17,000 Units per ml with a maximal cell density of 12 million cells/mL. At harvest, the culture was sampled for mycoplasma, bioburden, endotoxin and viral *in vitro* and *in vivo*, Transmission Electron Microscopy (TEM) and enzyme activity.

The culture was pumped by a peristaltic pump through four Millistak filtration system modules (Millipore) in parallel, each containing a layer of diatomaceous earth

graded to 4-8 μm and a layer of diatomaceous earth graded to 1.4-1.1 μm, followed by a cellulose membrane, then through a second single Millistak filtration system (Millipore) containing a layer of diatomaceous earth graded to 0.4-0.11 μm and a layer of diatomaceous earth graded to <0.1 μm, followed by a cellulose membrane, and then through a 0.22 μm final filter into a sterile single use flexible bag with a 350 L capacity. The harvested cell culture fluid was supplemented with 10 mM EDTA and 10 mM Tris to a pH of 7.5. The culture was concentrated 10× with a tangential flow filtration (TFF) apparatus using four Sartoslice TFF 30 kDa molecular weight cut-off (MWCO) polyether sulfone (PES) filter (Sartorius) , followed by a 10× buffer exchange with 10 mM Tris, 20 mM Na_2SO_4, pH 7.5 into a 0.22 μm final filter into a 50 L sterile storage bag.

The concentrated, diafiltered harvest was inactivated for virus. Prior to viral inactivation, a solution of 10 % Triton X-100, 3 % tri (n-butyl) phosphate (TNBP) was prepared. The concentrated, diafiltered harvest was exposed to 1 % Triton X-100, 0.3 % TNBP for 1 hour in a 36 L glass reaction vessel immediately prior to purification on the Q column.

B. Purification of Gen2 soluble rHuPH20

A Q Sepharose (Pharmacia) ion exchange column (9 L resin, H= 29 cm, D= 20 cm) was prepared. Wash samples were collected for a determination of pH, conductivity and endotoxin (LAL) assay. The column was equilibrated with 5 column volumes of 10 mM Tris, 20 mM Na_2SO_4, pH 7.5. Following viral inactivation, the concentrated, diafiltered harvest was loaded onto the Q column at a flow rate of 100 cm/hr. The column was washed with 5 column volumes of 10 mM Tris, 20 mM Na_2SO_4, pH 7.5 and 10 mM Hepes, 50 mM NaCl, pH 7.0. The protein was eluted with 10 mM Hepes, 400 mM NaCl, pH 7.0 into a 0.22 μm final filter into sterile bag. The eluate sample was tested for bioburden, protein concentration and hyaluronidase activity. A_{280} absorbance reading were taken at the beginning and end of the exchange.

Phenyl-Sepharose (Pharmacia) hydrophobic interaction chromatography was next performed. A Phenyl-Sepharose (PS) column (19-21 L resin, H=29 cm, D= 30 cm) was prepared. The wash was collected and sampled for pH, conductivity and endotoxin (LAL assay). The column was equilibrated with 5 column volumes of 5

mM potassium phosphate, 0.5 M ammonium sulfate, 0.1 mM CaCl2, pH 7.0. The protein eluate from the Q sepharose column was supplemented with 2M ammonium sulfate, 1 M potassium phosphate and 1 M $CaCl_2$ stock solutions to yield final concentrations of 5 mM, 0.5 M and 0.1 mM, respectively. The protein was loaded onto the PS column at a flow rate of 100 cm/hr and the column flow thru collected. The column was washed with 5 mM potassium phosphate, 0.5 M ammonium sulfate and 0.1 mM CaCl2 pH 7.0 at 100 cm/hr and the wash was added to the collected flow thru. Combined with the column wash, the flow through was passed through a 0.22 μm final filter into a sterile bag. The flow through was sampled for bioburden, protein concentration and enzyme activity.

An aminophenyl boronate column (ProMedics) was prepared. The wash was collected and sampled for pH, conductivity and endotoxin (LAL assay). The column was equilibrated with 5 column volumes of 5 mM potassium phosphate, 0.5 M ammonium sulfate. The PS flow through containing purified protein was loaded onto the aminophenyl boronate column at a flow rate of 100 cm/hr. The column was washed with 5 mM potassium phosphate, 0.5 M ammonium sulfate, pH 7.0. The column was washed with 20 mM bicine, 0.5 M ammonium sulfate, pH 9.0. The column was washed with 20 mM bicine, 100 mM sodium chloride, pH 9.0. The protein was eluted with 50 mM Hepes, 100 mM NaCl, pH 6.9 and passed through a sterile filter into a sterile bag. The eluted sample was tested for bioburden, protein concentration and enzyme activity.

The hydroxyapatite (HAP) column (Biorad) was prepared. The wash was collected and test for pH, conductivity and endotoxin (LAL assay). The column was equilibrated with 5 mM potassium phosphate, 100 mM NaCl, 0.1 mM $CaCl_2$, pH 7.0. The aminophenyl boronate purified protein was supplemented to final concentrations of 5 mM potassium phosphate and 0.1 mM $CaCl_2$ and loaded onto the HAP column at a flow rate of 100 cm/hr. The column was washed with 5 mM potassium phosphate, pH 7, 100 mM NaCl, 0.1 mM $CaCl_2$. The column was next washed with 10 mM potassium phosphate, pH 7, 100 mM NaCl, 0.1 mM $CaCl_2$. The protein was eluted with 70 mM potassium phosphate, pH 7.0 and passed through a 0.22μm sterile filter into a sterile bag. The eluted sample was tested for bioburden, protein concentration and enzyme activity.

The HAP purified protein was then passed through a viral removal filter. The sterilized Viosart filter (Sartorius) was first prepared by washing with 2 L of 70 mM potassium phosphate, pH 7.0. Before use, the filtered buffer was sampled for pH and conductivity. The HAP purified protein was pumped via a peristaltic pump through the 20 nM viral removal filter. The filtered protein in 70 mM potassium phosphate, pH 7.0 was passed through a 0.22 μm final filter into a sterile bag. The viral filtered sample was tested for protein concentration, enzyme activity, oligosaccharide, monosaccharide and sialic acid profiling. The sample also was tested for process related impurities.

The protein in the filtrate was then concentrated to 10 mg/mL using a 10 kD molecular weight cut off (MWCO) Sartocon Slice tangential flow filtration (TFF) system (Sartorius). The filter was first prepared by washing with 10 mM histidine, 130 mM NaCl, pH 6.0 and the permeate was sampled for pH and conductivity. Following concentration, the concentrated protein was sampled and tested for protein concentration and enzyme activity. A 6× buffer exchange was performed on the concentrated protein into the final buffer: 10 mM histidine, 130 mM NaCl, pH 6.0. Following buffer exchange, the concentrated protein was passed though a 0.22 μm filter into a 20 L sterile storage bag. The protein was sampled and tested for protein concentration, enzyme activity, free sulfhydryl groups, oligosaccharide profiling and osmolality.

The sterile filtered bulk protein was then asceptically dispensed at 20 mL into 30 mL sterile Teflon vials (Nalgene). The vials were then flash frozen and stored at -20 ± 5 °C.

Example 23

Effect of lysyl lysine as a stabilizer for rHuPH20

Lysyl lysine (Lys-Lys or dilysine) was tested for its ability to stabilize rHuPH20 at elevated temperatures. Eight formulations were prepared as described in Table 76 below. As a control, Hylenex™ (containing167 U/mL rHuPH20, 12.5 mM Na_2PO_4, 145 mM NaCl, 1 mg/mL HSA, 2.7 mM $CaCl_2$, 0.1% EDTA pH 7.4; designated F1) was tested. The remaining formulations were generated to contain 170 U/mL rHuPH20, 0.01% Tween 80 and various excipients/stabilizers as set forth in Table 76. To each tested formulation, Lys-Lys dihydrochloride (H-Lys-Lys-OH HCl;

RnD Chem #G-2675) was added at a concentration of either 50, 100 or 150 mM. Since Lys-Lys was used as a chloride salt, the osmolality varied between the compositions, as seen in Table 76 below.

All formulations were filled into 2 mL USP type I borosilicate glass vials with 13 mm 4432/50 gray rubber stoppers and sealed with alumina seals. The vials were incubated at three different temperatures, 5° C, 25° C and 40° C for up to 3 months. Samples were tested at time 0, 1 week, 2 weeks, 1 month, 2 months and 3 months, depending on the temperature (see Tables 77-79). At each testing point, samples were removed from the incubators and tested for hyaluronidase activity as described in Example 2.

Table 76. Formulations

Form.	PH20 U/mL	NaCl mM	Na_2PO_4 mM	Lys-Lys·2 HCl mM	TW80 %	Methionine mM	pH	mOsm
F1 Hylenex™	167 U/mL PH20, 12.5 mM Na_2PO_4, 145 mM NaCl, 1 mg/mL HSA, 2.7 mM $CaCl_2$, 0.1% EDTA pH 7.4							NA
F2	170	150	12.5	none	0.01	10	7.0	316
F3	170	100	12.5	50	0.01	10	7.0	364
F4	170	50	12.5	100	0.01	10	7.0	419
F5	170	NA	NA	100	0.01	10	7.0	444
F6	170	NA	NA	150	0.01	10	6.5	446
F7	170	NA	NA	150	0.01	none	7.0	449
F8	170	NA	NA	100	0.01	10	7.0	300

The results are shown in Tables 77-79 below, which set forth the hyaluronidase activity in U/mL. For samples stored at 5° C or 25° C, small decreases in the activity of rHuPH20 were observed, but all samples maintained at least 135 U/mL hyaluronidase activity (Tables 77 and 78). Further, no significant differences in hyaluronidase activity were observed between compositions containing Lys-Lys and compositions that did not contain Lys-Lys. For samples stored at 40° C, all samples had decreased enzymatic activity over time. Formulations F1 and F2 that did not contain Lys-Lys had the largest decrease in hyaluronidase activity. After 2 weeks at 40° C, the activity of formulations F1 and F2 was below 135 U/mL. Formulations containing Lys-Lys, at any concentration, all maintained their activity above 135 U/mL for at least 1 month at 40° C.

Table 77. Hyaluronidase activity (U/mL) of rHuPH20 at 5° C

Form.	Incubation Time, Months			
	0	1	2	3
F1	172	169	168	160

F2	170	162	162	150
F3	169	163	161	153
F4	165	158	158	152
F5	161	156	160	148
F6	170	163	164	157
F7	163	161	159	149
F8	177	174	169	161

Table 78. Hyaluronidase activity (U/mL) of rHuPH20 at 25° C

Form.	Incubation Time, Months				
	0	0.5	1	2	3
F1	172	176	168	157	161
F2	170	170	162	144	145
F3	169	162	162	151	152
F4	165	165	161	150	147
F5	161	161	162	160	159
F6	170	175	169	169	164
F7	163	159	155	152	156
F8	177	174	170	167	165

Table 79. Hyaluronidase activity (U/mL) of rHuPH20 at 40° C

Form.	Incubation Time, Months					
	0	0.25	0.5	1	2	3
F1	172	137	127	85	46	33
F2	170	133	114	98	63	53
F3	169	162	158	141	113	100
F4	165	160	155	138	110	96
F5	161	160	156	139	125	126
F6	170	167	162	158	141	137
F7	163	157	152	139	121	113
F8	177	171	170	149	117	128

Example 24

Effect of low concentrations of lysyl lysine on rHuPH20 stability

Various rHuPH20 formulations were tested for effects of low concentrations of lysyl lysine and pH on the activity of rHuPH20 at elevated temperatures. Three different Lys-Lys concentrations (10, 30 and 50 mM) and three different pH (6.5, 7.0 and 7.5) were evaluated, generating a total of 9 samples. The tested formulations are set forth in Table 80 below. As a control, Hylenex™ (set forth in Example 23; F1) was tested. Formulations F2 to F10 were all generated to target around 160 U/mL rHuPH20, 10 mM L-methionine (Spectrum #M1441), and 0.01% polysorbate 80

(Baker #4117-04) and various amounts of H-Lys-Lys-OH HCl (RnD Chem #G-2675) and NaCl (Baker #3628). 1N NaOH (EMD #SX0607H-6) was used to adjust the pH. The NaCl concentration was adjusted so all formulations had approximately the same osmolality (300 mOsm/Kg). All formulations were sterile filtered though a 0.2 micron filter.

All formulations were filled at 0.5 mL per vial into 2 mL USP type I borosilicate glass vials with 13 mm 4432/50 gray rubber stoppers and sealed with alumina seals. The vials were incubated at 40° C for up to 8 weeks. Samples were tested at time 0, and 1, 2, 3, 4, and 8 weeks. At each testing point, samples were removed from the incubators and tested for hyaluronidase activity as described in Example 2.

Table 80. Formulations

Form.	Lys-Lys mM	Met mM	NaCl mM	PS80 %	pH	Osmolality mOsm/Kg
F1 Hylenex™	167 U/mL PH20, 12.5 mM Na_2PO_4, 145 mM NaCl, 1 mg/mL HSA, 2.7 mM $CaCl_2$, 0.1% EDTA pH 7.4				7.40	ND
F2	50	10	97	0.01	7.01	302
F3	30	10	117	0.01	7.51	305
F4	50	10	99	0.01	6.54	304
F5	10	10	147	0.01	6.52	305
F6	50	10	91	0.01	7.52	306
F7	10	10	145	0.01	7.52	309
F8	30	10	121	0.01	6.54	306
F9	10	10	147	0.01	7.01	310
F10	30	10	122	0.01	7.02	304

The results are shown in Table 81 below, which sets forth the hyaluronidase activity in U/mL and the ratio of the activity as compared to time 0. Formulations F2-F9, all containing Lys-Lys, showed improved rHuPH20 stability over reference formula F1, regardless of pH. No significant difference was observed among samples containing 10, 30 or 50 mM Lys-Lys, indicating addition of 10 mM Lys-Lys is sufficient to improve rHuPH20 stability at 40° C. Formulations with a higher pH (7.5) had reduced enzymatic activity compared to all samples with lower pH (6.5 or 7.0). For example, for samples having a pH of 6.5 or 7.0, hyaluronidase activity after 4 week incubation at 40° C remained above 80% of the initial activity, whereas samples having a pH of 7.5 retained only about 70% of the initial activity. After 4 weeks, less than 50% of the rHuPH20 activity remained for the reference rHuPH20 sample (F1).

Table 81. Hyaluronidase activity at 40° C											
Form.	Activity (U/mL)						Activity Ratio (T_X/T_0)				
	0	1W	2W	3W	4W	8W	1W	2W	3W	4W	8W
F1 Hylenex™	169	127	105	84	73	43	0.75	0.62	0.50	0.43	0.25
F2	149	141	141	133	132	114	0.95	0.95	0.89	0.89	0.76
F3	146	123	128	110	113	84	0.84	0.87	0.75	0.77	0.58
F4	152	148	152	143	139	118	0.97	1.00	0.94	0.91	0.78
F5	159	151	148	141	140	107	0.95	0.93	0.89	0.88	0.67
F6	148	133	130	114	111	82	0.90	0.87	0.76	0.75	0.55
F7	156	130	129	110	101	87	0.84	0.83	0.71	0.95	0.56
F8	156	141	144	137	132	107	0.90	0.92	0.88	0.85	0.68
F9	155	140	136	134	127	109	0.91	0.88	0.86	0.82	0.70
F10	153	146	141	137	135	112	0.95	0.92	0.90	0.88	0.73

Example 25

Effect of lysyl lysine on rHuPH20 activity and insulin stability in rHuPH20/insulin co-formulations

Lysyl lysine was evaluated for its ability to stabilize co-formulations containing both rHuPH20 and an insulin analog. The formulations are set forth in Table 82 below. All formulations contained 600 U/mL rHuPH20, 3.5 mg/mL insulin glulisine, 30 mM Tris HCL, 5 mM methionine, 0.001% polysorbate 20, pH 7.2, and 0.1% phenol and 0.15% m-cresol as preservatives (EP-B preservative levels). Formulations F5, F1, F2 and F3 contained 50 mM NaCl and 0 mM, 20 mM, 40 mM and 80 mM Lys-Lys, respectively. Formulation F4 contained a higher concentration of NaCl (140 mM) and did not contain Lys-Lys. The osmolality of formulations F3 and F4 were adjusted with NaCl to be approximately the same.

Each formulation was aliquoted at 1.0 mL and filled into a 2 mL USP Type I borosilicate glass vial with 13 mm 4432/50 gray rubber stoppers and sealed with alumina seals. The vials were incubated at 5° C and 37° C. Samples were withdrawn at days 3 and 6 and tested for hyaluronidase enzymatic activity, pH, osmolality and appearance, as described in Examples 2 and 3.

Table 82. Formulations containing 600 U/mL rHuPH20 and 3.5 mg/mL glulisine								
Form.	pH	Buffer	Tonicity modifier	Stabilizers			Preservatives	
		Tris/Cl mM	NaCl mM	Methionine mM	PS20 %	Lys-Lys mM	Phenol %	m-Cresol %
F1	7.2	30	50	5	0.001	20	0.100	0.150
F2	7.2	30	50	5	0.001	40	0.100	0.150
F3	7.2	30	50	5	0.001	80	0.100	0.150
F4	7.2	30	140	5	0.001	0	0.100	0.150
F5	7.2	30	50	5	0.001	0	0.100	0.150

The addition of lysyl lysine did not affect the appearance as all formulations were clear and colorless with no visible particles indicating insulin stability. The hyaluronidase activity, as extrapolated from a standard curve, of the tested formulations is shown in Table 83 below. All formulations retained hyaluronidase activity after incubation for 6 days at 5° C. Only formula F3, which contained 80 mM Lys-Lys, retained some hyaluronidase enzymatic activity after 3 days at 37° C. After 6 days at 37° C, F3 had lost most of its hyaluronidase enzymatic activity. When osmolality was controlled to be roughly comparable, as in formulations F3 and F4, Lys-Lys was a better rHuPH20 stabilizer than NaCl, as evidenced by increased hyaluronidase activity.

Table 83. Hyaluronidase enzymatic activity (U/mL) and osmolality

Form.	Osmolality (mOsm)	5° C	37° C	37° C
		6d	3d	6d
F1	238	556	13	-18
F2	288	589	63	1
F3	396	563	165	79
F4	347	582	70	9
F5	182	541	5	-4

Example 26

Comparison of lysyl lysine and NaCl on rHuPH20 stability in rHuPH20/insulin co-formulations

Co-formulations of insulin and rHuPH20 were tested for the effects of lysyl lysine and NaCl on rHuPH20 stability. The tested formulations were generated to have equal ionic strength, but with one formulation containing lysyl lysine and NaCl and the other formulation containing only NaCl. Specifically, three sets of insulin glulisine and rHuPH20 formulations having different levels of ionic strength were prepared. The formulations are set forth in Table 84 below. All formulations contained 600 U/mL rHuPH20, 3.5 mg/mL insulin glulisine, 30 mM Tris HCL, 5 mM methionine, 0.001% polysorbate 20, pH 7.2, and 0.1% phenol and 0.15% m-cresol as preservatives (EP-B preservative levels). Formulations F1, F3 and F5 contained lysyl lysine and NaCl as set forth in Table 84 below. Formulations F2, F4 and F6 contained only NaCl. The formulations were tested for their actual pH and osmolality.

Each formulation was aliquotted at 1.0 mL and filled into a 2 mL USP Type I borosilicate glass vial with 13 mm 4432/50 gray rubber stoppers and sealed with alumina seals. The vials were incubated at 5° C and 30° C for up to 4 weeks. Samples were withdrawn on day 6 and after 2 and 4 weeks and tested for hyaluronidase enzymatic activity as described in Example 2.

Table 84. Formulations containing 600 U/mL rHuPH20 and 3.5 mg/mL glulisine

Form.	pH	Buffer	Tonicity modifier	Stabilizers			Preservatives	
		Tris/Cl mM	NaCl mM	Methionine mM	PS20 %	Lys-Lys mM	Phenol %	m-Cresol %
F1	7.2	30	40	5	0.001	20	0.100	0.150
F2	7.2	30	60	5	0.001	None	0.100	0.150
F3	7.2	30	80	5	0.001	40	0.100	0.150
F4	7.2	30	120	5	0.001	None	0.100	0.150
F5	7.2	30	160	5	0.001	80	0.100	0.150
F6	7.2	30	240	5	0.001	None	0.100	0.150

The results are shown in Table 85 below, which sets forth the hyaluronidase enzymatic activity (U/mL) at the tested days as well as the % hyaluronidase activity compared to the activity exhibited at T0 (% to T0). A multi-variable analysis of rHuPH20 enzymatic activity in response to incubation time, lysyl lysine concentration and ionic strength was performed based on relative activity to T0, and analyzed by JMP v. 8.02 (SAS Institutes). Formulations having higher ionic strength (F5 and F6) retained higher hyaluronidase enzymatic activity than formulations having lower ionic strength (F1 and F2) ($p<0.001$). Further, formulations containing lysyl lysine had significantly higher activity than those without ($p = 0.04$). Rate of hyaluronidase enzymatic activity loss correlated with ionic strength, indicating higher ionic strength could significantly reduce the rate of degradation. For solutions at the same ionic strength (*e.g.*, F5 and F6) no significant difference was observed after two weeks time, however after 4 weeks at 30° C, a significant difference was observed in hyaluronidase enzymatic activity, with F5 containing lysyl lysine retaining 91% activity but F6, which did not contain lysyl lysine, having only 75% activity remaining. The same trend was observed for formulations at lower ionic strength, with formulations containing lysyl lysine having increased activity as compared to those without lysyl lysine.

Table 85. pH, osmolality and rHuPH20 enzymatic activity at 30° C

Form.	pH	Osmolality (mOsm)	rHuPH20 activity, U/mL (% to T0)			
			T0	6d	2w	4w
F1	7.17	204	567 (100%)	505 (89%)	405 (71%)	288 (51%)
F2	7.22	210	523 (100%)	416 (80%)	334 (64%)	222 (42%)
F3	7.23	321	543 (100%)	517 (95%)	481 (88%)	400 (73%)
F4	7.20	312	528 (100%)	471 (89%)	438 (83%)	332 (63%)
F5	7.22	542	525 (100%)	522 (99%)	496 (94%)	476 (91%)
F6	7.19	531	511 (100%)	533 (104%)	498 (97%)	382 (75%)

Example 27

Effect of Lysyl lysine as a Buffer and Effects of Temperature on pH change of rHuPH20/insulin Co-Formulations

Lysyl lysine was evaluated for its ability to act as a buffer in co-formulations containing rHuPH20 and insulin by replacing Tris with lysyl lysine. The tested formulations are set forth in Table 86 below. All formulations were generated to contain 600 U/mL rHuPH20, 3.5 mg/mL insulin glulisine, 5 mM methionine, 0.001% polysorbate 20, pH 7.2, and 0.1% phenol and 0.15% m-cresol as preservatives (EP-B preservative levels). Each formulation contained 30, 50, 80 or 105 mM lysyl lysine (Biopeptide, G-2675, Lot#1037762). No formulation contained Tris HCl. NaCl was added to adjust the final osmolality to 380±30 mOsm.

Each formulation was aliquotted at 1.0 mL and filled into a 2 mL USP Type I borosilicate glass vial with 13 mm 4432/50 gray rubber stoppers and sealed with alumina seals. The vials were incubated at 5° C and 37° C. Samples were withdrawn at days 1, 4 and 6 and tested for hyaluronidase enzymatic activity, pH and osmolality, as described in Examples 2 and 3. Each formulation also was tested to evaluate the ability of lysyl lysine to act as a buffer at the target pH range of pH 6.5 to 8.0 by generating a titration curve of the pH as a function of the amount of titrant (1N NaOH; EMD, Cat No. SX0607H-6, Lot HC067239) that was added.

Table 86. Formulations containing 600 U/mL rHuPH20 and 3.5 mg/mL glulisine

Form.	pH	Tonicity modifier	Stabilizers			Preservatives	
		NaCl mM	Methionine mM	PS20 %	Lys-Lys mM	Phenol %	m-Cresol %
F1	7.2	0	5	0.001	105	0.100	0.150
F2	7.2	40	5	0.001	80	0.100	0.150
F3	7.2	85	5	0.001	50	0.100	0.150
F4	7.2	115	5	0.001	30	0.100	0.150

The results are shown in Table 87, which set forth the osmolality and hyaluronidase enzymatic activity. As shown in previous examples, Lys-Lys stabilized rHuPH20 in the formulations as hyaluronidase activity was decreased less with higher concentrations of Lys-Lys. Further, lysyl lysine was able to stabilize rHuPH20 more than NaCl at a similar level of ionic strength.

Table 87. Osmolality and hyaluronidase activity

Form.	Osmolality (mOsm)	rHuPH20 activity, U/mL			
		T0	1d	4d	6d
F1	373	507	408	263	134
F2	382	550	411	218	85
F3	410	535	403	213	81
F4	368	582	421	170	54

Table 88 below sets forth the pH at various temperatures and effect of temperature on the pH. Titration of lysyl lysine with NaOH revealed that lysyl lysine can serve as a buffer at a pH range of 6.5 to 8.0, especially at higher concentrations (*e.g.*, 80 or 105 mM). As shown in Table 88, the temperature effect (dpH/dT), or ratio of the change in pH to the change in temperature, remained constant, at -0.033 U/°C, which is slightly higher than reported for Tris (-0.028 U/°C). An ideal buffer has a larger temperature effect, as at lower storage temperatures (e.g., 2-8 °C) insulin benefits from a higher pH whereas at higher temperatures, rHuPH20 benefits from a lower pH. Thus, lysyl lysine is shown to be both an optimal buffer and a stabilizer in formulations containing both insulin and rHuPH20.

Table 88. Effect of temperature on the pH

Form.	Actual pH at temperature			Temperature Effect
	0° C	22° C	37° C	dpH/dT (U/°C)
F1	7.93	7.20	6.72	-0.033
F2	7.93	7.26	6.71	-0.033
F3	7.96	7.26	6.73	-0.033
F4	7.94	7.23	6.72	-0.033

Example 28

Stability of rHuPH20 in insulin co-formulations containing lysyl lysine over time at 5° C, 30° C and 37° C

Lysyl lysine was tested for its ability to stabilize insulin analog-rHuPH20 co-formulations containing USP levels of preservatives. The formulations are set forth in Table 89 below. Each formulation contains 600 U/mL rHuPH20, 3.5 mg/mL insulin analog (either insulin glulisine or insulin aspart), 5 mM methionine, 0.001%

surfactant (either poloxamer 188 or polysorbate 20), and USP levels of preservatives (0.125% phenol and 0.075% m-cresol). For each insulin analog, two formulations had a pH of 7.0 and two formulations had a pH of 7.2. Further, at each pH, one formulation contained only 100 mM lysyl lysine whereas the other formulation contained 30 mM NaCl and 80 mM lysyl lysine. As a comparison for insulin aspart, F9 was prepared containing 30 mM Tris/HCl.

Each formulation was aliquotted at 1.0 mL and filled into a 2 mL USP Type I borosilicate glass vial with 13 mm 4432/50 gray rubber stoppers and sealed with alumina seals. The vials were incubated at 5° C, 30° C and 37° C for up to 4 weeks. Samples were withdrawn as indicated in Tables 90 and 91 and tested for rHuPH20 enzymatic activity as described in Example 2. The results are shown in Tables 90 and 91 below, which set forth the hyaluronidase enzymatic activity in U/mL.

Table 89. Formulations containing 600 U/mL rHuPH20 and 3.5 mg/mL insulin analog

		Buffer	Tonicity modifier	Stabilizers		Surfactant		Preservatives		API
Form.	pH	Tris/Cl mM	NaCl mM	Methionine mM	Lys-Lys mM	F68 %	PS20 %	Phenol %	m-Cresol %	Insulin Analog
F1	7.0	0	0	5	100		0.001	0.125	0.075	glulisine
F2	7.0	0	30	5	80		0.001	0.125	0.075	glulisine
F3	7.2	0	0	5	100		0.001	0.125	0.075	glulisine
F4	7.2	0	30	5	80		0.001	0.125	0.075	glulisine
F5	7.0	0	0	5	100	0.01		0.125	0.075	aspart
F6	7.0	0	30	5	80	0.01		0.125	0.075	aspart
F7	7.2	0	0	5	100	0.01		0.125	0.075	aspart
F8	7.2	0	30	5	80	0.01		0.125	0.075	aspart
F9	7.2	30	100	5	0	0.01		0.125	0.075	aspart

*F68 = poloxamer 188 (Pluronic® F68); PS20 = polysorbate 20.

1. rHuPH20-insulin glulisine co-formulations

As shown in Table 90, for rHuPH20-insulin glulisine formulations, hyaluronidase activity remained constant for all formulations at 5° C and 30° C. At 37° C, a decrease in hyaluronidase activity was observed over time among all formulations. Over the time period tested, the formulations were determined to be stable, since none of the formulations had hyaluronidase activity below 375 U/mL.

Table 90. Hyaluronidase activity (rHuPH20-glulisine formulations)

Form.	5° C				30° C		37° C	
	T0	4w	2M	3M	2w	4w	3d	6d
F1	783	710	740	743	713	790	708	647
F2	811	770	776	745	741	834	726	678
F3	730	689	718	681	689	752	645	545
F4	688	655	718	646	665	676	581	491

2. rHuPH20-insulin aspart co-formulations

As shown in Table 91, for rHuPH20-insulin aspart formulations, all formulations were stable for up to 3 months at 5° C. At 30° C, all formulations containing lysyl lysine were stable for up to 4 weeks, whereas formulation F9 (containing no Lys-Lys) showed a loss in hyaluronidase activity. At 37° C, all formulations had decreased hyaluronidase activity over time, with the rate of decline depending on the formulation. For example, after 6 days at 37° C, formulation F5 (having 100 mM Lys-Lys and pH 7.0) had higher activity than formulation F8 (having 80 mM Lys-Lys and pH 7.2). At this time point and temperature, formulation F9 that did not contain lysyl lysine lost almost all hyaluronidase activity.

Table 91. Hyaluronidase activity (rHuPH20-aspart formulations)

Form.	5° C				30° C		37° C	
	T0	4w	2M	3M	2w	4w	3d	6d
F5	741	661	762	775	677	726	622	505
F6	749	676	795	672	687	739	600	495
F7	712	625	726	635	601	656	544	413
F8	703	608	729	621	605	638	499	292
F9	806	695	851	713	582	448	279	65

Example 29

Effect of Human Serum Albumin (HSA) or Lysyl Lysine on Stability of rHuPH20 under Agitation and Thermal Stress

rHuPH20 formulations were tested for stability under accelerated conditions, including agitation and thermal stress. The formulations are set forth in Table 92 below. Each formulation contained 160 U/mL rHuPH20, 12.5 mM Sodium phosphate, pH 7.0 and 145 mM NaCl. Each formulation additionally contained one or more of human serum albumin (HSA), $CaCl_2$, EDTA, polysorbate 80, methionine and lysyl lysine, as set forth in Table 92 below.

Each formulation was aliquotted at 1.5 mL and filled into a 2 mL USP Type I borosilicate glass vial with 13 mm 4432/50 gray rubber stoppers and sealed with alumina seals. Vials containing visible particles were excluded from the study. The formulations were tested under both agitation and thermal stress. For the agitation study, 2 samples of each formulation were incubated at 25° C and agitated at 650 rpm for 72 hours. For the thermal stress study, 4 samples of each formulation were incubated at 40° C for up to 4 weeks, with samples withdrawn every week.

Table 92. rHuPH20 Formulations									
Form.	PH20 U/mL	Buffer, pH	NaCl mM	HSA mg/mL	$CaCl_2$ mM	EDTA %	PS80 %	Met mM	Lys-Lys mM
F1	160	12.5 mM $NaPO_4$, 7.0	145	1	2.7	0.1	0	0	0
F2	160	12.5 mM $NaPO_4$, 7.0	145	1	0	0	0	0	0
F3	160	12.5 mM $NaPO_4$, 7.0	145	1	0	0	0.02	0	0
F4	160	12.5 mM $NaPO_4$, 7.0	145	0	0	0	0.02	10	0
F5	160	12.5 mM $NaPO_4$, 7.0	145	0	0	0	0.04	10	0
F6	160	12.5 mM $NaPO_4$, 7.0	145	0	0	0	0.06	10	0
F7	160	12.5 mM $NaPO_4$, 7.0	145	0	0	0	0.02	10	10

All samples were tested for hyaluronidase activity as described in Example 2. The presence of insoluble and soluble particles were tested using a MicroFlow Imaging (MFI). For this, each rHuPH20 formulation or control sample was measured with a Micro-Flow Imaging instrument Model 4200 (Brightwell Technologies, Inc., Ottawa, Canada). Prior to each run, blank buffer was filtered through a 0.22 micron filter and flushed through the system to provide a clean background and optimize illumination. To equilibrate the system, at least 2 mL of sample was dispensed before analysis. One (1) ml samples were run at a flow rate of 170 µL/min using a peristaltic pump. The magnification setting was 5 X to allow detection of particles within the range of 1 to 100 µm with an analysis depth field of 100 µm. Particles were counted and recorded automatically by the machine and reported as particle number/mL. Buffer and or formulations that contained rHuPH20 but were not subjected to agitation were used as controls. Size exclusion chromatography also was performed as described in Example 4. The results are shown in Tables 93-95 below.

1. Hyaluronidase Activity

Table 93 sets forth the hyaluronidase activity in U/mL and the % of activity as compared to time 0 (T0). The results show that after 8 days at 40° C, formulations that did not contain HSA (F4, F5 and F6) had reduced hyaluronidase activity compared to formulations F1, F2 and F3 that contained HSA. Formulation F7, containing Lys-Lys, retained the highest level of hyaluronidase activity after 15 days at 40° C.

After agitation stress, formulations containing at least 0.04% polysorbate 80 and 10 mM methionine retained hyaluronidase activity, as did the formulation containing Lys-Lys. Formulations F1-F4 had a slight decrease in hyaluronidase activity. Overall, formulation F7 (containing Lys-Lys) exhibited the greatest retention in rHuPH20 enzymatic activity under both the thermal stress test and agitation test.

Table 93. Hyaluronidase activity under agitation and thermal stress

Form.	Time 0	Agitation 25° C, 72h U/mL (% t0)	40° C, 8 days U/mL (% t0)	40° C, 15 days U/mL (% t0)
F1	155	146 (94)	149 (96)	139 (90)
F2	154	144 (94)	150 (97)	134 (87)
F3	153	142 (93)	153 (100)	132 (86)
F4	155	148 (96)	122 (79)	100 (65)
F5	153	158 (103)	110 (72)	105 (69)
F6	153	157 (103)	117 (76)	107 (70)
F7	151	153 (101)	147 (97)	147 (97)

2. Presence of Aggregated Protein or Insoluble Aggregates

Table 94 sets forth the particulate counts (insoluble aggregates) in particle number/mL for each micron size range. For example, micron size ranges that were tested using MFI were particles (p) greater than 5 micrometers (μm) but less than 10 μm ($5 \leq p < 10$), $10 \leq p < 25$, $25 \leq p < 50$ and $p \geq 50$. Control formulations for F1 and F4 were generated that did not contain rHuPH20, and control formulations for F2, F4 and F6 samples were the same formulations that were not agitated.

The presence or absence of HSA did not affect control non-agitated samples, with each having approximately equal particle counts. Once agitated, formulations containing HSA but lacking polysorbate 80 (F1 and F2) had significantly more particles than all other formulations, including F3 that only differed from F2 by addition of polysorbate 80. Addition of more polysorbate 80 (F5 and F6 compared to F4) did not have a significant effect in reducing aggregate formation. Formulation F7, containing Lys-Lys, had the smallest numbers of insoluble aggregates after agitation.

Table 94. Particulate counts as measured by MFI (particle number/mL)

	Agitation				Control (non-agitated samples)				Buffer (no rHuPH20)			
	5≤p <10	10≤p <25	25≤p <50	p ≥50	5≤p <10	10≤p <25	25≤p <50	p ≥50	5≤p <10	10≤p <25	25≤p <50	p ≥50
1	9770	5385	1090	248	---	---	---	---	403	58	7	0
2	10858	6930	795	139	1257	397	15	0	---	---	---	---

3	2895	382	39	10	---	---	---	---	---	---	---	---
4	2380	267	27	7	1356	167	0	3	31	8.0	0	0
5	2313	211	5	0	---	---	---	---	---	---	---	---
6	2610	432	41	8	1667	150	6	0	---	---	---	---
7	1299	129	5	0	---	---	---	---	---	---	---	---

p - particle size (microns)

3. Size Exclusion Chromatography

Soluble aggregates were also measured by size exclusion chromatography. Table 95 sets forth the soluble aggregates (high molecular weight aggregates) in mAu*min and the percentage of total peak area. Formulations F4 to F7 contained too little protein to detect by size exclusion chromatography. Formulations F1-F3, containing HSA, showed low levels of high molecular weight aggregates after agitation at 25° C. Addition of polysorbate 80 reduced the overall number of aggregates (F3 compared to F1 or F2).

Table 95. Soluble aggregates as measured by size exclusion chromatography in mAu*min (% total peak area)

Form.	Control		Agitation	
	5° C, T0		72 hr @650 rpm (25° C)	
SEC	Main	HMW	Main	HMW
F1	4066 (97.9)	86 (2.1)	4020 (97.1)	120 (2.9)
F2	4023 (98.0)	81 (2.0)	3913 (97.3)	108 (2.7)
F3	3970 (98.0)	82 (2.0)	3950 (98.0)	79 (2.0)
F4	20	ND	19	ND
F5	21	ND	19	ND
F6	22	ND	21	ND
Fs7	21	ND	20	ND

HMW = high molecular weight

Since modifications will be apparent to those of skill in this art, it is intended that this invention be limited only by the scope of the appended claims.

WHAT IS CLAIMED:

1. A stable co-formulation composition, wherein:

the composition has a pH of between or about between 6.8 to 7.8; and

the composition comprises:

a therapeutically effective amount of a fast-acting insulin;

a hyaluronan-degrading enzyme in an amount sufficient to render the composition super-fast acting;

NaCl at a concentration between or about between 50 mM to 200 mM;

an anti-microbially effective amount of a preservative or mixture of preservatives; and

a stabilizing agent or agents,

whereby the composition is stable for at least 6 months at a temperature from or from about 2°C to 8°C and/or for at least 14 days at a temperature from or from about 20°C to 30°C.

2. The stable co-formulation of claim 1, wherein:

the hyaluronan-degrading enzyme in the co-formulation retains at least 50% of the initial hyaluronidase activity for at least 6 months at a temperature from or from about 2°C to 8°C and/or for at least 14 days at a temperature from or from about 20°C to 30°C; and/or

the insulin in the composition retains:

at least 90% potency or recovery of the initial level of insulin in the composition for at least 6 months at a temperature from or from about 2°C to 8°C and/or for at least 14 days at a temperature from or from about 20°C to 30°C; and/or

at least 90% of the initial insulin purity for at least 6 months at a temperature from or from about 2°C to 8°C and/or for at least 14 days at a temperature from or from about 20°C to 30°C; and/or

less than 2% high molecular weight (HMWt) insulin species for at least 6 months at a temperature from or from about 2°C to 8°C and/or for at least 14 days at a temperature from or from about 20°C to 30°C.

3. The stable co-formulation of claim 1 or claim 2, wherein the NaCl concentration is between or about between 80 mM to 200 mM, 80 mM to 140 mM, 50

mM to 100 mM, 80 mM to 100 mM, 50 mM to 80 mM, 100 mM to 140 mM or 120 mM to 140 mM.

4. The stable co-formulation of any of claims 1-3, comprising a sufficient amount of a buffering agent to maintain the pH range of between or about between 6.8 to 7.8.

5. A stable co-formulation composition, wherein:

the composition has a pH of between or about between 6.5 to 7.5;

the composition comprises:

a therapeutically effective amount of a fast-acting insulin;

a hyaluronan-degrading enzyme in an amount sufficient to render the composition super-fast acting;

NaCl at a concentration between or about between 120 mM to 200 mM;

an anti-microbially effective amount of a preservative or mixture of preservatives; and

a stabilizing agent or agents; and

whereby the composition is stable for at least 3 days at a temperature from or from about 32°C to 40°C and/or is stable for at least 3 hours under agitation.

6. The stable co-formulation of claim 5, wherein the stabilizing agent comprises an effective amount of a hyaluronidase inhibitor.

7. The stable co-formulation of claim 6, wherein the hyaluronidase inhibitor is selected from among a protein, a substrate of a hyaluronan-degrading enzyme, polysaccharides, fatty acids, lanostanoids, antibiotics, anti-nematodes, synthetic organic compounds and a plant-derived bioactive component.

8. The stable co-formulation of claim 6 or claim 7, wherein the hyaluronidase inhibitor is selected from among a glycosaminoglycan (GAG), serum hyaluronidase inhibitor, *Withania somnifera* glycoprotein (WSG), heparin, heparin sulfate, dermatan sulfate, chitosans, β-(1,4)-galacto-oligosaccharides, sulphated verbascose, sulphated planteose, pectin, poly(styrene-4-sulfonate), dextran sulfate, sodium alginate, polysaccharide from *Undaria pinnatifida*, mandelic acid condensation polymer, eicosatrienoic acid, nervonic acid, oleanolic acid, aristolochic acid, ajmaline, reserpine, flavone, desmethoxycentauredine, quercetin, apigenin,

kaempferol, silybin, luteolin, luteolin-7-glucoside, phloretin, apiin, hesperidin, sulphonated hesperidin, calycosin-7-O-β-D-glucopyranoside, sodium flavone-7-sulphate, flavone 7-fluoro-4'-hydroxyflavone, 4'-chloro-4,6-dimethoxychalcone, sodium 5-hydroxyflavone 7-sulphate, myricetin, rutin, morin, glycyrrhizin, vitamin C, D-isoascorbic acid, D-saccharic 1,4-lactone, L-ascorbic acid-6-hexadecanoate (Vcpal), 6-O-acylated vitamin C, catechin, nordihydroguaiaretic acid, curcumin, N-propyl gallate, tannic acid, ellagic acid, gallic acid, phlorofucofuroeckol A, dieckol, 8,8'-bieckol, procyanidine, gossypol, celecoxib, nimesulide, dexamethasone, indomethcin, fenoprofen, phenylbutazone, oxyphenbutazone, salysylates, disodium cromoglycate, sodium aurothiomalate, transilist, traxanox, ivermectin, linocomycin and spectinomycin, sulfamethoxazole and trimerthoprim, neomycin sulphate, 3α-acetylpolyporenic acid A, (25S)-(+)-12α-hydroxy-3α-methylcarboxyacetate-24-methyllanosta-8,24(31)-diene-26-oic acid, lanostanoid, polyporenic acid c, PS53 (hydroquinone-sulfonic acid-formaldehyde polymer), polymer of poly (styrene-4-sulfonate), VERSA-TL 502, 1-tetradecane sulfonic acid, mandelic acid condensation polymer (SAMMA), 1,3-diacetylbenzimidazole-2-thione, N-monoacylated benzimidazol-2thione, N,N'-diacylated benzimidazol-2-thione, alkyl-2-phenylindole derivate, 3-propanoylbenzoxazole-2-thione, N-alkylated indole derivative, N-acylated indole derivate, benzothiazole derivative, N-substituted indole-2- and 3-carboxamide derivative, halogenated analogs (chloro and fluoro) of N-substituted indole-2- and 3-carboxamide derivative, 2-(4-hydroxyphenyl)-3-phenylindole, indole carboxamides, indole acetamides, 3-benzolyl-1-methyl-4-phenyl-4-piperidinol, benzoyl phenyl benzoate derivative, L-arginine derivative, guanidium HCL, L-NAME, HCN, linamarin, amygdalin, hederagenin, aescin, CIS-hinokiresinol and 1,3-di-p-hydroxyphenyl-4-penten-1-one.

9. The stable co-formulation of any of claims 6-8, wherein the hyaluronidase inhibitor is a hyaluronidase substrate that is a hyaluronan (HA) oligosaccharide.

10. The stable co-formulation of claim 9, wherein the concentration of HA is between or about between 1 mg/mL to 20 mg/mL.

11. The stable co-formulation of any of claims 5-10, wherein:

the hyaluronan-degrading enzyme in the co-formulation retains at least 50% of the initial hyaluronidase activity for at least 3 days at a temperature from or from about 32°C to 40°C or is stable for at least 3 days under agitation; and

the insulin in the composition retains:

at least 90% potency or recovery of the initial level of insulin in the composition for at least 3 days at a temperature from or from about 32°C to 40°C or is stable for at least 3 days under agitation; and/or

at least 90% of the initial insulin purity for at least 3 days at a temperature from or from about 32°C to 40°C or is stable for at least 3 hours under agitation; and/or

less than 2% high molecular weight (HMWt) insulin species for at least 3 days at a temperature from or from about 32°C to 40°C or is stable for at least 3 days under agitation.

12. The stable co-formulation of any of claims 5-11, wherein the NaCl concentration is between or about between 150 mM to 200 mM or 160 mM to 180 mM.

13. The stable co-formulation of any of claims 5-12, comprising a sufficient amount of a buffering agent to maintain the pH range of between or about between 6.5 to 7.5.

14. The stable co-formulation of any of claims 1-13, wherein the hyaluronan-degrading enzyme is a hyaluronidase or a chondroitinase.

15. The stable co-formulation of any of claims 1-14, wherein the hyaluronan-degrading enzyme is a hyaluronidase that is active at neutral pH.

16. The stable co-formulation of any of claims 1-15, wherein:

the hyaluronan-degrading enzyme lacks a glycosylphosphatidylinositol (GPI) anchor or is not membrane-associated when expressed from a cell; or

the hyaluronan-degrading enzyme contains C-terminal truncations of one or more amino acid residues to remove all or part of a GPI anchor.

17. The stable co-formulation of any of claims 1-16, wherein the hyaluronan-degrading enzyme is a hyaluronidase that is a PH20 or a C-terminal truncated form thereof.

18. The stable co-formulation of any of claims 1-17, wherein the hyaluronan-degrading enzyme is a PH20 polypeptide that has the sequence of amino acids set forth in any of SEQ ID NOS: 4-9, 47-48, 234-254, and 267-273, or a sequence of amino acids that exhibits at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 4-9, 47-48, 234-254, and 267-273

19. The stable co-formulation of any of claims 1-18, wherein the hyaluronan-degrading enzyme is a C-terminal truncated PH20 that comprises a sequence of amino acids set forth in any of SEQ ID NOS: 4-9.

20. The stable co-formulation of any of claims 1-19, wherein the amount of a hyaluronan-degrading enzyme is between or about between 10 U/mL to 20000 U/mL, 10 U/mL to 15000 U/mL, 10 U/mL to 10000 U/mL, 10 U/mL to 5000 U/mL, 50 U/mL to 10000 U/mL, 50 U/mL to 4000 U/mL, 100 U/mL to 2000 U/mL, 300 U/mL to 2000 U/mL, 600 U/mL to 2000 U/mL, or 100 U/mL to 1000 U/mL.

21. The stable co-formulation of any of claims 1-20, wherein the amount of a hyaluronan-degrading enzyme is at least or is about or is 30 U/mL, 35 U/mL, 40 U/mL, 50 U/mL, 100 U/mL, 150 U/mL, 200 U/mL, 250 U/mL, 300 U/mL, 350 U/mL, 400 U/mL, 450 U/mL, 500 U/mL, 600 U/mL, 700 U/mL, 800 U/mL, 900 U/mL, 1000 U/mL, 2000 U/mL, 3000 U/mL, 4000 U/mL, 5000 U/mL, 10000 U/mL, 15000 U/mL or 20000 U/mL.

22. The stable co-formulation of any of claims 1-21, wherein the fast-acting insulin is a regular insulin.

23. The stable co-formulation of claim 22, wherein the regular insulin is a human insulin or pig insulin.

24. The stable co-formulation of claim 22 or claim 23, wherein the regular insulin is an insulin with an A chain having a sequence of amino acids set forth in SEQ ID NO:103 and a B chain having a sequence of amino acids set forth in SEQ ID NO:104; or an insulin with an A chain having a sequence of amino acids set forth as amino acid residue positions 88-108 of SEQ ID NO:123 and a B chain having a sequence of amino acids set forth as amino acid residue positions 25-54 of SEQ ID NO:123.

25. The stable co-formulation of any of claims 1-21, wherein the fast-acting insulin is an insulin analog.

26. The stable co-formulation of any of claims 1-25, wherein the fast-acting insulin is an insulin analog selected from among insulin lispro, insulin aspart and insulin glulisine.

27. The stable co-formulation of claim 25 or claim 26, wherein the insulin analog is selected from among an insulin having an A chain with a sequence of amino acids set forth in SEQ NOS:103 and a B chain having a sequence of amino acids set forth in any of SEQ NOS:147-149.

28. The stable co-formulation of any of claims 1-27, wherein the amount of fast-acting insulin is 10 U/mL to 1000 U/mL, 50 U/mL to 500 U/mL, 100 U/mL to 1000 U/mL or 500 U/mL to 1000 U/mL, inclusive.

29. The stable co-formulation of any of claims 4 and 13-28, wherein the buffering agent is selected from among Tris, histidine, phosphate and citrate.

30. The stable co-formulation of any of claims 4 and 13-29, wherein the buffering agent is Tris.

31. The stable co-formulation of any of claims 4 and 13-30, wherein the concentration of the buffering agent is between or is between about 1 mM to 100 mM, 10 mM to 50 mM or 20 mM to 40 mM.

32. The stable co-formulation of any of claims 1-31, wherein the preservative(s) in the formulation comprises one or more of a phenolic preservative(s), a non-phenolic preservative(s) or a phenolic preservative(s) and a non-phenolic preservative(s).

33. The stable co-formulation of any of claims 1-32, wherein the preservative(s) is(are) selected from among phenol, m-cresol, methylparaben, benzyl alcohol, thimerosal, benzalkonium chloride, 4-chloro-1-butanol, chlorhexidine dihydrochloride, chlorhexidine digluconate, L-phenylalanine, EDTA, bronopol, phenylmercuric acetate, glycerol, imidurea, chlorhexidine, sodium dehydroacetate, o-cresol, p-cresol, chlorocresol, cetrimide, benzethonium chloride, ethyl paraben, propylparaben, butylparaben and any combinations thereof.

34. The stable co-formulation of any of claims 1-33, wherein the formulation contains a single preservative or a mixture of preservatives that contains 2, 3 or 4 different preservatives.

35. The stable co-formulation of any of claims 1-34, comprising at least one phenolic preservative.

36. The stable co-formulation of any of claims 1-35, wherein the preservative(s) is(are) phenol, m-cresol or phenol and m-cresol.

37. The stable co-formulation of any of claims 1-36, wherein the total amount of the one or more preservative agents as a percentage (%) of mass concentration (w/v) in the formulation is or is between 0.1% and 0.4%, 0.1% to 0.3%, 0.15% to 0.325%, 0.15% to 0.25%, 0.1% to 0.2%, 0.2%to 0.3% or 0.3% to 0.4% inclusive.

38. The stable co-formulation of any of claims 1-37, wherein the preservatives are phenol and m-cresol and the amount as a % of mass concentration (w/v) in the formulation is between or about between 0.1% to 0.25% phenol and between or about between 0.05% to 0.2% m-cresol, is between or about between 0.10% to 0.2% phenol and between or about between 0.6% to 01.8% m-cresol, between or about between 0.1% to 0.15% phenol and 0.8% t 0.15% m-cresol, is between or about between 0.10% to 0.15% phenol and between or about between 0.06 to 0.09% m-cresol or is between or about between 0.12% to 0.18% phenol and between or about between 0.14 to 0.22% m-cresol.

39. The stable co-formulation of any of claims 1-38, comprising a stabilizing agent that is selected from among an amino acid, an amino acid derivative, an amine, a sugar, a polyols, a salt and a surfactant.

40. The stable co-formulation of claim 39, wherein the stabilizing agent is an amino acid that is glycine or proline.

41. The stable co-formulation of claim 39 or claim 40, wherein the concentration of the amino acid is between or between about 0.01 M to 1 M, 0.01 M to 0.1 M, 0.1M to 0.75M or 0.2M to 0.5M, inclusive.

42. The stable co-formulation of claim 39, wherein the stabilizing agent is an amino acid is selected from among arginine, glutamic acid, and/or salts thereof and/or combinations thereof.

43. The stable co-formulation of claim 42, wherein the concentration of the amino acids are 1 to 100 mM, 10 to 100 mM, or of or of about 30 mM, 50 mM or 80 mM.

44. The stable co-formulation of any of claims 1-43, wherein the stabilizing agent is a surfactant and the amount of surfactant as a % of mass concentration (w/v) in the formulation is between or about between 0.005% to 1.0%, 0.01% to 0.5%, 0.01% to 0.1%, 0.01% to 0.05%, or 0.01% to 0.02%.

45. The stable co-formulation of claim 44, wherein the surfactant is selected from among a polypropylene glycol, polyethylene glycol, glycerin, sorbitol, poloxamer and polysorbate.

46. The stable co-formulation of claim 44 or claim 45, wherein the surfactant is selected from among poloxamer 188, polysorbate 20 and polysorbate 80.

47. The stable co-formulations of any of claims 1-46, wherein:

the stabilizing agent is a surfactant that is poloxamer 188 and is provided in an amount as a % of mass concentration (w/v) of between or about between 0.01% to 0.05%; or

the stabilizing agent is a surfactant that is polysorbate 20 and is provided in an amount as a % of mass concentration (w/v) of between or about between 0.01% to 0.05%; or

the stabilizing agent is a surfactant that is polysorbate 80 and is provided in an amount as a % of mass concentration (w/v) of between or about between 0.01% to 0.05%.

48. The stable co-formulations of any of claims 1-47, comprising a tonicity modifier to maintain the osmolarity of between or about between 245 mOsm/kg to 305 mOsm/kg.

49. The stable co-formulation of claim 48, wherein the tonicity modifier is selected from among glycerin, salt, amino acids, polyalcohols and trehalose.

50. The stable co-formulation of claim 48 or claim 49, comprising glycerin in a concentration less than 60 mM, less than 55 mM, less than 50 mM, less than 45 mM, less than 40 mM, less than 35 mM, less than 30 mM, less than 25 mM, less than 20 mM, less than 15 mM, less than 10 mM.

51. The stable co-formulation of any of claims 48-50, wherein:

the fast-acting insulin is an insulin analog that is insulin aspart and the formulation comprises glycerin in a concentration between or about between 20 mM to 50 mM, inclusive; or

the fast acting insulin is a regular insulin or is insulin lispro and the formulation comprises glycerin in a concentration between or about between 40 mM to 60 mM, inclusive.

52. The stable co-formulation of any of claims 1-51, comprising an antioxidant.

53. The stable co-formulation of claim 52, wherein the antioxidant is selected from among cysteine, tryptophan and methionine.

54. The stable co-formulation of claim 52 or claim 53, wherein the antioxidant is at a concentration from between or from about between 5 mM to 50 mM, 5 mM to 40 mM, 5 mM to 20 mM or 10 mM to 20 mM, inclusive.

55. The stable co-formulation of claim 53, wherein the antioxidant is methionine and the concentration is between or about between 10 mM to 20 mM.

56. The stable co-formulation of any of claims 1-55, comprising zinc.

57. The stable co-formulation of claim 56, wherein the concentration of zinc is between or about between 0.001 to 0.1 mg per 100 units of insulin (mg/100U), 0.001 to 0.05 mg/100U or 0.01 to 0.05 mg/100U.

58. The stable co-formulation of any of claims 1-57, comprising a nicotinic compound.

59. The stable co-formulation of claim 58, wherein the nicotinic compound is selected from among nicotinamide, nicotinic acid, niacin, niacinamide, vitamin B3 and/or salts thereof and/or any combination thereof.

60. The stable co-formulation of claim 58 or claim 59, wherein the nicotinic compound(s) are present in a concentration from or from about 1 mm to 150 mM, 10 mM to 150 mM, 50 mM to 100 mM or of or of about 80 mM.

61. The stable co-formulation of any of claims 1-60, wherein the formulation has a pH of between or about between 7.0 to 7.6, and comprises:

a hyaluronan-degrading enzyme that is a PH20 in an amount between or about between 100 U/mL to 1000 U/mL, inclusive;

a fast-acting insulin analog in an amount between or about between 10 U/mL to 1000 U/mL, inclusive;

a Tris buffering agent at a concentration of between or about between 10 mM to 50 mM, inclusive;

NaCl at a concentration of between or about between 50 to 200 mM, inclusive;

methionine at a concentration between or about between 5 mM to 50 mM, inclusive;

glycerin at a concentration from between or about between 0 mM to 50 mM, inclusive;

a surfactant that is poloxamer 188, polysorbate 20 or polysorbate 80 at a percentage (%) of mass concentration (w/v) of between or about between 0.01% to 0.5%; and

a preservative(s) that comprises phenol at a percentage (%) of mass concentration (w/v) of between or between about between 0.1% to 0.25% and m-cresol at a % w/v of between or between about 0.05% to 0.2%.

62. The stable co-formulation of claim 61, comprising zinc at a concentration of 0.001 to 0.1 mg per 100 units of insulin (mg/100U).

63. The stable co-formulation of claim 61 or claim 62, wherein:

the fast-acting insulin is a fast-acting insulin analog that is insulin lispro,

the formulation has a pH between or about between 7.0 to 7.6; and

the formulation comprises:

a hyaluronan-degrading enzyme that is a PH20 in an amount between or about between 100 U/mL to 1000 U/mL, inclusive;

insulin lispro in an amount between or about between 10 U/mL to 1000 U/mL, inclusive;

a Tris buffering agent at a concentration of between or about between 25 mM to 35 mM, inclusive;

NaCl at a concentration of between or about between 50 mM to 120 mM, inclusive;

methionine at a concentration between or about between 10 mM to 30 mM, inclusive;

glycerin at a concentration from between or about between 40 mM to 60 mM, inclusive;

a surfactant that is poloxamer 188, polysorbate 20 or polysorbate 80 at a percentage (%) of mass concentration (w/v) of between or about between 0.01% to 0.05%, inclusive;

zinc at a concentration of 0.017 to 0.024 mg per 100 units of insulin (mg/100U); and

a preservative(s) that comprises a percentage (%) of mass concentration(w/v) of between or about between 0.08% to 0.17% phenol, inclusive, and between or about between 0.07% to 0.17% m-cresol.

64. The stable co-formulation of claim 63, wherein the NaCl concentration is between or about between 70 mM to 100 mM.

65. The stable co-formulation of claim 61 or claim 62, wherein:

the fast-acting insulin is a fast-acting insulin analog that is insulin aspart,

the formulation has a pH between or about between 7.0 to 7.6; and

the formulation comprises:

a hyaluronan-degrading enzyme that is a PH20 in an amount between or about between 100 U/mL to 1000 U/mL, inclusive;

insulin aspart in an amount between or about between 10 U/mL to 1000 U/mL, inclusive;

a Tris buffering agent at a concentration of between or about between 25 mM to 35 mM, inclusive;

NaCl at a concentration of between or about between 80 mM to 160 mM, inclusive;

methionine at a concentration between or about between 10 mM to 30 mM, inclusive;

glycerin at a concentration from between or about between 20 mM to 50 mM, inclusive;

a surfactant that is poloxamer 188, polysorbate 20 or polysorbate 80 at a percentage (%) of mass concentration (w/v) of between or about between 0.01% to 0.05%, inclusive;

zinc at a concentration of 0.017 to 0.024 mg per 100 units of insulin (mg/100U); and

a preservative(s) that comprises a percentage (%) of mass concentration(w/v) of between or about between 0.08% to 0.17% phenol, inclusive, and between or about between 0.07% to 0.17% m-cresol.

66. The stable co-formulation of claim 65, wherein the NaCl concentration is or is about between 70 mM to 100 mM.

67. The stable co-formulation of claim 61 or claim 62, wherein:

the fast-acting insulin is a fast-acting insulin analog that is insulin glulisine;

the formulation has a pH between or about between 7.0 to 7.6; and

the formulation comprises:

a hyaluronan-degrading enzyme that is a PH20 in an amount between or about between 100 U/mL to 1000 U/mL, inclusive;

insulin glulisine in an amount between or about between 10 U/mL to 1000 U/mL, inclusive;

a Tris buffering agent at a concentration of between or about between 25 mM to 35 mM, inclusive;

NaCl at a concentration of between or about between 80 mM to 200 mM, inclusive;

methionine at a concentration between or about between 10 mM to 30 mM, inclusive;

glycerin at a concentration from between or about between 40 mM to 60 mM, inclusive;

a surfactant that is poloxamer 188 at a percentage (%) of mass concentration (w/v) of between or about between 0.01% to 0.05%, inclusive; and

a preservative(s) that comprises a percentage (%) of mass concentration(w/v) of between or about between 0.08% to 0.17% phenol, inclusive, and between or about between 0.07% to 0.17% m-cresol.

68. The stable co-formulation of claim 67, wherein the NaCl concentration is between or about between 100 mM to 150 mM.

69. The stable co-formulation of any of claims 1-68 that is formulated for multi-dose administration.

70. A composition, comprising:

a therapeutically effective amount of a hyaluronan-degrading enzyme; and

lysyl lysine (Lys-Lys) in an amount to render the hyaluronan-degrading enzyme stable.

71. The composition of claim 70, wherein the amount of Lys-Lys is sufficient such that the hyaluronan-degrading enzyme retains at least 50% of the initial hyaluronidase activity for at least three (3) days at 37°C.

72. The composition of claim 70 or claim 71, wherein the hyaluronan-degrading enzyme retains at least 50% of the initial hyaluronidase activity at 37°C for at least 4 days, 5 days, 6 days, one week, two weeks, three weeks, one month, two months, three months, four months, five months, six months or more.

73. The composition of any of claims 70-72, wherein the hyaluronan-degrading enzyme retains at least 50% of the initial hyaluronidase activity for at least one month at 37°C.

74. The composition of any of claims 70-73 wherein the Lys-Lys concentration is between or about between 5 mM to 120 mM, 10 mM to 100 mM, 10 mM to 50 mM, 30 mM to 110 mM, 30 mM to 80 mM, 50 mM to 100 mM or 100 mM to 120 mM.

75. The composition of any of claims 70-74, wherein the pH of the formulation is between or about between 6.5 to 8.0, 6.5 to 7.4, 6.8 to 7.8, 7.0 to 7.6 or 6.8 to 7.2, inclusive.

76. The composition of any of claims 70-75, wherein the formulation further comprises a stabilizing agent.

77. The composition of claim 76, wherein the stabilizing agent is selected from among an amino acid, an amino acid derivative, an amine, a sugar, a polyol, a salt and a surfactant.

78. The composition of claim 76 or claim 77, wherein the stabilizing agent is a surfactant and the amount of surfactant, as a % of mass concentration (w/v) in the formulation, is between or about between 0.0005% to 1.0%, 0.0005% to 0.005%, 0.001% to 0.01%, 0.01% to 0.5%, 0.01% to 0.1%, 0.01% to 0.05%, or 0.01% to 0.02%, inclusive.

79. The composition of claim 77 or claim 78, wherein the surfactant is selected from among a polypropylene glycol, polyethylene glycol, glycerin, sorbitol, poloxamer and polysorbate.

80. The composition of any of claims 77-79, wherein the surfactant is selected from among poloxamer 188, polysorbate 20 and polysorbate 80.

81. The composition of any of claims 70-80, wherein the composition comprises an antioxidant.

82. The composition of claim 81, wherein the antioxidant is selected from among cysteine, tryptophan and methionine.

83. The composition of claim 82, wherein the antioxidant is methionine.

84. The composition of any of claims 81-83, wherein the antioxidant is at a concentration from between or from about between 5 mM to 50 mM, 5 mM to 40 mM, 5 mM to 20 mM or 10 mM to 20 mM, inclusive.

85. The composition of any of claims 70-84, comprising a tonicity modifier to maintain the osmolarity of between or about between 245 mOsm/kg to 500 mOsm/kg, inclusive.

86. The composition of claim 85, wherein the tonicity modifier is selected from among glycerin, salt, amino acids, polyalcohols and trehalose.

87. The composition of claim 85 or claim 86, wherein the tonicity modifier is a salt that is NaCl and the concentration of NaCl is or is about between 20 mM to 200 mM, 40 mM to 160 mM, 80 mM to 120 mM, 20 mM to 80 mM or 50 mM to 150 mM, inclusive.

88. The composition of any of claims 70-87, comprising NaCl at a concentration of less than 150 mM, less than 140 mM, less than 130 mM, less than 120 mM, less than 110 mM, less than 100 mM, less than 90 mM, less than 80 mM, less than 70 mM, less than 60 mM, less than 50 mM, less than 40 mM, less than 30 mM, less than 20 mM, less than 10 mM, or less.

89. The composition of any of claims 70-88, comprising a sufficient amount of a buffering agent to maintain the pH range of between or about between 6.5 to 8.0, 6.8 to 7.8, 7.0 to 7.6, 6.5 to 7.2, or 6.8 to 7.4, inclusive.

90. The composition of claim 89, wherein the buffering agent is selected from among Tris, histidine, phosphate and citrate.

91. The composition of claim 89 or claim 90, wherein the buffering agent is a phosphate that is sodium phosphate.

92. The composition of any of claims 89-91, wherein the concentration of the buffering agent is between or is between about 1 mM to 100 mM, 10 mM to 80 mM, 5 mM to 50 mM or 20 mM to 40 mM, inclusive.

93. The composition of any of claims 70-92, wherein the hyaluronan-degrading enzyme is a hyaluronidase or a chondroitinase.

94. The composition of any of claims 70-93, wherein the hyaluronan-degrading enzyme is a hyaluronidase that is active at neutral pH.

95. The composition of any of claims 70-94, wherein:

the hyaluronan-degrading enzyme lacks a glycosylphosphatidylinositol (GPI) anchor or is not membrane-associated when expressed from a cell; or

the hyaluronan-degrading contains C-terminal truncations of one or more amino acid residues to remove all or part of a GPI anchor.

96. The composition of any of claims 70-95, wherein the hyaluronan-degrading enzyme is a hyaluronidase that is a PH20 or a C-terminally truncated fragment thereof.

97. The composition of any of claims 70-96, wherein the hyaluronan-degrading enzyme is a PH20 polypeptide that has the sequence of amino acids set forth in any of SEQ ID NOS: 4-9, 47-48, 234-254, and 267-273, or a sequence of amino acids that exhibits at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 4-9, 47-48, 234-254, and 267-273.

98. The composition of any of claims 70-97, wherein the hyaluronan-degrading enzyme is a C-terminal truncated PH20 that comprises a sequence of amino acids set forth in any of SEQ ID NOS: 4-9.

99. The composition of any of claims 70-98, wherein the amount of a hyaluronan-degrading enzyme is between or about between 10 U/mL to 20,000 U/mL, 50 U/mL to 15,000 U/mL, 50 U/mL to 10,000 U/mL, 50 U/mL to 5000 U/mL, 50 U/mL to 4000 U/mL, 100 U/mL to 10000 U/mL, 100 U/mL to 5000 U/mL, 100 U/mL to 2000 U/mL, 300 U/mL to 5000 U/mL, 300 U/mL to 2000 U/mL, 600 U/mL

to 5000 U/mL, 600 U/mL to 2000 U/mL, 100 U/mL to 1000 U/mL, 200 U/mL to 800 U/mL, 100 U/mL to 500 U/mL, or 150 U/mL to 300 U/ml, inclusive.

100. The composition of any of claims 70-99, wherein the concentration of Lys-Lys is 5 mM to 50 mM, inclusive.

101. The composition of any of claim 70-100, wherein:

the pH of the composition is between or about between 6.5 to 7.2;

and the composition comprises:

a hyaluronan-degrading enzyme in an amount that is between or about between 100 U/mL to 500 U/mL, inclusive;

Lys-Lys at a concentration that is between or about between 5 mM to 30 mM, inclusive;

NaCl at a concentration less than 140 mM NaCl;

a surfactant that is polysorbate 80 at a percentage (%) of mass concentration (w/v) of between or about between 0.01% to 0.05%, inclusive;

methionine at a concentration that is between or about between 5 mM to 20 mM, inclusive; and

sodium phosphate at a concentration that is between or about between 5 mM to 50 mM, inclusive.

102. The composition of any of claims 70-101, wherein the hyaluronan-degrading enzyme is a PH20 or a C-terminally truncated fragment thereof.

103. The composition of any of claims 70-102, further comprising a fast-acting insulin.

104. The composition of claim 103, wherein the concentration of Lys-Lys is 30 mM to 120 mM, 50 mM to 105 mM or 80 mM to 100 mM, inclusive.

105. The composition of claim 103 or claim 104, wherein the fast-acting insulin is a regular insulin.

106. The composition of claim 105, wherein the regular insulin is a human insulin or pig insulin.

107. The composition of claim 105 or claim 106, wherein the regular insulin is an insulin with an A chain having a sequence of amino acids set forth in SEQ ID NO:103 and a B chain having a sequence of amino acids set forth in SEQ ID NO:104; or an insulin with an A chain having a sequence of amino acids set forth as amino

acid residue positions 88-108 of SEQ ID NO:123 and a B chain having a sequence of amino acids set forth as amino acid residue positions 25-54 of SEQ ID NO:123.

108. The composition of any of claims 103-107, wherein the fast-acting insulin is an insulin analog.

109. The composition of claim 108, wherein the insulin analog is selected from among insulin aspart, insulin lispro and insulin glulisine.

110. The composition of claim 108 or claim 109, wherein the insulin analog is selected from among an insulin having an A chain with a sequence of amino acids set forth in SEQ NOS:103 and a B chain having a sequence of amino acids set forth in any of SEQ NOS:147-149.

111. The composition of any of claims 103-110, wherein the amount of fast-acting insulin is between or is about between 10 U/mL to 1000 U/mL, 20 U/mL to 500 U/mL, 50 U/mL to 300 U/mL or 200 U/mL to 800 U/mL, inclusive.

112. The composition of any of claims 103-111, wherein the fast-acting insulin is an insulin analog and the hyaluronan-degrading enzyme is a PH20 or a C-terminally truncated fragment thereof.

113. The composition of any of claims 103-112, wherein:

the fast-acting insulin is an insulin analog that is glulisine and the concentration of Lys-Lys is 50 to 105 mM; or

the fast-acting insulin is an insulin analog that is insulin aspart or insulin lispro and the concentration of Lys-Lys is 80 to 100 mM.

114. The composition of any of claims 70-113 that is for single dosage administration or is for multiple dosage administration.

115. The composition of claim 114, wherein the composition is for multiple dosage administration and comprises an anti-microbially effective amount of a preservative or mixture of preservatives.

116. The composition of claim 115, wherein the preservative(s) in the formulation comprises one or more of a phenolic preservative(s), a non-phenolic preservative(s) or a phenolic preservative(s) and a non-phenolic preservative(s).

117. The composition of claim 115 or claim 116, wherein the preservative(s) is(are) selected from among phenol, m-cresol, methylparaben, benzyl alcohol, thimerosal, benzalkonium chloride, 4-chloro-1-butanol, chlorhexidine

dihydrochloride, chlorhexidine digluconate, L-phenylalanine, EDTA, bronopol, phenylmercuric acetate, glycerol, imidurea, chlorhexidine, sodium dehydroacetate, o-cresol, p-cresol, chlorocresol, cetrimide, benzethonium chloride, ethyl paraben, propylparaben, butylparaben and any combinations thereof.

118. The composition of any of claims 115-117, wherein the formulation contains a single preservative; or

contains a mixture of preservatives that contains 2, 3 or 4 different preservatives.

119. The composition of any of claims 115-118, comprising at least one phenolic preservative.

120. The composition of any of claims 115-119, wherein the preservative(s) is(are) phenol, m-cresol or phenol and m-cresol.

121. The composition of any of claims 115-120, wherein the total amount of the one or more preservative agents as a percentage (%) of mass concentration (w/v) in the formulation is or is between 0.1% and 0.4%, 0.1% to 0.3%, 0.15% to 0.325%, 0.15% to 0.25%, 0.1% to 0.2%, 0.2% to 0.3% or 0.3% to 0.4%, inclusive.

122. The composition of any of claims 115-121, wherein the preservatives are phenol and m-cresol and the amount as a % of mass concentration (w/v) in the formulation is between or about between 0.1% to 0.25% phenol and between or about between 0.05% to 0.2% m-cresol, is between or about between 0.10% to 0.2% phenol and between or about between 0.06% to 0.18% m-cresol, is between or about between 0.1% to 0.15% phenol and 0.08% to 0.15% m-cresol, is between or about between 0.10% to 0.15% phenol and between or about between 0.06 to 0.09% m-cresol or is between or about between 0.12% to 0.18% phenol and between or about between 0.14 to 0.22% m-cresol, inclusive.

123. The composition of any of claims 70-122, wherein:

the pH of the composition is between or about between 6.8 to 7.4; and

the composition comprises:

a hyaluronan-degrading enzyme that is a PH20 in an amount between or about between 100 U/mL to 1000 U/mL, inclusive;

a fast-acting insulin analog that is insulin glulisine is an amount between or about between 10 U/mL to 1000 U/mL, inclusive;

Lys-Lys at a concentration between or about between 50 mM to 105 mM, inclusive;

NaCl at a concentration of less than 100 mM;

a surfactant that is polysorbate 20 at a percentage (%) of mass concentration (w/v) of between or about between 0.0005% to 0.005%, inclusive;

methionine at a concentration between or about between 5 mM to 20 mM, inclusive; and

a preservative(s) that comprises phenol at a percentage (%) of mass concentration (w/v) of between or between about between 0.1% to 0.25% and m-cresol at a % w/v of between or between about 0.05% to 0.2%.

124. The composition of any of claims 70-122, wherein:

the pH of the composition is between or about between 6.8 to 7.4; and

the composition comprises:

a hyaluronan-degrading enzyme that is a PH20 in an amount between or about between 100 U/mL to 1000 U/mL, inclusive;

a fast-acting insulin analog that is insulin aspart or insulin lispro is an amount between or about between 10 U/mL to 1000 U/mL, inclusive;

Lys-Lys at a concentration between or about between 80 mM to 100 mM, inclusive;

NaCl at a concentration of less than 30 mM;

a surfactant that is polysorbate 20 at a percentage (%) of mass concentration (w/v) of between or about between 0.0005% to 0.005%, inclusive;

methionine at a concentration between or about between 5 mM to 20 mM, inclusive; and

a preservative(s) that comprises phenol at a percentage (%) of mass concentration (w/v) of between or between about between 0.1% to 0.25% and m-cresol at a % w/v of between or between about 0.05% to 0.2%.

125. A composition, comprising:

a therapeutically effective amount of a hyaluronan-degrading enzyme; and

$MgCl_2$ in a sufficient such that the hyaluronan-degrading enzyme retains at least 50% of the initial hyaluronidase activity for at least three (3) days at 37°C.

126. The composition of claim 125, wherein the $MgCl_2$ concentration is between or about between 50 mM to 150 mM, 75 mM to 125 mM or 80 mM to 100 mM, inclusive.

127. The composition of claim 125 or claim 126, wherein the hyaluronan-degrading enzyme retains at least 50% of the initial hyaluronidase activity at 37°C for at least 4 days, 5 days, 6 days, one week, two weeks, three weeks, one month, two months, three months, four months, five months, six months or more.

128. The composition of any of claims 125-127, wherein the pH of the composition is between or about between 6.5 to 8.0, 6.5 to 7.4, 6.8 to 7.8, 7.0 to 7.6 or 6.8 to 7.2.

129. The composition of any of claims 125-128, wherein the composition further comprises a stabilizing agent.

130. The composition of any of claims 125-129, wherein the stabilizing agent is selected from among an amino acid, an amino acid derivative, an amine, a sugar, a polyol, a salt and a surfactant.

131. The composition of claim 130, wherein the stabilizing agent is a surfactant and the amount of surfactant, as a % of mass concentration (w/v) in the formulation, is between or about between 0.0005% to 1.0%, 0.0005% to 0.005%, 0.001% to 0.01%, 0.01% to 0.5%, 0.01% to 0.1%, 0.01% to 0.05%, or 0.01% to 0.02%, inclusive.

132. The composition of claim 130 or claim 131, wherein the surfactant is selected from among a polypropylene glycol, polyethylene glycol, glycerin, sorbitol, poloxamer and polysorbate.

133. The composition of any of claims 130-132, wherein the surfactant is selected from among poloxamer 188, polysorbate 20 and polysorbate 80.

134. The composition of any of claims 125-133, wherein the composition comprises an antioxidant.

135. The composition of claim 134, wherein the antioxidant is selected from among cysteine, tryptophan and methionine.

136. The composition of claim 135, wherein the antioxidant is methionine.

137. The composition of any of claims 134-136, wherein the antioxidant is at a concentration from between or from about between 5 mM to 50 mM, 5 mM to 40 mM, 5 mM to 20 mM or 10 mM to 20 mM, inclusive.

138. The composition of any of claims 125-137, comprising a sufficient amount of a buffering agent to maintain the pH range of between or about between 6.5 to 8.0, 6.8 to 7.8, 7.0 to 7.6, 6.5 to 7.2, or 6.8 to 7.4.

139. The composition of claim 138, wherein the buffering agent is selected from among Tris, histidine, phosphate and citrate.

140. The composition of claim 138 or claim 139, wherein the buffering agent is histidine hydrochloride.

141. The composition of any of claims 138-140, wherein the concentration of the buffering agent is between or is between about 1 mM to 100 mM, 10 mM to 80 mM, 5 mM to 50 mM or 20 mM to 40 mM.

142. The composition of any of claims 125-141, wherein the hyaluronan-degrading enzyme is a hyaluronidase or a chondroitinase.

143. The composition of any of claims 125-142, wherein the hyaluronan-degrading enzyme is a hyaluronidase that is active at neutral pH.

144. The composition of any of claims 125-143, wherein the hyaluronan-degrading enzyme lacks a glycosylphosphatidylinositol (GPI) anchor or is not membrane-associated when expressed from a cell; or the hyaluronan-degrading enzyme is a hyaluronan-degrading enzyme that contains C-terminal truncations of one or more amino acid residues to remove all or part of a GPI anchor.

145. The composition of any of claims 125-144, wherein the hyaluronan-degrading enzyme is a hyaluronidase that is a PH20 or a C-terminally truncated fragment thereof.

146. The composition of any of claims 125-145, wherein the hyaluronan-degrading enzyme is a PH20 polypeptide that has the sequence of amino acids set forth in any of SEQ ID NOS: 4-9, 47-48, 234-254, and 267-273, or a sequence of amino acids that exhibits at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%,

91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 4-9, 47-48, 234-254, and 267-273.

147. The composition of any of claims 125-146, wherein the hyaluronan-degrading enzyme is a C-terminal truncated PH20 that comprises a sequence of amino acids set forth in any of SEQ ID NOS: 4-9.

148. The composition of any of claims 125-147, wherein the amount of a hyaluronan-degrading enzyme is between or about between 10 U/mL to 20,000 U/mL, 50 U/mL to 15,000 U/mL, 50 U/mL to 10,000 U/mL, 50 U/mL to 5000 U/mL, 50 U/mL to 4000 U/mL, 100 U/mL to 10000 U/mL, 100 U/mL to 5000 U/mL, 100 U/mL to 2000 U/mL, 300 U/mL to 5000 U/mL, 300 U/mL to 2000 U/mL, 600 U/mL to 5000 U/mL, 600 U/mL to 2000 U/mL, 100 U/mL to 1000 U/mL, 200 U/mL to 800 U/mL, 100 U/mL to 500 U/mL, or 150 U/mL to 300 U/ml, inclusive.

149. The composition of any of claims 125-148, wherein:

the pH of the composition is between or about between 6.5 to 7.2; and

the composition comprises:

a hyaluronan-degrading enzyme in an amount that is between or about between 100 U/mL to 500 U/mL, inclusive;

$MgCl_2$ at a concentration that is between or about between 50 mM to 150 mM, inclusive;

a surfactant that is polysorbate 80 at a percentage (%) of mass concentration (w/v) of between or about between 0.01% to 0.05%, inclusive;

methionine at a concentration that is between or about between 5 mM to 20 mM, inclusive; and

histidine/HCl at a concentration that is between or about between 5 mM to 50 mM, inclusive.

150. The stable co-formulation or composition of any of claims 1-149, wherein the volume of the composition is between or about between 0.5 mL to 50 mL, 1 mL to 40 mL, 1 mL to 20 mL, 1 mL to 10 mL, or 3 mL to 10 mL, inclusive.

151. The stable co-formulation or composition of any of claims 1-150 that is formulated for delivery using a vial, syringe, pen, reservoir for a pump or a closed loop system.

152. The stable co-formulation or composition of any of claims 1-151 that is formulated for delivery using a continuous subcutaneous insulin infusion.

153. The stable co-formulation or composition of any of claims 1-152, wherein the pH is or is about between 6.8 to 7.2

154. A syringe or vial, comprising the stable co-formulation or composition of any of claims 1-153.

155. A closed loop system, comprising the stable co-formulation or composition of any of claims 1-153.

156. An insulin pump, comprising the stable co-formulation or composition of any of claims 1-153.

157. An insulin pen, comprising the stable co-formulation or composition of any of claims 1-153.

158. A method of treating diabetes, comprising administering to a subject a therapeutically effective amount of a stable co-formulation or composition of any of claims 1-153.

159. The method of claim 158, wherein the diabetes is selected from among type 1 diabetes mellitus, type 2 diabetes mellitus and gestational diabetes.

160. A method for controlling blood glucose levels in a subject, comprising administering to a subject a therapeutically effective amount of a stable co-formulation or composition of any of claims 1-153.

161. The method of any of claims 158-160, further comprising administering a different anti-diabetic drug.

162. A stable co-formulation or composition of any of claims 1-153 for use in treating diabetes or for use in controlling blood glucose levels in a subject.

163. Use of a stable co-formulation or composition of any of claims 1-153 for treating diabetes or for controlling blood glucose levels in a subject.

164. Use of stable co-formulation or composition of any of claims 1-153 for the manufacture of a medicament for treating diabetes or for controlling blood glucose levels in a subject.

165. The stable co-formulation or composition or use of any of claims 162-164, wherein the diabetes is selected from among type 1 diabetes mellitus, type 2 diabetes mellitus and gestational diabetes.

SEQUENCE LISTING

<110> Halozyme, Inc.
Yang, Tzung-Horng
LaBarre, Michael James
Vaughn, Daniel Edward
Caster, Christopher L.
Nicol, Francois
Kim, Donghyun

<120> STABLE FORMULATIONS OF A HYALURONAN-DEGRADING ENZYME

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<140> Not yet assigned
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Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
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Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
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Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
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Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
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Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
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    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
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Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
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Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
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Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
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                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
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    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
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Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
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Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
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            180                 185                 190
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        195                 200                 205
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Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
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        275                 280                 285
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    370                 375                 380
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385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
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Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
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    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
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            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
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Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
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385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
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Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
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        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
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225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
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            260                 265                 270
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                325                 330                 335
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            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
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            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
```

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                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
        435                 440                 445


<210> 7
<211> 444
<212> PRT
<213> Homo sapiens

<220>
<223> soluble rHuPH20 1-444

<400> 7
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
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Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln
        435                 440


<210> 8
<211> 443
<212> PRT
<213> Homo sapiens

<220>
<223> soluble rHuPH20 1-443

<400> 8
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
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```
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp

                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro
        435                 440


<210> 9
<211> 442
<212> PRT
<213> Homo sapiens

<220>
<223> soluble rHuPH20 1-442

<400> 9
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
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```
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu
        435                 440


<210> 10
<211> 450
<212> PRT
<213> Bos taurus

<220>
<223> hyaluronidase

<400> 10
Met Arg Pro Phe Ser Leu Glu Val Ser Leu His Leu Pro Trp Ala Met
 1               5                  10                  15
Ala Ala His Leu Leu Pro Val Cys Thr Leu Phe Leu Asn Leu Leu Ser
            20                  25                  30
Met Thr Gln Gly Ser Arg Asp Pro Val Val Pro Asn Gln Pro Phe Thr
        35                  40                  45
Thr Ile Trp Asn Ala Asn Thr Glu Trp Cys Met Lys Lys His Gly Val
    50                  55                  60
Asp Val Asp Ile Ser Ile Phe Asp Val Val Thr Asn Pro Gly Gln Thr
65                  70                  75                  80
Phe Arg Gly Pro Asn Met Thr Ile Phe Tyr Ser Ser Gln Leu Gly Thr
                85                  90                  95
Tyr Pro Tyr Tyr Thr Ser Ala Gly Glu Pro Val Phe Gly Gly Leu Pro
            100                 105                 110
Gln Asn Ala Ser Leu Asn Ala His Leu Ala Arg Thr Phe Gln Asp Ile
        115                 120                 125
Leu Ala Ala Met Pro Glu Pro Arg Phe Ser Gly Leu Ala Val Ile Asp
    130                 135                 140
Trp Glu Ala Trp Arg Pro Arg Trp Ala Phe Asn Trp Asp Thr Lys Asp
145                 150                 155                 160
Ile Tyr Arg Gln Arg Ser Arg Ala Leu Val Gln Lys Gln His Pro Asp
                165                 170                 175
Trp Leu Ala Pro Arg Val Glu Ala Ala Ala Gln Asp Gln Phe Glu Gly
            180                 185                 190
```

```
Ala Ala Glu Glu Trp Met Ala Gly Thr Leu Lys Leu Gly Gln Ala Leu
        195                 200                 205
Arg Pro Gln Gly Leu Trp Gly Phe Tyr Asn Phe Pro Glu Cys Tyr Asn
    210                 215                 220
Tyr Asp Phe Lys Ser Pro Asn Tyr Thr Gly Arg Cys Pro Leu Asn Ile
225                 230                 235                 240
Cys Ala Gln Asn Asp Gln Leu Gly Trp Leu Trp Gly Gln Ser Arg Ala
                245                 250                 255
Leu Tyr Pro Ser Ile Tyr Leu Pro Ala Ala Leu Glu Gly Thr Lys Lys
            260                 265                 270
Thr Gln Met Phe Val Gln His Arg Val Ala Glu Ala Phe Arg Val Ala
        275                 280                 285
Ala Gly Ala Gly Asp Pro Lys Leu Pro Val Leu Pro Tyr Met Gln Leu
    290                 295                 300
Phe Tyr Asp Met Thr Asn His Phe Leu Pro Ala Glu Glu Leu Glu His
305                 310                 315                 320
Ser Leu Gly Glu Ser Ala Ala Gln Gly Ala Ala Gly Val Val Leu Trp
                325                 330                 335
Val Ser Trp Leu Ser Thr Ser Thr Lys Glu Ser Cys Gln Ala Ile Lys
            340                 345                 350
Glu Tyr Val Asp Thr Thr Leu Gly Pro Ser Ile Leu Asn Val Thr Ser
        355                 360                 365
Gly Ala Arg Leu Cys Ser Gln Val Leu Cys Ser Gly His Gly Arg Cys
    370                 375                 380
Ala Arg Arg Pro Ser Tyr Pro Lys Ala Arg Leu Ile Leu Asn Ser Thr
385                 390                 395                 400
Ser Phe Ser Ile Lys Pro Thr Pro Gly Gly Gly Pro Leu Thr Leu Gln
                405                 410                 415
Gly Ala Leu Ser Leu Glu Asp Arg Leu Arg Met Ala Val Glu Phe Glu
            420                 425                 430
Cys Arg Cys Tyr Arg Gly Trp Arg Gly Thr Arg Cys Glu Gln Trp Gly
        435                 440                 445
Met Trp
    450


<210> 11
<211> 553
<212> PRT
<213> Bos taurus

<220>
<223> PH20

<400> 11
Met Arg Met Leu Arg Arg His His Ile Ser Phe Arg Ser Phe Ala Gly
 1               5                  10                  15
Ser Ser Gly Thr Pro Gln Ala Val Phe Thr Phe Leu Leu Leu Pro Cys
            20                  25                  30
Cys Leu Ala Leu Asp Phe Arg Ala Pro Pro Leu Ile Ser Asn Thr Ser
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Val Glu Arg Cys Val Asn Arg Arg
    50                  55                  60
Phe Gln Leu Pro Pro Asp Leu Arg Leu Phe Ser Val Lys Gly Ser Pro
65                  70                  75                  80
Gln Lys Ser Ala Thr Gly Gln Phe Ile Thr Leu Phe Tyr Ala Asp Arg
                85                  90                  95
Leu Gly Tyr Tyr Pro His Ile Asp Glu Lys Thr Gly Lys Thr Val Phe
            100                 105                 110
Gly Gly Ile Pro Gln Leu Gly Asn Leu Lys Ser His Met Glu Lys Ala
        115                 120                 125
Lys Asn Asp Ile Ala Tyr Tyr Ile Pro Asn Asp Ser Val Gly Leu Ala
    130                 135                 140
Val Ile Asp Trp Glu Asn Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys
145                 150                 155                 160
Pro Lys Asp Val Tyr Arg Asp Glu Ser Val Glu Leu Val Leu Gln Lys
                165                 170                 175
Asn Pro Gln Leu Ser Phe Pro Glu Ala Ser Lys Ile Ala Lys Val Asp
```

```
            180                 185                 190
Phe Glu Thr Ala Gly Lys Ser Phe Met Gln Glu Thr Leu Lys Leu Gly
        195                 200                 205
Lys Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp
    210                 215                 220
Cys Tyr Asn His Asn His Asn Gln Pro Thr Tyr Asn Gly Asn Cys Pro
225                 230                 235                 240
Asp Val Glu Lys Arg Arg Asn Asp Asp Leu Glu Trp Leu Trp Lys Glu
                245                 250                 255
Ser Thr Ala Leu Phe Pro Ser Val Tyr Leu Asn Ile Arg Leu Lys Ser
            260                 265                 270
Thr Gln Asn Ala Ala Leu Tyr Val Arg Asn Arg Val Gln Glu Ala Ile
        275                 280                 285
Arg Leu Ser Lys Ile Ala Ser Val Glu Ser Pro Leu Pro Val Phe Val
    290                 295                 300
Tyr Ala Arg Pro Val Phe Thr Asp Gly Ser Ser Thr Tyr Leu Ser Gln
305                 310                 315                 320
Gly Asp Leu Val Asn Ser Val Gly Glu Ile Val Ser Leu Gly Ala Ser
                325                 330                 335
Gly Ile Ile Met Trp Gly Ser Leu Asn Leu Ser Leu Ser Met Gln Ser
            340                 345                 350
Cys Met Asn Leu Gly Thr Tyr Leu Asn Thr Thr Leu Asn Pro Tyr Ile
        355                 360                 365
Ile Asn Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys His
    370                 375                 380
Asn Glu Gly Val Cys Thr Arg Lys His Trp Asn Ser Ser Asp Tyr Leu
385                 390                 395                 400
His Leu Asn Pro Met Asn Phe Ala Ile Gln Thr Gly Glu Gly Gly Lys
                405                 410                 415
Tyr Thr Val Pro Gly Thr Val Thr Leu Glu Asp Leu Gln Lys Phe Ser
            420                 425                 430
Asp Thr Phe Tyr Cys Ser Cys Tyr Ala Asn Ile His Cys Lys Lys Arg
        435                 440                 445
Val Asp Ile Lys Asn Val His Ser Val Asn Val Cys Met Ala Glu Asp
    450                 455                 460
Ile Cys Ile Asp Ser Pro Val Lys Leu Gln Pro Ser Asp His Ser Ser
465                 470                 475                 480
Ser Gln Glu Ala Ser Thr Thr Thr Phe Ser Ser Ile Ser Pro Ser Thr
                485                 490                 495
Thr Thr Ala Thr Val Ser Pro Cys Thr Pro Glu Lys His Ser Pro Glu
            500                 505                 510
Cys Leu Lys Val Arg Cys Ser Glu Val Ile Pro Asn Val Thr Gln Lys
        515                 520                 525
Ala Cys Gln Ser Val Lys Leu Lys Asn Ile Ser Tyr Gln Ser Pro Ile
    530                 535                 540
Gln Asn Ile Lys Asn Gln Thr Thr Tyr
545                 550


<210> 12
<211> 331
<212> PRT
<213> Vespula vulgaris

<220>
<223> hyaluronidase A

<400> 12
Ser Glu Arg Pro Lys Arg Val Phe Asn Ile Tyr Trp Asn Val Pro Thr
 1               5                  10                  15
Phe Met Cys His Gln Tyr Asp Leu Tyr Phe Asp Glu Val Thr Asn Phe
            20                  25                  30
Asn Ile Lys Arg Asn Ser Lys Asp Asp Phe Gln Gly Asp Lys Ile Ala
        35                  40                  45
Ile Phe Tyr Asp Pro Gly Glu Phe Pro Ala Leu Leu Ser Leu Lys Asp
    50                  55                  60
Gly Lys Tyr Lys Lys Arg Asn Gly Gly Val Pro Gln Glu Gly Asn Ile
65                  70                  75                  80
```

```
Thr Ile His Leu Gln Lys Phe Ile Glu Asn Leu Asp Lys Ile Tyr Pro
                85                  90                  95
Asn Arg Asn Phe Ser Gly Ile Gly Val Ile Asp Phe Glu Arg Trp Arg
            100                 105                 110
Pro Ile Phe Arg Gln Asn Trp Gly Asn Met Lys Ile His Lys Asn Phe
        115                 120                 125
Ser Ile Asp Leu Val Arg Asn Glu His Pro Thr Trp Asn Lys Lys Met
    130                 135                 140
Ile Glu Leu Glu Ala Ser Lys Arg Phe Glu Lys Tyr Ala Arg Phe Phe
145                 150                 155                 160
Met Glu Glu Thr Leu Lys Leu Ala Lys Lys Thr Arg Lys Gln Ala Asp
                165                 170                 175
Trp Gly Tyr Tyr Gly Tyr Pro Tyr Cys Phe Asn Met Ser Pro Asn Asn
            180                 185                 190
Leu Val Pro Glu Cys Asp Val Thr Ala Met His Glu Asn Asp Lys Met
        195                 200                 205
Ser Trp Leu Phe Asn Asn Gln Asn Val Leu Leu Pro Ser Val Tyr Val
    210                 215                 220
Arg Gln Glu Leu Thr Pro Asp Gln Arg Ile Gly Leu Val Gln Gly Arg
225                 230                 235                 240
Val Lys Glu Ala Val Arg Ile Ser Asn Asn Leu Lys His Ser Pro Lys
                245                 250                 255
Val Leu Ser Tyr Trp Trp Tyr Val Tyr Gln Asp Glu Thr Asn Thr Phe
            260                 265                 270
Leu Thr Glu Thr Asp Val Lys Lys Thr Phe Gln Glu Ile Val Ile Asn
        275                 280                 285
Gly Gly Asp Gly Ile Ile Ile Trp Gly Ser Ser Ser Asp Val Asn Ser
    290                 295                 300
Leu Ser Lys Cys Lys Arg Leu Gln Asp Tyr Leu Leu Thr Val Leu Gly
305                 310                 315                 320
Pro Ile Ala Ile Asn Val Thr Glu Ala Val Asn
                325                 330
```

<210> 13
<211> 340
<212> PRT
<213> Vespula vulgaris

<220>
<223> hyaluronidase B

<400> 13

```
Asp Arg Thr Ile Trp Pro Lys Lys Gly Phe Ser Ile Tyr Trp Asn Ile
 1               5                  10                  15
Pro Thr His Phe Cys His Asn Phe Gly Val Tyr Phe Lys Glu Leu Lys
            20                  25                  30
Gln Phe Asn Ile Lys Tyr Asn Ser Met Asn Asn Phe Arg Gly Glu Thr
        35                  40                  45
Ile Ser Leu Phe Tyr Asp Pro Gly Asn Phe Pro Ser Met Val Leu Leu
    50                  55                  60
Lys Asn Gly Thr Tyr Glu Ile Arg Asn Glu Gly Val Pro Gln Lys Gly
65                  70                  75                  80
Asn Leu Thr Ile His Leu Glu Gln Phe Thr Lys Glu Leu Asp Glu Ile
                85                  90                  95
Tyr Pro Lys Lys Ile Ala Gly Gly Ile Gly Val Ile His Phe His Asn
            100                 105                 110
Trp Arg Pro Ile Phe Arg Arg Asn Val Asp Asn Leu Lys Ile Asn Lys
        115                 120                 125
Asp Ile Ser Ile Asp Leu Val Arg Lys Glu His Pro Lys Trp Asp Lys
    130                 135                 140
Ser Met Ile Glu Lys Glu Ala Ser Asn Arg Phe Glu Thr Ser Ala Lys
145                 150                 155                 160
Ile Phe Met Glu Lys Thr Leu Lys Leu Ala Lys Glu Ile Arg Lys Lys
                165                 170                 175
Thr Glu Trp Gly Tyr His Gly Tyr Pro His Cys Leu Ser Gly Ser Thr
            180                 185                 190
Asp Lys Pro Ser Phe Asp Cys Asp Ala Leu Ser Met Ser Glu Asn Asp
```

```
        195                 200                 205
Lys Met Ser Trp Leu Phe Asn Asn Gln Asn Val Leu Leu Pro Ser Ile
    210                 215                 220
Tyr Leu Lys Asn Val Leu Lys Pro Asp Glu Lys Ile His Leu Val Gln
225                 230                 235                 240
Glu Arg Leu Lys Glu Ala Ile Arg Ile Ser Lys Asn Phe Lys His Leu
                245                 250                 255
Pro Lys Val Leu Pro Tyr Trp Trp Tyr Thr Tyr Gln Asp Lys Glu Ser
            260                 265                 270
Ile Phe Leu Thr Glu Ala Asp Val Lys Asn Thr Phe Lys Glu Ile Leu
        275                 280                 285
Thr Asn Gly Ala Asp Gly Ile Ile Ile Trp Gly Val Ser Tyr Glu Leu
    290                 295                 300
Thr Asp Arg Lys Arg Cys Glu Lys Leu Lys Glu Tyr Leu Met Lys Ile
305                 310                 315                 320
Leu Gly Pro Ile Ala Phe Lys Val Thr Lys Ala Val Lys Glu Asn Thr
                325                 330                 335
Pro Leu Asn Phe
            340


<210> 14
<211> 382
<212> PRT
<213> Apis mellifera

<220>
<223> hyaluronidase

<400> 14
Met Ser Arg Pro Leu Val Ile Thr Glu Gly Met Met Ile Gly Val Leu
 1               5                  10                  15
Leu Met Leu Ala Pro Ile Asn Ala Leu Leu Leu Gly Phe Val Gln Ser
            20                  25                  30
Thr Pro Asp Asn Asn Lys Thr Val Arg Glu Phe Asn Val Tyr Trp Asn
        35                  40                  45
Val Pro Thr Phe Met Cys His Lys Tyr Gly Leu Arg Phe Glu Glu Val
    50                  55                  60
Ser Glu Lys Tyr Gly Ile Leu Gln Asn Trp Met Asp Lys Phe Arg Gly
65                  70                  75                  80
Glu Glu Ile Ala Ile Leu Tyr Asp Pro Gly Met Phe Pro Ala Leu Leu
                85                  90                  95
Lys Asp Pro Asn Gly Asn Val Val Ala Arg Asn Gly Gly Val Pro Gln
            100                 105                 110
Leu Gly Asn Leu Thr Lys His Leu Gln Val Phe Arg Asp His Leu Ile
        115                 120                 125
Asn Gln Ile Pro Asp Lys Ser Phe Pro Gly Val Gly Val Ile Asp Phe
    130                 135                 140
Glu Ser Trp Arg Pro Ile Phe Arg Gln Asn Trp Ala Ser Leu Gln Pro
145                 150                 155                 160
Tyr Lys Lys Leu Ser Val Glu Val Val Arg Arg Glu His Pro Phe Trp
                165                 170                 175
Asp Asp Gln Arg Val Glu Gln Glu Ala Lys Arg Arg Phe Glu Lys Tyr
            180                 185                 190
Gly Gln Leu Phe Met Glu Glu Thr Leu Lys Ala Ala Lys Arg Met Arg
        195                 200                 205
Pro Ala Ala Asn Trp Gly Tyr Tyr Ala Tyr Pro Tyr Cys Tyr Asn Leu
    210                 215                 220
Thr Pro Asn Gln Pro Ser Ala Gln Cys Glu Ala Thr Thr Met Gln Glu
225                 230                 235                 240
Asn Asp Lys Met Ser Trp Leu Phe Glu Ser Glu Asp Val Leu Leu Pro
                245                 250                 255
Ser Val Tyr Leu Arg Trp Asn Leu Thr Ser Gly Glu Arg Val Gly Leu
            260                 265                 270
Val Gly Gly Arg Val Lys Glu Ala Leu Arg Ile Ala Arg Gln Met Thr
        275                 280                 285
Thr Ser Arg Lys Lys Val Leu Pro Tyr Tyr Trp Tyr Lys Tyr Gln Asp
    290                 295                 300
```

Arg Arg Asp Thr Asp Leu Ser Arg Ala Asp Leu Glu Ala Thr Leu Arg
305 310 315 320
Lys Ile Thr Asp Leu Gly Ala Asp Gly Phe Ile Ile Trp Gly Ser Ser
325 330 335
Asp Asp Ile Asn Thr Lys Ala Lys Cys Leu Gln Phe Arg Glu Tyr Leu
340 345 350
Asn Asn Glu Leu Gly Pro Ala Val Lys Arg Ile Ala Leu Asn Asn Asn
355 360 365
Ala Asn Asp Arg Leu Thr Val Asp Val Ser Val Asp Gln Val
370 375 380

<210> 15
<211> 331
<212> PRT
<213> Dolichovespula maculata

<220>
<223> hyaluronidase

<400> 15
Ser Glu Arg Pro Lys Arg Val Phe Asn Ile Tyr Trp Asn Val Pro Thr
1 5 10 15
Phe Met Cys His Gln Tyr Gly Leu Tyr Phe Asp Glu Val Thr Asn Phe
20 25 30
Asn Ile Lys His Asn Ser Lys Asp Asp Phe Gln Gly Asp Lys Ile Ser
35 40 45
Ile Phe Tyr Asp Pro Gly Glu Phe Pro Ala Leu Leu Pro Leu Lys Glu
50 55 60
Gly Asn Tyr Lys Ile Arg Asn Gly Gly Val Pro Gln Glu Gly Asn Ile
65 70 75 80
Thr Ile His Leu Gln Arg Phe Ile Glu Asn Leu Asp Lys Thr Tyr Pro
85 90 95
Asn Arg Asn Phe Asn Gly Ile Gly Val Ile Asp Phe Glu Arg Trp Arg
100 105 110
Pro Ile Phe Arg Gln Asn Trp Gly Asn Met Met Ile His Lys Lys Phe
115 120 125
Ser Ile Asp Leu Val Arg Asn Glu His Pro Phe Trp Asp Lys Lys Met
130 135 140
Ile Glu Leu Glu Ala Ser Lys Arg Phe Glu Lys Tyr Ala Arg Leu Phe
145 150 155 160
Met Glu Glu Thr Leu Lys Leu Ala Lys Lys Thr Arg Lys Gln Ala Asp
165 170 175
Trp Gly Tyr Tyr Gly Tyr Pro Tyr Cys Phe Asn Met Ser Pro Asn Asn
180 185 190
Leu Val Pro Asp Cys Asp Ala Thr Ala Met Leu Glu Asn Asp Lys Met
195 200 205
Ser Trp Leu Phe Asn Asn Gln Asn Val Leu Leu Pro Ser Val Tyr Ile
210 215 220
Arg His Glu Leu Thr Pro Asp Gln Arg Val Gly Leu Val Gln Gly Arg
225 230 235 240
Val Lys Glu Ala Val Arg Ile Ser Asn Asn Leu Lys His Ser Pro Lys
245 250 255
Val Leu Ser Tyr Trp Trp Tyr Val Tyr Gln Asp Asp Thr Asn Thr Phe
260 265 270
Leu Thr Glu Thr Asp Val Lys Lys Thr Phe Gln Glu Ile Ala Ile Asn
275 280 285
Gly Gly Asp Gly Ile Ile Ile Trp Gly Ser Ser Ser Asp Val Asn Ser
290 295 300
Leu Ser Lys Cys Lys Arg Leu Arg Glu Tyr Leu Leu Thr Val Leu Gly
305 310 315 320
Pro Ile Thr Val Asn Val Thr Glu Thr Val Asn
325 330

<210> 16
<211> 367
<212> PRT

<213> Polistes annularis

<220>
<223> hyaluronidase

<400> 16
Tyr Val Ser Leu Ser Pro Asp Ser Val Phe Asn Ile Ile Thr Asp Asp
 1   5   10   15
Ile Ser His Gln Ile Leu Ser Arg Ser Asn Cys Glu Arg Ser Lys Arg
            20   25   30
Pro Lys Arg Val Phe Ser Ile Tyr Trp Asn Val Pro Thr Phe Met Cys
        35   40   45
His Gln Tyr Gly Met Asn Phe Asp Glu Val Thr Asp Phe Asn Ile Lys
    50   55   60
His Asn Ser Lys Asp Asn Phe Arg Gly Glu Thr Ile Ser Ile Tyr Tyr
65   70   75   80
Asp Pro Gly Lys Phe Pro Ala Leu Met Pro Leu Lys Asn Gly Asn Tyr
                85   90   95
Glu Glu Arg Asn Gly Gly Val Pro Gln Arg Gly Asn Ile Thr Ile His
            100   105   110
Leu Gln Gln Phe Asn Glu Asp Leu Asp Lys Met Thr Pro Asp Lys Asn
        115   120   125
Phe Gly Gly Ile Gly Val Ile Asp Phe Glu Arg Trp Lys Pro Ile Phe
    130   135   140
Arg Gln Asn Trp Gly Asn Thr Glu Ile His Lys Lys Tyr Ser Ile Glu
145   150   155   160
Leu Val Arg Lys Glu His Pro Lys Trp Ser Glu Ser Met Ile Glu Ala
                165   170   175
Glu Ala Thr Lys Lys Phe Glu Lys Tyr Ala Arg Tyr Phe Met Glu Glu
            180   185   190
Thr Leu Lys Leu Ala Lys Lys Thr Arg Lys Arg Ala Lys Trp Gly Tyr
        195   200   205
Tyr Gly Phe Pro Tyr Cys Tyr Asn Val Thr Pro Asn Asn Pro Gly Pro
    210   215   220
Asp Cys Asp Ala Lys Ala Thr Ile Glu Asn Asp Arg Leu Ser Trp Met
225   230   235   240
Tyr Asn Asn Gln Glu Ile Leu Phe Pro Ser Val Tyr Val Arg His Glu
                245   250   255
Gln Lys Pro Glu Glu Arg Val Tyr Leu Val Gln Gly Arg Ile Lys Glu
            260   265   270
Ala Val Arg Ile Ser Asn Asn Leu Glu His Ser Pro Ser Val Leu Ala
        275   280   285
Tyr Trp Trp Tyr Val Tyr Gln Asp Lys Met Asp Ile Tyr Leu Ser Glu
    290   295   300
Thr Asp Val Glu Lys Thr Phe Gln Glu Ile Val Thr Asn Gly Gly Asp
305   310   315   320
Gly Ile Ile Ile Trp Gly Ser Ser Ser Asp Val Asn Ser Leu Ser Lys
                325   330   335
Cys Lys Arg Leu Arg Glu Tyr Leu Leu Asn Thr Leu Gly Pro Phe Ala
            340   345   350
Val Asn Val Thr Glu Thr Val Asn Gly Arg Ser Ser Leu Asn Phe
        355   360   365

<210> 17
<211> 462
<212> PRT
<213> Mus musculus

<220>
<223> hyaluronidase

<400> 17
Met Leu Gly Leu Thr Gln His Ala Gln Lys Val Trp Arg Met Lys Pro
 1   5   10   15
Phe Ser Pro Glu Val Ser Pro Gly Ser Ser Pro Ala Thr Ala Gly His
            20   25   30
Leu Leu Arg Ile Ser Thr Leu Phe Leu Thr Leu Leu Glu Leu Ala Gln

```
        35                  40                  45
Val Cys Arg Gly Ser Val Val Ser Asn Arg Pro Phe Ile Thr Val Trp
    50                  55                  60
Asn Gly Asp Thr His Trp Cys Leu Thr Glu Tyr Gly Val Asp Val Asp
65                  70                  75                  80
Val Ser Val Phe Asp Val Val Ala Asn Lys Glu Gln Ser Phe Gln Gly
                85                  90                  95
Ser Asn Met Thr Ile Phe Tyr Arg Glu Glu Leu Gly Thr Tyr Pro Tyr
            100                 105                 110
Tyr Thr Pro Thr Gly Glu Pro Val Phe Gly Gly Leu Pro Gln Asn Ala
        115                 120                 125
Ser Leu Val Thr His Leu Ala His Thr Phe Gln Asp Ile Lys Ala Ala
    130                 135                 140
Met Pro Glu Pro Asp Phe Ser Gly Leu Ala Val Ile Asp Trp Glu Ala
145                 150                 155                 160
Trp Arg Pro Arg Trp Ala Phe Asn Trp Asp Ser Lys Asp Ile Tyr Arg
                165                 170                 175
Gln Arg Ser Met Glu Leu Val Gln Ala Glu His Pro Asp Trp Pro Glu
            180                 185                 190
Thr Leu Val Glu Ala Ala Ala Lys Asn Gln Phe Gln Glu Ala Ala Glu
        195                 200                 205
Ala Trp Met Ala Gly Thr Leu Gln Leu Gly Gln Val Leu Arg Pro Arg
    210                 215                 220
Gly Leu Trp Gly Tyr Tyr Gly Phe Pro Asp Cys Tyr Asn Asn Asp Phe
225                 230                 235                 240
Leu Ser Leu Asn Tyr Thr Gly Gln Cys Pro Val Phe Val Arg Asp Gln
                245                 250                 255
Asn Asp Gln Leu Gly Trp Leu Trp Asn Gln Ser Tyr Ala Leu Tyr Pro
            260                 265                 270
Ser Ile Tyr Leu Pro Ala Ala Leu Met Gly Thr Gly Lys Ser Gln Met
        275                 280                 285
Tyr Val Arg His Arg Val Gln Glu Ala Leu Arg Val Ala Ile Val Ser
    290                 295                 300
Arg Asp Pro His Val Pro Val Met Pro Tyr Val Gln Ile Phe Tyr Glu
305                 310                 315                 320
Met Thr Asp Tyr Leu Leu Pro Leu Glu Glu Leu Glu His Ser Leu Gly
                325                 330                 335
Glu Ser Ala Ala Gln Gly Val Ala Gly Ala Val Leu Trp Leu Ser Ser
            340                 345                 350
Asp Lys Thr Ser Thr Lys Glu Ser Cys Gln Ala Ile Lys Ala Tyr Met
        355                 360                 365
Asp Ser Thr Leu Gly Pro Phe Ile Val Asn Val Thr Ser Ala Ala Leu
    370                 375                 380
Leu Cys Ser Glu Ala Leu Cys Ser Gly His Gly Arg Cys Val Arg His
385                 390                 395                 400
Pro Ser Tyr Pro Glu Ala Leu Leu Thr Leu Asn Pro Ala Ser Phe Ser
                405                 410                 415
Ile Glu Leu Thr His Asp Gly Arg Pro Pro Ser Leu Lys Gly Thr Leu
            420                 425                 430
Ser Leu Lys Asp Arg Ala Gln Met Ala Met Lys Phe Arg Cys Arg Cys
        435                 440                 445
Tyr Arg Gly Trp Arg Gly Lys Trp Cys Asp Lys Arg Gly Met
    450                 455                 460


<210> 18
<211> 473
<212> PRT
<213> Mus musculus

<220>
<223> Hyaluronidase 2

<400> 18
Met Arg Ala Gly Leu Gly Pro Ile Ile Thr Leu Ala Leu Val Leu Glu
 1               5                  10                  15
Val Ala Trp Ala Gly Glu Leu Lys Pro Thr Ala Pro Pro Ile Phe Thr
            20                  25                  30
```

```
Gly Arg Pro Phe Val Val Ala Trp Asn Val Pro Thr Gln Glu Cys Ala
        35                  40                  45
Pro Arg His Lys Val Pro Leu Asp Leu Arg Ala Phe Asp Val Lys Ala
    50                  55                  60
Thr Pro Asn Glu Gly Phe Phe Asn Gln Asn Ile Thr Thr Phe Tyr Tyr
65                  70                  75                  80
Asp Arg Leu Gly Leu Tyr Pro Arg Phe Asp Ala Ala Gly Thr Ser Val
                85                  90                  95
His Gly Gly Val Pro Gln Asn Gly Ser Leu Cys Ala His Leu Pro Met
            100                 105                 110
Leu Lys Glu Ser Val Glu Arg Tyr Ile Gln Thr Gln Glu Pro Gly Gly
        115                 120                 125
Leu Ala Val Ile Asp Trp Glu Glu Trp Arg Pro Val Trp Val Arg Asn
    130                 135                 140
Trp Gln Glu Lys Asp Val Tyr Arg Gln Ser Ser Arg Gln Leu Val Ala
145                 150                 155                 160
Ser Arg His Pro Asp Trp Pro Ser Asp Arg Val Met Lys Gln Ala Gln
                165                 170                 175
Tyr Glu Phe Glu Phe Ala Ala Arg Gln Phe Met Leu Asn Thr Leu Arg
            180                 185                 190
Tyr Val Lys Ala Val Arg Pro Gln His Leu Trp Gly Phe Tyr Leu Phe
        195                 200                 205
Pro Asp Cys Tyr Asn His Asp Tyr Val Gln Asn Trp Glu Ser Tyr Thr
    210                 215                 220
Gly Arg Cys Pro Asp Val Glu Val Ala Arg Asn Asp Gln Leu Ala Trp
225                 230                 235                 240
Leu Trp Ala Glu Ser Thr Ala Leu Phe Pro Ser Val Tyr Leu Asp Glu
                245                 250                 255
Thr Leu Ala Ser Ser Val His Ser Arg Asn Phe Val Ser Phe Arg Val
            260                 265                 270
Arg Glu Ala Leu Arg Val Ala His Thr His His Ala Asn His Ala Leu
        275                 280                 285
Pro Val Tyr Val Phe Thr Arg Pro Thr Tyr Thr Arg Gly Leu Thr Gly
    290                 295                 300
Leu Ser Gln Val Asp Leu Ile Ser Thr Ile Gly Glu Ser Ala Ala Leu
305                 310                 315                 320
Gly Ser Ala Gly Val Ile Phe Trp Gly Asp Ser Glu Asp Ala Ser Ser
                325                 330                 335
Met Glu Thr Cys Gln Tyr Leu Lys Asn Tyr Leu Thr Gln Leu Leu Val
            340                 345                 350
Pro Tyr Ile Val Asn Val Ser Trp Ala Thr Gln Tyr Cys Ser Trp Thr
        355                 360                 365
Gln Cys His Gly His Gly Arg Cys Val Arg Arg Asn Pro Ser Ala Asn
    370                 375                 380
Thr Phe Leu His Leu Asn Ala Ser Ser Phe Arg Leu Val Pro Gly His
385                 390                 395                 400
Thr Pro Ser Glu Pro Gln Leu Arg Pro Glu Gly Gln Leu Ser Glu Ala
                405                 410                 415
Asp Leu Asn Tyr Leu Gln Lys His Phe Arg Cys Gln Cys Tyr Leu Gly
            420                 425                 430
Trp Gly Gly Glu Gln Cys Gln Arg Asn Tyr Lys Gly Ala Ala Gly Asn
        435                 440                 445
Ala Ser Arg Ala Trp Ala Gly Ser His Leu Thr Ser Leu Leu Gly Leu
    450                 455                 460
Val Ala Val Ala Leu Thr Trp Thr Leu
465                 470
```

<210> 19
<211> 412
<212> PRT
<213> Mus musculus

<220>
<223> hyalurinidase 3

<400> 19

```
Met Ile Met His Leu Gly Leu Met Met Val Val Gly Leu Thr Leu Cys
```

```
 1               5                  10                  15
Leu Met His Gly Gln Ala Leu Leu Gln Val Pro Glu His Pro Phe Ser
            20                  25                  30
Val Val Trp Asn Val Pro Ser Ala Arg Cys Lys Ala His Phe Gly Val
        35                  40                  45
His Leu Pro Leu Asp Ala Leu Gly Ile Val Ala Asn His Gly Gln His
    50                  55                  60
Phe His Gly Gln Asn Ile Ser Ile Phe Tyr Lys Asn Gln Phe Gly Leu
65                  70                  75                  80
Tyr Pro Tyr Phe Gly Pro Arg Gly Thr Ala His Asn Gly Gly Ile Pro
                85                  90                  95
Gln Ala Val Ser Leu Asp His His Leu Ala Arg Ala Ala His Gln Ile
            100                 105                 110
Leu His Ser Leu Gly Ser Ser Phe Ala Gly Leu Ala Val Leu Asp Trp
        115                 120                 125
Glu Glu Trp Tyr Pro Leu Trp Ala Gly Asn Trp Gly Pro His Arg Gln

    130                 135                 140
Val Tyr Leu Ala Ala Ser Trp Val Trp Thr Gln Gln Met Phe Pro Gly
145                 150                 155                 160
Leu Asp Pro Gln Glu Gln Leu His Lys Ala His Thr Ser Phe Glu Gln
                165                 170                 175
Ala Ala Arg Ala Leu Met Glu Tyr Thr Leu Gln Leu Gly Arg Thr Leu
            180                 185                 190
Arg Pro Ser Gly Leu Trp Gly Phe Tyr Arg Tyr Pro Ala Cys Gly Asn
        195                 200                 205
Gly Trp His Lys Met Ala Ser Asn Tyr Thr Gly His Cys His Ala Ala
    210                 215                 220
Ile Thr Thr Gln Asn Thr Gln Leu Arg Trp Leu Trp Ala Ala Ser Ser
225                 230                 235                 240
Ala Leu Phe Pro Ser Ile Tyr Leu Pro Pro Arg Leu Pro Leu Ala Tyr
                245                 250                 255
Arg Gln Ala Phe Val Arg His Arg Leu Glu Glu Ala Phe Arg Val Ala
            260                 265                 270
Leu Leu Glu His Ser His Pro Leu Pro Val Leu Ala Tyr Ser Arg Leu
        275                 280                 285
Thr His Arg Ser Ser Gly Arg Phe Leu Ser Leu Asp Asp Leu Met Gln
    290                 295                 300
Thr Ile Gly Val Ser Ala Ala Leu Gly Thr Ala Gly Val Val Leu Trp
305                 310                 315                 320
Gly Asp Leu Ser Phe Ser Ser Ser Glu Glu Lys Cys Trp Arg Leu His
                325                 330                 335
Asp Tyr Leu Val Gly Thr Leu Gly Pro Tyr Val Ile Asn Val Thr Lys
            340                 345                 350
Ala Asp Met Ala Cys Ser His Gln Arg Cys His Gly His Gly Arg Cys
        355                 360                 365
Ala Arg Lys Asp Pro Gly Gln Met Glu Ala Phe Leu His Leu Gln Pro
    370                 375                 380
Asp Asp Ser Leu Gly Ala Trp Asn Ser Phe Arg Cys His Cys Tyr Ser
385                 390                 395                 400
Gly Trp Ala Gly Pro Thr Cys Leu Glu Pro Lys Pro
                405                 410
```

<210> 20
<211> 435
<212> PRT
<213> Sus scrofa

<220>
<223> hyalauronidase

<400> 20

```
Met Ala Ala His Leu Leu Pro Ile Cys Thr Leu Phe Leu Asn Leu Leu
 1               5                  10                  15
Ser Val Ala Gln Gly Ser Arg Asp Pro Val Val Leu Asn Arg Pro Phe
            20                  25                  30
Thr Thr Ile Trp Asn Ala Asn Thr Gln Trp Cys Leu Lys Arg His Gly
```

```
        35                  40                  45
Val Asp Val Asp Val Ser Val Phe Glu Val Val Asn Pro Gly Gln
    50                  55                  60
Thr Phe Arg Gly Pro Asn Met Thr Ile Phe Tyr Ser Ser Gln Leu Gly
65                  70                  75                  80
Thr Tyr Pro Tyr Tyr Thr Ser Ala Gly Glu Pro Val Phe Gly Gly Leu
                85                  90                  95
Pro Gln Asn Ala Ser Leu Asp Val His Leu Asn Arg Thr Phe Lys Asp
            100                 105                 110
Ile Leu Ala Ala Met Pro Glu Ser Asn Phe Ser Gly Leu Ala Val Ile
        115                 120                 125
Asp Trp Glu Ala Trp Arg Pro Arg Trp Ala Phe Asn Trp Asp Ala Lys
    130                 135                 140
Asp Ile Tyr Arg Gln Arg Ser Arg Ala Leu Val Gln Lys Gln His Pro
145                 150                 155                 160
Asp Trp Pro Ala Pro Trp Val Glu Ala Ala Ala Gln Asp Gln Phe Gln
                165                 170                 175
Glu Ala Ala Gln Thr Trp Met Ala Gly Thr Leu Lys Leu Gly Gln Thr
            180                 185                 190
Leu Arg Pro His Gly Leu Trp Gly Phe Tyr Gly Phe Pro Asp Cys Tyr
        195                 200                 205
Asn Tyr Asp Phe Gln Ser Ser Asn Tyr Thr Gly Gln Cys Pro Pro Gly
    210                 215                 220
Val Ser Ala Gln Asn Asp Gln Leu Gly Trp Leu Trp Gly Gln Ser Arg
225                 230                 235                 240
Ala Leu Tyr Pro Ser Ile Tyr Leu Pro Ser Ala Leu Glu Gly Thr Asn
                245                 250                 255
Lys Thr Gln Leu Tyr Val Gln His Arg Val Asn Glu Ala Phe Arg Val
            260                 265                 270
Ala Ala Ala Ala Gly Asp Pro Asn Leu Pro Val Leu Pro Tyr Ala Gln
        275                 280                 285
Ile Phe His Asp Met Thr Asn Arg Leu Leu Ser Arg Glu Glu Leu Glu
    290                 295                 300
His Ser Leu Gly Glu Ser Ala Ala Gln Gly Ala Ala Gly Val Val Leu
305                 310                 315                 320
Trp Val Ser Trp Glu Asn Thr Arg Thr Lys Glu Ser Cys Gln Ser Ile
                325                 330                 335
Lys Glu Tyr Val Asp Thr Thr Leu Gly Pro Phe Ile Leu Asn Val Thr
            340                 345                 350
Ser Gly Ala Leu Leu Cys Ser Gln Ala Val Cys Ser Gly His Gly Arg
        355                 360                 365
Cys Val Arg Arg Pro Ser His Thr Glu Ala Leu Pro Ile Leu Asn Pro
    370                 375                 380
Ser Ser Phe Ser Ile Lys Pro Thr Pro Gly Gly Gly Pro Leu Thr Leu
385                 390                 395                 400
Gln Gly Ala Leu Ser Leu Lys Asp Arg Val Gln Met Ala Glu Glu Phe
                405                 410                 415
Gln Cys Arg Cys Tyr Pro Gly Trp Arg Gly Thr Trp Cys Glu Gln Gln
            420                 425                 430
Gly Thr Arg
        435


<210> 21
<211> 419
<212> PRT
<213> Sus scrofa

<220>
<223> hyaluronidase 3

<400> 21
Met Thr Met Gln Leu Gly Leu Ala Leu Val Leu Gly Val Ala Met Cys
 1               5                  10                  15
Leu Gly Cys Gly Gln Pro Leu Leu Arg Ala Pro Glu Arg Pro Phe Cys
            20                  25                  30
Val Leu Trp Asn Val Pro Ser Ala Arg Cys Lys Ala Arg Phe Gly Val
        35                  40                  45
```

```
His Leu Pro Leu Glu Ala Leu Gly Ile Thr Ala Asn His Gly Gln Arg
    50                  55                  60
Phe His Gly Gln Asn Ile Thr Ile Phe Tyr Lys Ser Gln Leu Gly Leu
65                  70                  75                  80
Tyr Pro Tyr Phe Gly Pro Arg Gly Thr Ala His Asn Gly Gly Ile Pro
                85                  90                  95
Gln Ala Val Ser Leu Asp His His Leu Ala Arg Ala Ala Tyr Gln Ile
            100                 105                 110
His Arg Ser Leu Arg Pro Gly Phe Thr Gly Leu Ala Val Leu Asp Trp
        115                 120                 125
Glu Glu Trp Cys Pro Leu Trp Ala Gly Asn Trp Gly Arg Arg Gln Ala
    130                 135                 140
Tyr Gln Ala Ala Ser Cys Ala Trp Ala Gln Arg Val Tyr Pro Asn Leu
145                 150                 155                 160
Asp Pro Gln Glu Gln Leu Cys Lys Ala Arg Ala Gly Phe Glu Glu Ala
                165                 170                 175
Ala Arg Ala Leu Met Glu Asp Thr Leu Arg Leu Gly Arg Met Leu Arg
            180                 185                 190
Pro His Gly Leu Trp Gly Phe Tyr His Tyr Pro Ala Cys Gly Asn Gly
        195                 200                 205
Trp His Gly Thr Ala Ser Asn Tyr Thr Gly His Cys His Ala Ala Ala
    210                 215                 220
Leu Ala Arg Asn Thr Gln Leu Tyr Trp Leu Trp Ala Ala Ser Ser Ala
225                 230                 235                 240
Leu Phe Pro Ser Ile Tyr Leu Pro Pro Gly Leu Pro Pro Ala Tyr His
                245                 250                 255
Gln Ala Phe Val Arg Tyr Arg Leu Glu Glu Ala Phe Arg Val Ala Leu
            260                 265                 270
Val Gly His Pro His Pro Leu Pro Val Leu Ala Tyr Ala Arg Leu Thr
        275                 280                 285
His Arg Asn Ser Gly Arg Phe Leu Ser Gln Asp Glu Leu Val Gln Thr
    290                 295                 300
Ile Gly Val Ser Ala Ala Leu Gly Ala Ser Gly Val Val Leu Trp Gly
305                 310                 315                 320
Asp Leu Ser Phe Ser Ser Ser Glu Glu Glu Cys Trp His Leu Arg Gly
                325                 330                 335
Tyr Leu Val Gly Thr Leu Gly Pro Tyr Val Ile Asn Val Thr Arg Ala
            340                 345                 350
Ala Met Ala Cys Ser His Gln Arg Cys His Gly His Gly Arg Cys Ala
        355                 360                 365
Trp Gln Asp Pro Gly Gln Leu Lys Val Phe Leu His Leu His Pro Gly
    370                 375                 380
Gly Ser Pro Gly Ala Trp Glu Ser Phe Ser Cys Arg Cys Tyr Trp Gly
385                 390                 395                 400
Trp Ala Gly Pro Thr Cys Gln Glu Pro Arg Pro Glu Leu Gly Pro Glu
                405                 410                 415
Glu Ala Thr
```

<210> 22
<211> 449
<212> PRT
<213> Rattus norvegicus

<220>
<223> hyaluronidase 1

<400> 22

```
Met Lys Pro Phe Ser Pro Glu Val Ser Pro Asp Pro Cys Pro Ala Thr
 1               5                  10                  15
Ala Ala His Leu Leu Arg Thr Tyr Thr Leu Phe Leu Thr Leu Leu Glu
            20                  25                  30
Leu Ala Gln Gly Cys Arg Gly Ser Met Val Ser Asn Arg Pro Phe Ile
        35                  40                  45
Thr Val Trp Asn Ala Asp Thr His Trp Cys Leu Lys Asp His Gly Val
    50                  55                  60
Asp Val Asp Val Ser Val Phe Asp Val Val Ala Asn Lys Glu Gln Asn
65                  70                  75                  80
```

```
Phe Gln Gly Pro Asn Met Thr Ile Phe Tyr Arg Glu Glu Leu Gly Thr
                85                  90                  95
Tyr Pro Tyr Tyr Thr Pro Thr Gly Glu Pro Val Phe Gly Gly Leu Pro
            100                 105                 110
Gln Asn Ala Ser Leu Val Thr His Leu Ala His Ala Phe Gln Asp Ile
        115                 120                 125
Lys Ala Ala Met Pro Glu Pro Asp Phe Ser Gly Leu Ala Val Ile Asp
    130                 135                 140
Trp Glu Ala Trp Arg Pro Arg Trp Ala Phe Asn Trp Asp Ser Lys Asp
145                 150                 155                 160
Ile Tyr Gln Gln Arg Ser Met Glu Leu Val Arg Ala Glu His Pro Asp
                165                 170                 175
Trp Pro Glu Thr Leu Val Glu Ala Glu Ala Gln Gly Gln Phe Gln Glu
            180                 185                 190
Ala Ala Glu Ala Trp Met Ala Gly Thr Leu Gln Leu Gly Gln Val Leu
        195                 200                 205
Arg Pro Arg Gly Leu Trp Gly Tyr Tyr Gly Phe Pro Asp Cys Tyr Asn
    210                 215                 220
Tyr Asp Phe Leu Ser Pro Asn Tyr Thr Gly Gln Cys Ser Leu Ser Ile
225                 230                 235                 240
His Asp Gln Asn Asp Gln Leu Gly Trp Leu Trp Asn Gln Ser Tyr Ala
                245                 250                 255
Leu Tyr Pro Ser Ile Tyr Leu Pro Ala Ala Leu Met Gly Thr Gly Lys
            260                 265                 270
Ser Gln Met Tyr Val Arg Tyr Arg Val Gln Glu Ala Phe Arg Leu Ala
        275                 280                 285
Leu Val Ser Arg Asp Pro His Val Pro Ile Met Pro Tyr Val Gln Ile
    290                 295                 300
Phe Tyr Glu Lys Thr Asp Tyr Leu Leu Pro Leu Glu Glu Leu Glu His
305                 310                 315                 320
Ser Leu Gly Glu Ser Ala Ala Gln Gly Ala Ala Gly Ala Val Leu Trp
                325                 330                 335
Ile Ser Ser Glu Lys Thr Ser Thr Lys Glu Ser Cys Gln Ala Ile Lys
            340                 345                 350
Ala Tyr Met Asp Ser Thr Leu Gly Pro Phe Ile Leu Asn Val Thr Ser
        355                 360                 365
Ala Ala Leu Leu Cys Ser Glu Ala Leu Cys Ser Gly Arg Gly Arg Cys
    370                 375                 380
Val Arg His Pro Ser Tyr Pro Glu Ala Leu Leu Thr Leu Ser Pro Ala
385                 390                 395                 400
Ser Phe Ser Ile Glu Pro Thr His Asp Gly Arg Pro Leu Ser Leu Lys
                405                 410                 415
Gly Thr Leu Ser Leu Lys Asp Arg Ala Gln Met Ala Met Lys Phe Lys
            420                 425                 430
Cys Arg Cys Tyr Arg Gly Trp Ser Gly Glu Trp Cys Lys Lys Gln Asp
        435                 440                 445
Met
```

<210> 23
<211> 473
<212> PRT
<213> Rattus norvegicus

<220>
<223> hyaluronidase 2

<400> 23

```
Met Arg Ala Gly Leu Gly Pro Ile Ile Thr Leu Ala Leu Val Leu Glu
 1               5                  10                  15
Val Ala Trp Ala Ser Glu Leu Lys Pro Thr Ala Pro Pro Ile Phe Thr
            20                  25                  30
Gly Arg Pro Phe Val Val Ala Trp Asn Val Pro Thr Gln Glu Cys Ala
        35                  40                  45
Pro Arg His Lys Val Pro Leu Asp Leu Arg Ala Phe Asp Val Glu Ala
    50                  55                  60
Thr Pro Asn Glu Gly Phe Phe Asn Gln Asn Ile Thr Thr Phe Tyr Tyr
65                  70                  75                  80
```

```
Asp Arg Leu Gly Leu Tyr Pro Arg Phe Asp Ala Ala Gly Met Ser Val
                85                  90                  95
His Gly Gly Val Pro Gln Asn Gly Ser Leu Cys Ala His Leu Pro Met
            100                 105                 110
Leu Lys Glu Ala Val Glu Arg Tyr Ile Gln Thr Gln Glu Pro Ala Gly
        115                 120                 125
Leu Ala Val Ile Asp Trp Glu Glu Trp Arg Pro Val Trp Val Arg Asn
    130                 135                 140
Trp Gln Glu Lys Asp Val Tyr Arg Gln Ser Ser Arg Gln Leu Val Ala
145                 150                 155                 160
Ser Arg His Pro Asp Trp Pro Ser Asp Arg Ile Val Lys Gln Ala Gln
                165                 170                 175
Tyr Glu Phe Glu Phe Ala Ala Arg Gln Phe Met Leu Asn Thr Leu Arg
            180                 185                 190
Tyr Val Lys Ala Val Arg Pro Gln His Leu Trp Gly Phe Tyr Leu Phe
        195                 200                 205
Pro Asp Cys Tyr Asn His Asp Tyr Val Gln Asn Trp Asp Ser Tyr Thr
    210                 215                 220
Gly Arg Cys Pro Asp Val Glu Val Ala Gln Asn Asp Gln Leu Ala Trp
225                 230                 235                 240
Leu Trp Ala Glu Asn Thr Ala Leu Phe Pro Ser Val Tyr Leu Asp Lys
                245                 250                 255
Thr Leu Ala Ser Ser Lys His Ser Arg Asn Phe Val Ser Phe Arg Val
            260                 265                 270
Gln Glu Ala Leu Arg Val Ala His Thr His His Ala Asn His Ala Leu
        275                 280                 285
Pro Val Tyr Val Phe Thr Arg Pro Thr Tyr Thr Arg Arg Leu Thr Glu
    290                 295                 300
Leu Asn Gln Met Asp Leu Ile Ser Thr Ile Gly Glu Ser Ala Ala Leu
305                 310                 315                 320
Gly Ser Ala Gly Val Ile Phe Trp Gly Asp Ser Val Tyr Ala Ser Ser
                325                 330                 335
Met Glu Asn Cys Gln Asn Leu Lys Lys Tyr Leu Thr Gln Thr Leu Val
            340                 345                 350
Pro Tyr Ile Val Asn Val Ser Trp Ala Thr Gln Tyr Cys Ser Trp Thr
        355                 360                 365
Gln Cys His Gly His Gly Arg Cys Val Arg Arg Asn Pro Ser Ala Ser
    370                 375                 380
Thr Phe Leu His Leu Ser Pro Ser Ser Phe Arg Leu Val Pro Gly Arg
385                 390                 395                 400
Thr Pro Ser Glu Pro Gln Leu Arg Pro Glu Gly Glu Leu Ser Glu Asp
                405                 410                 415
Asp Leu Ser Tyr Leu Gln Met His Phe Arg Cys His Cys Tyr Leu Gly
            420                 425                 430
Trp Gly Gly Glu Gln Cys Gln Trp Asn His Lys Arg Ala Ala Gly Asp
        435                 440                 445
Ala Ser Arg Ala Trp Ala Gly Ala His Leu Ala Ser Leu Leu Gly Leu
    450                 455                 460
Val Ala Met Thr Leu Thr Trp Thr Leu
465                 470


<210> 24
<211> 412
<212> PRT
<213> Rattus norvegicus

<220>
<223> hyaluronidase 3

<400> 24
Met Ile Thr Gln Leu Gly Leu Thr Leu Val Val Gly Leu Thr Leu Cys
 1                5                  10                  15
Leu Val His Val Gln Ala Leu Leu Gln Val Pro Glu Phe Pro Phe Ser
            20                  25                  30
Val Leu Trp Asn Val Pro Ser Ala Arg Cys Lys Thr Arg Phe Gly Val
        35                  40                  45
His Leu Pro Leu Asp Ala Leu Gly Ile Ile Ala Asn His Gly Gln Arg
```

```
    50                  55                  60
Phe His Gly Gln Asn Ile Thr Ile Phe Tyr Lys Asn Gln Phe Gly Leu
65                  70                  75                  80
Tyr Pro Tyr Phe Gly Pro Arg Gly Thr Ala His Asn Gly Gly Ile Pro
                85                  90                  95
Gln Ala Val Ser Leu Asp His His Leu Ala Gln Ala Ala His Gln Ile
            100                 105                 110
Leu His Asn Leu Gly Ser Ser Phe Ala Gly Leu Ala Val Leu Asp Trp
        115                 120                 125
Glu Glu Trp Tyr Pro Leu Trp Ala Gly Asn Trp Gly Thr His Arg Gln
    130                 135                 140
Val Tyr Gln Ala Ala Ser Trp Ala Trp Ala Gln Gln Met Phe Pro Asp
145                 150                 155                 160
Leu Asn Pro Gln Glu Gln Leu His Lys Ala Gln Thr Gly Phe Glu Gln
                165                 170                 175
Ala Ala Arg Ala Leu Met Glu His Thr Leu Arg Leu Gly Gln Met Leu
            180                 185                 190
Arg Pro His Gly Leu Trp Gly Phe Tyr Arg Tyr Pro Val Cys Gly Asn
        195                 200                 205
Gly Trp His Asn Met Ala Ser Asn Tyr Thr Gly His Cys His Pro Ala
    210                 215                 220
Ile Ile Thr Arg Asn Thr Gln Leu Arg Trp Leu Trp Ala Ala Ser Ser
225                 230                 235                 240
Ala Leu Phe Pro Ser Ile Tyr Leu Pro Pro Arg Leu Pro Pro Ala Tyr
                245                 250                 255
His Gln Thr Phe Val Arg His Arg Leu Glu Glu Ala Phe Arg Val Ala
            260                 265                 270
Leu Thr Gly His Ala His Pro Leu Pro Val Leu Ala Tyr Val Arg Leu
        275                 280                 285
Thr His Arg Ser Ser Gly Arg Phe Leu Ser Leu Asp Asp Leu Met Gln
    290                 295                 300
Thr Ile Gly Val Ser Ala Ala Leu Gly Ala Ala Gly Val Val Leu Trp
305                 310                 315                 320
Gly Asp Leu Ser Val Ser Ser Ser Glu Glu Glu Cys Trp Arg Leu His
                325                 330                 335
Asp Tyr Leu Val Gly Thr Leu Gly Pro Tyr Val Ile Asn Val Thr Lys
            340                 345                 350
Ala Ala Thr Ala Cys Ser His Gln Arg Cys His Gly His Gly Arg Cys
        355                 360                 365
Ser Trp Lys Asp Pro Gly Gln Met Glu Ala Phe Leu His Leu Gln Pro
    370                 375                 380
Asp Asp Asn Leu Gly Ala Trp Lys Ser Phe Arg Cys Arg Cys Tyr Leu
385                 390                 395                 400
Gly Trp Ser Gly Pro Thr Cys Leu Glu Pro Lys Pro
                405                 410
```

<210> 25
<211> 545
<212> PRT

<213> Oryctolagus cuniculus

<220>
<223> PH20

<400> 25

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Gly Ser Ala Val Glu
 1               5                  10                  15
Leu Ser Gly Val Phe Gln Ile Val Phe Ile Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Ala Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Thr Glu Phe Cys Leu Gly Lys Ser
    50                  55                  60
Gly Glu Pro Leu Asp Met Ser Leu Phe Ser Leu Phe Gly Ser Pro Arg
65                  70                  75                  80
Lys Asn Lys Thr Gly Gln Gly Ile Thr Ile Phe Tyr Val Asp Arg Leu
```

```
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Pro His Thr Gly Ala Ile Val His Gly
            100                 105                 110
Arg Ile Pro Gln Leu Gly Pro Leu Gln Gln His Leu Thr Lys Leu Arg
        115                 120                 125
Gln Glu Ile Leu Tyr Tyr Met Pro Lys Asp Asn Val Gly Leu Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Leu Pro Thr Trp Leu Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Ile Tyr Arg Ile Lys Ser Ile Glu Leu Val Lys Ser Gln His
                165                 170                 175
Pro Gln Tyr Asn His Ser Tyr Ala Thr Glu Lys Ala Lys Arg Asp Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Met Glu Glu Thr Leu Lys Leu Gly Arg
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Asp Lys Pro Asn Leu Tyr Lys Gly Ser Cys Phe
225                 230                 235                 240
Asp Ile Glu Lys Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Lys Glu
                245                 250                 255
Ser Thr Ala Leu Phe Pro Ser Val Tyr Leu Thr Ser Arg Ala Arg Ser
            260                 265                 270
Ala Thr Ala Leu Ser Lys Leu Tyr Val Val Arg Asn Arg Val His Glu
        275                 280                 285
Ala Ile Arg Val Ser Lys Ile Pro Asp Asp Lys Ser Pro Leu Pro Asn
    290                 295                 300
Phe Val Tyr Thr Arg Leu Val Phe Thr Asp Gln Ile Phe Gln Phe Leu
305                 310                 315                 320
Ser His His Asp Leu Val Tyr Thr Ile Gly Glu Ile Val Ala Leu Gly
                325                 330                 335
Ala Ser Gly Ile Val Val Trp Gly Ser Gln Ser Leu Ala Arg Ser Met
            340                 345                 350
Lys Ser Cys Leu His Leu Asp Asn Tyr Met Lys Thr Ile Leu Asn Pro
        355                 360                 365
Tyr Leu Ile Asn Val Thr Leu Ala Ala Lys Met Cys Asn Gln Val Leu
    370                 375                 380
Cys Gln Glu Gln Gly Val Cys Thr Arg Lys Asn Trp Asn Pro Asn Asp
385                 390                 395                 400
Tyr Leu His Leu Asn Pro Gly Asn Phe Ala Ile Gln Leu Gly Ser Asn
                405                 410                 415
Gly Thr Tyr Lys Val Asp Gly Lys Pro Thr Leu Thr Asp Leu Glu Gln
            420                 425                 430
Phe Ser Lys Asn Phe Gln Cys Ser Cys Tyr Thr Asn Leu Asn Cys Lys
        435                 440                 445
Glu Arg Thr Asp Met Asn Asn Val Arg Thr Val Asn Val Cys Ala Val
    450                 455                 460
Glu Asn Val Cys Ile Asp Thr Asn Val Gly Pro Gln Ala Val Thr Tyr
465                 470                 475                 480
Ala Pro Lys Glu Lys Lys Asp Val Ala His Ile Leu Ser Asn Thr Thr
                485                 490                 495
Ser Ile Asn Ser Ser Thr Thr Met Ser Leu Pro Phe Pro Arg Lys His
            500                 505                 510
Val Ser Gly Cys Leu Leu Val Leu Cys Met Tyr Ser Gln Tyr Leu Asn
        515                 520                 525
Ile Cys Tyr Arg Leu Val Ala Ile Gly Ile Gln His Gly Tyr Tyr Leu
    530                 535                 540
Lys
545
```

<210> 26
<211> 476
<212> PRT
<213> Ovis aries

<220>
<223> hyaluronidase 2

<400> 26
Met Trp Thr Gly Leu Gly Pro Ala Val Thr Leu Ala Leu Val Leu Val
 1   5   10   15
Val Ala Trp Ala Thr Glu Leu Lys Pro Thr Ala Pro Pro Ile Phe Thr
            20   25   30
Gly Arg Pro Phe Val Val Ala Trp Asp Val Pro Thr Gln Asp Cys Gly
        35   40   45
Pro Arg His Lys Met Pro Leu Asp Pro Lys Asp Met Lys Ala Phe Asp
    50   55   60
Val Gln Ala Ser Pro Asn Glu Gly Phe Val Asn Gln Asn Ile Thr Ile
65   70   75   80
Phe Tyr Arg Asp Arg Leu Gly Met Tyr Pro His Phe Asn Ser Val Gly
                85   90   95
Arg Ser Val His Gly Gly Val Pro Gln Asn Gly Ser Leu Trp Val His
            100   105   110
Leu Glu Met Leu Lys Gly His Val Glu His Tyr Ile Arg Thr Gln Glu
        115   120   125
Pro Ala Gly Leu Ala Val Ile Asp Trp Glu Asp Trp Arg Pro Val Trp
    130   135   140
Val Arg Asn Trp Gln Asp Lys Asp Val Tyr Arg Arg Leu Ser Arg Gln
145   150   155   160
Leu Val Ala Ser His His Pro Asp Trp Pro Pro Glu Arg Ile Val Lys
                165   170   175
Glu Ala Gln Tyr Glu Phe Glu Phe Ala Ala Arg Gln Phe Met Leu Glu
            180   185   190
Thr Leu Arg Phe Val Lys Ala Phe Arg Pro Arg His Leu Trp Gly Phe
        195   200   205
Tyr Leu Phe Pro Asp Cys Tyr Asn His Asp Tyr Val Gln Asn Trp Glu
    210   215   220
Thr Tyr Thr Gly Arg Cys Pro Asp Val Glu Val Ser Arg Asn Asp Gln
225   230   235   240
Leu Ser Trp Leu Trp Ala Glu Ser Thr Ala Leu Phe Pro Ser Val Tyr
                245   250   255
Leu Glu Glu Thr Leu Ala Ser Ser Thr His Gly Arg Asn Phe Val Ser
            260   265   270
Phe Arg Val Gln Glu Ala Leu Arg Val Ala Asp Val His His Ala Asn
        275   280   285
His Ala Leu Pro Val Tyr Val Phe Thr Arg Pro Thr Tyr Ser Arg Gly
    290   295   300
Leu Thr Gly Leu Ser Glu Met Asp Leu Ile Ser Thr Ile Gly Glu Ser
305   310   315   320
Ala Ala Leu Gly Ala Ala Gly Val Ile Leu Trp Gly Asp Ala Gly Phe
                325   330   335
Thr Thr Ser Asn Glu Thr Cys Arg Arg Leu Lys Asp Tyr Leu Thr Arg
            340   345   350
Ser Leu Val Pro Tyr Val Val Asn Val Ser Trp Ala Ala Gln Tyr Cys
        355   360   365
Ser Trp Ala Gln Cys His Gly His Gly Arg Cys Val Arg Arg Asp Pro
    370   375   380
Asn Ala His Thr Phe Leu His Leu Ser Ala Ser Ser Phe Arg Leu Val
385   390   395   400
Pro Ser His Ala Pro Asp Glu Pro Arg Leu Arg Pro Glu Gly Glu Leu
                405   410   415
Ser Trp Ala Asp Arg Asn His Leu Gln Thr His Phe Arg Cys Gln Cys
            420   425   430
Tyr Leu Gly Trp Gly Gly Glu Gln Cys Gln Trp Asp Arg Arg Arg Ala
        435   440   445
Ala Gly Gly Ala Ser Gly Ala Trp Ala Gly Ser His Leu Thr Gly Leu
    450   455   460
Leu Ala Val Ala Val Leu Ala Phe Thr Trp Thr Ser
465   470   475

<210> 27
<211> 114
<212> PRT
<213> Ovis aries

<220>
<223> PH20 partial sequence

<400> 27
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Leu Ser Lys Ile
 1   5   10   15
Ala Ser Val Glu Ser Pro Leu Pro Val Phe Val Tyr His Arg Pro Val
            20   25   30
Phe Thr Asp Gly Ser Ser Thr Tyr Leu Ser Gln Gly Asp Leu Val Asn
        35   40   45
Ser Val Gly Glu Ile Val Ala Leu Gly Ala Ser Gly Ile Ile Met Trp
    50   55   60
Gly Ser Leu Asn Leu Ser Leu Thr Met Gln Ser Cys Met Asn Leu Gly
65   70   75   80
Asn Tyr Leu Asn Thr Thr Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                85   90   95
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            100   105   110
Ile Arg

<210> 28
<211> 414
<212> PRT
<213> Pongo pygmaeus

<220>
<223> hyaluronidase 3

<400> 28
Met Thr Thr Arg Leu Gly Pro Ala Leu Val Leu Gly Val Ala Leu Cys
 1   5   10   15
Leu Gly Cys Gly Gln Pro Leu Pro Gln Val Pro Glu Arg Pro Phe Ser
            20   25   30
Val Leu Trp Asn Val Pro Ser Ala His Cys Lys Ser Arg Phe Gly Val
        35   40   45
His Leu Pro Leu Asn Ala Leu Gly Ile Ile Ala Asn Arg Gly Gln His
    50   55   60
Phe His Gly Gln Asn Met Thr Ile Phe Tyr Lys Asn Gln Leu Gly Leu
65   70   75   80
Tyr Pro Tyr Phe Gly Pro Lys Gly Thr Ala His Asn Gly Gly Ile Pro
                85   90   95
Gln Ala Leu Pro Leu Asp Arg His Leu Ala Leu Ala Ala Tyr Gln Ile
            100   105   110
His His Ser Leu Arg Pro Gly Phe Ala Gly Pro Ala Val Leu Asp Trp
        115   120   125
Glu Glu Trp Cys Pro Leu Trp Ala Gly Asn Trp Gly Arg Arg Arg Ala
    130   135   140
Tyr Gln Ala Ala Ser Trp Ala Trp Ala Gln Gln Val Phe Pro Asp Leu
145   150   155   160
Asp Pro Gln Glu Gln Leu Tyr Lys Ala Tyr Thr Gly Phe Glu Gln Ala
                165   170   175
Ala Arg Ala Leu Met Glu Asp Thr Leu Arg Val Ala Gln Ala Leu Arg
            180   185   190
Pro His Gly Leu Trp Gly Phe Tyr His Tyr Pro Ala Cys Gly Asn Gly
        195   200   205
Trp His Ser Met Ala Ser Asn Tyr Thr Gly Arg Cys His Ala Ala Thr
    210   215   220
Leu Ala Arg Asn Thr Gln Leu His Trp Leu Trp Ala Ala Ser Ser Ala
225   230   235   240
Leu Phe Pro Ser Ile Tyr Leu Pro Pro Arg Leu Pro Pro Ala His His
                245   250   255
Gln Ala Phe Val Arg His Arg Leu Glu Glu Ala Phe Arg Val Ala Leu
            260   265   270
Val Gly His Leu Pro Val Leu Ala Tyr Val Arg Leu Thr His Arg Arg
        275   280   285
Ser Gly Arg Phe Leu Ser Gln Asp Asp Leu Val Gln Thr Ile Gly Val

290 295 300
Ser Ala Ala Leu Gly Ala Ala Gly Val Val Leu Trp Gly Asp Leu Ser
305 310 315 320
Leu Ser Ser Ser Glu Glu Glu Cys Trp His Leu His Asp Tyr Leu Val
325 330 335
Asp Thr Leu Gly Pro Tyr Gly Ile Asn Val Thr Arg Ala Ala Met Ala
340 345 350
Cys Ser His Gln Arg Cys His Gly His Gly Arg Cys Ala Arg Arg Asp
355 360 365
Pro Gly Gln Met Glu Ala Phe Leu His Leu Trp Pro Asp Gly Ser Leu
370 375 380
Gly Asp Trp Lys Ser Phe Ser Cys His Cys Tyr Trp Gly Trp Ala Gly
385 390 395 400
Pro Thr Cys Gln Glu Pro Arg Leu Gly Pro Lys Glu Ala Val
405 410

<210> 29
<211> 510
<212> PRT
<213> Macaca fascicularis

<220>
<223> PH20

<400> 29
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
1 5 10 15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
20 25 30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Ile Ile Pro Asn Val Pro
35 40 45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
50 55 60
Asn Glu Pro Leu Asp Met Ser Leu Phe Thr Leu Met Gly Ser Pro Arg
65 70 75 80
Ile Asn Val Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
85 90 95
Gly Tyr Tyr Pro Tyr Ile Asp Leu Thr Thr Gly Val Thr Val His Gly
100 105 110
Gly Ile Pro Gln Lys Val Ser Leu Gln Asp His Leu Asp Lys Ser Lys
115 120 125
Gln Asp Ile Leu Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
130 135 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145 150 155 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
165 170 175
Val Gln Leu Ser Leu Pro Gln Ala Thr Asp Lys Ala Lys Gln Glu Phe
180 185 190
Glu Lys Ala Gly Lys Asp Phe Met Leu Glu Thr Ile Lys Leu Gly Arg
195 200 205
Ser Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
210 215 220
Tyr Asn His His Tyr Arg Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asp
225 230 235 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
245 250 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Val Val
260 265 270
Val Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
275 280 285
Ser Lys Ile Pro Asp Ala Lys Asn Pro Leu Pro Val Phe Val Tyr Ala
290 295 300
Arg Leu Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Arg Glu Glu
305 310 315 320
Leu Val Ser Thr Leu Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
325 330 335

```
Val Ile Trp Gly Ser Leu Ser Ile Thr Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Thr Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asp Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Asp Ile Arg Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val His Gly Lys Pro Thr Val Glu Asp Leu Glu Glu Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Thr Asn Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Ser Leu Lys Pro Pro Val Glu Thr Glu Gly Ser Pro Pro
465                 470                 475                 480
Ile Phe Tyr Asn Thr Ser Ser Ser Thr Val Ser Thr Thr Met Phe Ile
                485                 490                 495
Val Asn Ile Leu Phe Leu Ile Ile Ser Ser Val Ala Ser Leu
            500                 505                 510


<210> 30
<211> 529
<212> PRT
<213> Cavia porcellus

<220>
<223> PH20

<400> 30
Met Gly Ala Phe Thr Phe Lys His Ser Phe Phe Gly Ser Phe Val Glu
 1               5                  10                  15
Cys Ser Gly Val Leu Gln Thr Val Phe Ile Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Ala Asp Lys Arg Ala Pro Pro Leu Ile Pro Asn Val Pro Leu
        35                  40                  45
Leu Trp Val Trp Asn Ala Pro Thr Glu Phe Cys Ile Gly Gly Thr Asn
    50                  55                  60
Gln Pro Leu Asp Met Ser Phe Phe Ser Ile Val Gly Thr Pro Arg Lys
65                  70                  75                  80
Asn Ile Thr Gly Gln Ser Ile Thr Leu Tyr Tyr Val Asp Arg Leu Gly
                85                  90                  95
Tyr Tyr Pro Tyr Ile Asp Pro His Thr Gly Ala Ile Val His Gly Gly
            100                 105                 110
Leu Pro Gln Leu Met Asn Leu Gln Gln His Leu Arg Lys Ser Arg Gln
        115                 120                 125
Asp Ile Leu Phe Tyr Met Pro Thr Asp Ser Val Gly Leu Ala Val Ile
    130                 135                 140
Asp Trp Glu Glu Trp Arg Pro Thr Trp Thr Arg Asn Trp Arg Pro Lys
145                 150                 155                 160
Asp Ile Tyr Arg Asn Lys Ser Ile Glu Leu Val Lys Ser Gln His Pro
                165                 170                 175
Gln Tyr Asn His Ser Tyr Ala Val Ala Val Ala Lys Arg Asp Phe Glu
            180                 185                 190
Arg Thr Gly Lys Ala Phe Met Leu Glu Thr Leu Lys Leu Gly Lys Ser
        195                 200                 205
Leu Arg Pro Ser Ser Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr
    210                 215                 220
Asn Thr His Phe Thr Lys Pro Asn Tyr Asp Gly His Cys Pro Pro Ile
225                 230                 235                 240
Glu Leu Gln Arg Asn Asn Asp Leu Gln Trp Leu Trp Asn Asp Ser Thr
                245                 250                 255
Ala Leu Tyr Pro Ser Val Tyr Leu Thr Ser Arg Val Arg Ser Ser Gln
            260                 265                 270
Asn Gly Ala Leu Tyr Val Arg Asn Arg Val His Glu Ser Ile Arg Val
```

275 280 285
Ser Lys Leu Met Asp Asp Lys Asn Pro Leu Pro Ile Tyr Val Tyr Ile
290 295 300
Arg Leu Val Phe Thr Asp Gln Thr Thr Thr Phe Leu Glu Leu Asp Asp
305 310 315 320
Leu Val His Ser Val Gly Glu Ile Val Pro Leu Gly Val Ser Gly Ile
325 330 335
Ile Ile Trp Gly Ser Leu Ser Leu Thr Arg Ser Leu Val Ser Cys Ile
340 345 350
Gly Leu Glu Asn Tyr Met Lys Gly Thr Leu Leu Pro Tyr Leu Ile Asn
355 360 365
Val Thr Leu Ala Ala Lys Met Cys Gly Gln Val Leu Cys Lys Asn Gln
370 375 380
Gly Ile Cys Thr Arg Lys Asp Trp Asn Thr Asn Thr Tyr Leu His Leu
385 390 395 400
Asn Ala Thr Asn Phe Asp Ile Glu Leu Gln Gln Asn Gly Lys Phe Val
405 410 415
Val His Gly Lys Pro Ser Leu Glu Asp Leu Gln Glu Phe Ser Lys Asn
420 425 430
Phe His Cys Ser Cys Tyr Thr Asn Val Ala Cys Lys Asp Arg Leu Asp
435 440 445
Val His Asn Val Arg Ser Val Asn Val Cys Thr Ala Asn Asn Ile Cys
450 455 460
Ile Asp Ala Val Leu Asn Phe Pro Ser Leu Asp Asp Asp Asp Glu Pro
465 470 475 480
Pro Ile Thr Asp Asp Thr Ser Gln Asn Gln Asp Ser Ile Ser Asp Ile
485 490 495
Thr Ser Ser Ala Pro Pro Ser Ser His Ile Leu Pro Lys Asp Leu Ser
500 505 510
Trp Cys Leu Phe Leu Leu Ser Ile Phe Ser Gln His Trp Lys Tyr Leu
515 520 525
Leu

<210> 31
<211> 512
<212> PRT
<213> Rattus norvegicus

<220>
<223> PH20

<400> 31
Met Gly Glu Leu Gln Phe Lys Trp Leu Phe Trp Arg Ser Phe Ala Glu
1 5 10 15
Ser Gly Gly Thr Phe Gln Thr Val Leu Ile Phe Leu Phe Ile Pro Tyr
20 25 30
Ser Leu Thr Val Asp Tyr Arg Ala Thr Pro Val Leu Ser Asp Thr Thr
35 40 45
Phe Val Trp Val Trp Asn Val Pro Thr Glu Ala Cys Val Glu Asn Val
50 55 60
Thr Glu Pro Ile Asp Leu Ser Phe Phe Ser Leu Ile Gly Ser Pro Arg
65 70 75 80
Lys Thr Ala Ile Gly Gln Pro Val Thr Leu Phe Tyr Val Asp Arg Leu
85 90 95
Gly Asn Tyr Pro His Ile Asp Ala Gln Gln Thr Glu His His Gly Gly
100 105 110
Ile Pro Gln Lys Gly Asp Leu Thr Thr His Leu Val Lys Ala Lys Glu
115 120 125
Asp Val Glu Arg Tyr Ile Pro Thr Asp Lys Leu Gly Leu Ala Ile Ile
130 135 140
Asp Trp Glu Glu Trp Arg Pro Thr Trp Met Arg Asn Trp Thr Pro Lys
145 150 155 160
Asp Ile Tyr Arg Asn Lys Ser Ile Glu Leu Val Gln Ala Ala Asp Pro
165 170 175
Ala Ile Asn Ile Thr Glu Ala Thr Val Arg Ala Lys Ala Gln Phe Glu
180 185 190
Gly Ala Ala Lys Glu Phe Met Glu Gly Thr Leu Lys Leu Gly Lys His

```
        195                 200                 205
Ile Arg Pro Lys His Leu Trp Gly Phe Tyr Leu Phe Pro Asp Cys Tyr
    210                 215                 220
Asn Asn Lys Phe Gln Val Asp Asn Tyr Asp Gly Gln Cys Pro Asp Val
225                 230                 235                 240
Glu Lys Lys Arg Asn Asp Asp Leu Asp Trp Leu Trp Lys Glu Ser Thr
                245                 250                 255
Gly Leu Tyr Pro Ser Val Tyr Leu Lys Lys Asp Leu Lys Ser Ser Arg
            260                 265                 270
Lys Ala Thr Leu Tyr Val Arg Tyr Arg Val Leu Glu Ser Ile Arg Val
        275                 280                 285
Ser Lys Val Ser Asp Glu Ser Asn Pro Val Pro Ile Phe Val Tyr Ile
    290                 295                 300
Arg Leu Val Phe Thr Asp His Val Ser Glu Tyr Leu Leu Glu Asp Asp
305                 310                 315                 320
Leu Val Asn Thr Ile Gly Glu Ile Val Ala Gln Gly Thr Ser Gly Ile
                325                 330                 335
Ile Ile Trp Asp Ala Met Ser Leu Ala Gln Arg Ser Ala Gly Cys Pro
            340                 345                 350
Ile Leu Arg Gln Tyr Met Lys Thr Thr Leu Asn Pro Tyr Ile Val Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Thr Leu Cys Lys Glu Lys
    370                 375                 380
Gly Met Cys Ser Arg Lys Thr Glu Ser Ser Asp Ala Tyr Leu His Leu
385                 390                 395                 400
Asp Pro Ser Ser Phe Ser Ile Asn Val Thr Glu Ala Gly Lys Tyr Glu
                405                 410                 415
Val Leu Gly Lys Pro Glu Val Lys Asp Leu Glu Tyr Phe Ser Glu His
            420                 425                 430
Phe Lys Cys Ser Cys Phe Ser Lys Met Thr Cys Glu Glu Thr Ser Asp
        435                 440                 445
Met Arg Ser Ile Gln Asp Val Asn Val Cys Met Gly Asp Asn Val Cys
    450                 455                 460
Ile Lys Ala Thr Leu Gly Pro Asn Ser Ala Phe His Leu Leu Pro Gly
465                 470                 475                 480
Lys Gly Leu Leu Leu Met Thr Thr Leu Ala His Ile Leu His His Leu
                485                 490                 495
Pro His Asp Ile Phe Val Phe Pro Trp Lys Met Leu Val Ser Thr Pro
            500                 505                 510
```

<210> 32
<211> 512
<212> PRT
<213> Mus musculus

<220>

<223> PH20

<400> 32

```
Met Gly Glu Leu Arg Phe Lys His Leu Phe Trp Gly Ser Phe Val Glu
 1               5                  10                  15
Ser Gly Gly Thr Phe Gln Thr Val Leu Ile Phe Leu Leu Ile Pro Cys
            20                  25                  30
Ser Leu Thr Val Asp Tyr Arg Ala Ala Pro Ile Leu Ser Asn Thr Thr
        35                  40                  45
Phe Leu Trp Ile Trp Asn Val Pro Thr Glu Arg Cys Val Gly Asn Val
    50                  55                  60
Asn Asp Pro Ile Asp Leu Ser Phe Phe Ser Leu Ile Gly Ser Pro Arg
65                  70                  75                  80
Lys Thr Ala Thr Gly Gln Pro Val Thr Leu Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Leu Tyr Pro His Ile Asp Ala Asn Gln Ala Glu His Tyr Gly Gly
            100                 105                 110
Ile Pro Gln Arg Gly Asp Tyr Gln Ala His Leu Arg Lys Ala Lys Thr
        115                 120                 125
Asp Ile Glu His Tyr Ile Pro Asp Asp Lys Leu Gly Leu Ala Ile Ile
```

```
    130                 135                 140
Asp Trp Glu Glu Trp Arg Pro Thr Trp Leu Arg Asn Trp Lys Pro Lys
145                 150                 155                 160
Asp Asn Tyr Arg Asn Lys Ser Ile Glu Leu Val Gln Ser Thr Asn Pro
                165                 170                 175
Gly Leu Ser Ile Thr Glu Ala Thr Gln Lys Ala Ile Gln Gln Phe Glu
            180                 185                 190
Glu Ala Gly Arg Lys Phe Met Glu Gly Thr Leu His Leu Gly Lys Phe
        195                 200                 205
Leu Arg Pro Asn Gln Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr
    210                 215                 220
Asn Asn Lys Phe Gln Asp Pro Lys Tyr Asp Gly Gln Cys Pro Ala Val
225                 230                 235                 240
Glu Lys Lys Arg Asn Asp Asn Leu Lys Trp Leu Trp Lys Ala Ser Thr
                245                 250                 255
Gly Leu Tyr Pro Ser Val Tyr Leu Lys Lys Asp Leu Lys Ser Asn Arg
            260                 265                 270
Gln Ala Thr Leu Tyr Val Arg Tyr Arg Val Val Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Val Gly Asn Ala Ser Asp Pro Val Pro Ile Phe Val Tyr Ile
    290                 295                 300
Arg Leu Val Phe Thr Asp Arg Thr Ser Glu Tyr Leu Leu Glu Asp Asp
305                 310                 315                 320
Leu Val Asn Thr Ile Gly Glu Ile Val Ala Leu Gly Thr Ser Gly Ile
                325                 330                 335
Ile Ile Trp Asp Ala Met Ser Leu Ala Gln Arg Ala Ala Gly Cys Pro
            340                 345                 350
Ile Leu His Lys Tyr Met Gln Thr Thr Leu Asn Pro Tyr Ile Val Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Thr Leu Cys Asn Glu Lys
    370                 375                 380
Gly Met Cys Ser Arg Arg Lys Glu Ser Ser Asp Val Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Ser His Phe Asp Ile Met Leu Thr Glu Thr Gly Lys Tyr Glu
                405                 410                 415
Val Leu Gly Asn Pro Arg Val Gly Asp Leu Glu Tyr Phe Ser Glu His
            420                 425                 430
Phe Lys Cys Ser Cys Phe Ser Arg Met Thr Cys Lys Glu Thr Ser Asp
        435                 440                 445
Val Lys Asn Val Gln Asp Val Asn Val Cys Val Gly Asp Asn Val Cys
    450                 455                 460
Ile Lys Ala Lys Val Glu Pro Asn Pro Ala Phe Tyr Leu Leu Pro Gly
465                 470                 475                 480
Lys Ser Leu Leu Phe Met Thr Thr Leu Gly His Val Leu Tyr His Leu
                485                 490                 495
Pro Gln Asp Ile Phe Val Phe Pro Arg Lys Thr Leu Val Ser Thr Pro
            500                 505                 510


<210> 33
<211> 807
<212> PRT
<213> Staphylococcus aureus

<220>
<223> hyaluronidase

<400> 33
Met Thr Tyr Arg Ile Lys Lys Trp Gln Lys Leu Ser Thr Ile Thr Leu
 1               5                  10                  15
Leu Met Ala Gly Val Ile Thr Leu Asn Gly Gly Glu Phe Arg Ser Val
            20                  25                  30
Asp Lys His Gln Ile Ala Val Ala Asp Thr Asn Val Gln Thr Pro Asp
        35                  40                  45
Tyr Glu Lys Leu Arg Asn Thr Trp Leu Asp Val Asn Tyr Gly Tyr Asp
    50                  55                  60
Lys Tyr Asp Glu Asn Asn Pro Asp Met Lys Lys Lys Phe Asp Ala Thr
65                  70                  75                  80
```

```
Glu Lys Glu Ala Thr Asn Leu Leu Lys Glu Met Lys Thr Glu Ser Gly
                85                  90                  95
Arg Lys Tyr Leu Trp Ser Gly Ala Glu Thr Leu Glu Thr Asn Ser Ser
            100                 105                 110
His Met Thr Arg Thr Tyr Arg Asn Ile Glu Lys Ile Ala Glu Ala Met
        115                 120                 125
Arg Asn Pro Lys Thr Thr Leu Asn Thr Asp Glu Asn Lys Lys Lys Val
    130                 135                 140
Lys Asp Ala Leu Glu Trp Leu His Lys Asn Ala Tyr Gly Lys Glu Pro
145                 150                 155                 160
Asp Lys Lys Val Lys Glu Leu Ser Glu Asn Phe Thr Lys Thr Thr Gly
                165                 170                 175
Lys Asn Thr Asn Leu Asn Trp Trp Asp Tyr Glu Ile Gly Thr Pro Lys
            180                 185                 190
Ser Leu Thr Asn Thr Leu Ile Leu Leu Asn Asp Gln Phe Ser Asn Glu
        195                 200                 205
Glu Lys Lys Lys Phe Thr Ala Pro Ile Lys Thr Phe Ala Pro Asp Ser
    210                 215                 220
Asp Lys Ile Leu Ser Ser Val Gly Lys Ala Glu Leu Ala Lys Gly Gly
225                 230                 235                 240
Asn Leu Val Asp Ile Ser Lys Val Lys Leu Leu Glu Cys Ile Ile Glu
                245                 250                 255
Glu Asp Lys Asp Met Met Lys Lys Ser Ile Asp Ser Phe Asn Lys Val
            260                 265                 270
Phe Thr Tyr Val Gln Asp Ser Ala Thr Gly Lys Glu Arg Asn Gly Phe
        275                 280                 285
Tyr Lys Asp Gly Ser Tyr Ile Asp His Gln Asp Val Pro Tyr Thr Gly
    290                 295                 300
Ala Tyr Gly Val Val Leu Leu Glu Gly Ile Ser Gln Met Met Pro Met
305                 310                 315                 320
Ile Lys Glu Thr Pro Phe Asn Asp Lys Thr Gln Asn Asp Thr Thr Leu
                325                 330                 335
Lys Ser Trp Ile Asp Asp Gly Phe Met Pro Leu Ile Tyr Lys Gly Glu
            340                 345                 350
Met Met Asp Leu Ser Arg Gly Arg Ala Ile Ser Arg Glu Asn Glu Thr
        355                 360                 365
Ser His Ser Ala Ser Ala Thr Val Met Lys Ser Leu Leu Arg Leu Ser
    370                 375                 380
Asp Ala Met Asp Asp Ser Thr Lys Ala Lys Tyr Lys Lys Ile Val Lys
385                 390                 395                 400
Ser Ser Val Glu Ser Asp Ser Ser Tyr Lys Gln Asn Asp Tyr Leu Asn
                405                 410                 415
Ser Tyr Ser Asp Ile Asp Lys Met Lys Ser Leu Met Thr Asp Asn Ser
            420                 425                 430
Ile Ser Lys Asn Gly Leu Thr Gln Gln Leu Lys Ile Tyr Asn Asp Met
        435                 440                 445
Asp Arg Val Thr Tyr His Asn Lys Asp Leu Asp Phe Ala Phe Gly Leu
    450                 455                 460
Ser Met Thr Ser Lys Asn Val Ala Arg Tyr Glu Ser Ile Asn Gly Glu
465                 470                 475                 480
Asn Leu Lys Gly Trp His Thr Gly Ala Gly Met Ser Tyr Leu Tyr Asn
                485                 490                 495
Ser Asp Val Lys His Tyr His Asp Asn Phe Trp Val Thr Ala Asp Met
            500                 505                 510
Lys Arg Leu Ser Gly Thr Thr Thr Leu Asp Asn Glu Ile Leu Lys Asp
        515                 520                 525
Thr Asp Asp Lys Lys Ser Ser Lys Thr Phe Val Gly Gly Thr Lys Val
    530                 535                 540
Asp Asp Gln His Ala Ser Ile Gly Met Asp Phe Glu Asn Gln Asp Lys
545                 550                 555                 560
Thr Leu Thr Ala Lys Lys Ser Tyr Phe Ile Leu Asn Asp Lys Ile Val
                565                 570                 575
Phe Leu Gly Thr Gly Ile Lys Ser Thr Asp Ser Ser Lys Asn Pro Val
            580                 585                 590
Thr Thr Ile Glu Asn Arg Lys Ala Asn Gly Tyr Thr Leu Tyr Thr Asp
        595                 600                 605
Asp Lys Gln Thr Thr Asn Ser Asp Asn Gln Glu Asn Asn Ser Val Phe
    610                 615                 620
```

```
Leu Glu Ser Thr Asp Thr Lys Lys Asn Ile Gly Tyr His Phe Leu Asn
625                 630                 635                 640
Lys Pro Lys Ile Thr Val Lys Lys Glu Ser His Thr Gly Lys Trp Lys
                645                 650                 655
Glu Ile Asn Lys Ser Gln Lys Asp Thr Gln Lys Thr Asp Glu Tyr Tyr
            660                 665                 670
Glu Val Thr Gln Lys His Ser Asn Ser Asp Asn Lys Tyr Gly Tyr Val
        675                 680                 685
Leu Tyr Pro Gly Leu Ser Lys Asp Val Phe Lys Thr Lys Lys Asp Glu
    690                 695                 700
Val Thr Val Val Lys Gln Glu Asp Asp Phe His Val Val Lys Asp Asn
705                 710                 715                 720
Glu Ser Val Trp Ala Gly Val Asn Tyr Ser Asn Ser Thr Gln Thr Phe
                725                 730                 735
Asp Ile Asn Asn Thr Lys Val Glu Val Lys Ala Lys Gly Met Phe Ile
            740                 745                 750
Leu Lys Lys Lys Asp Asp Asn Thr Tyr Glu Cys Ser Phe Tyr Asn Pro
        755                 760                 765
Glu Ser Thr Asn Ser Ala Ser Asp Ile Glu Ser Lys Ile Ser Met Thr
    770                 775                 780
Gly Tyr Ser Ile Thr Asn Lys Asn Thr Ser Thr Ser Asn Glu Ser Gly
785                 790                 795                 800
Val His Phe Glu Leu Thr Lys
                805
```

<210> 34
<211> 371
<212> PRT
<213> Streptococcus pyogenes bacteriophage H4489A

<220>
<223> hyaluronidase

<400> 34

```
Met Thr Glu Asn Ile Pro Leu Arg Val Gln Phe Lys Arg Met Ser Ala
 1               5                  10                  15
Asp Glu Trp Ala Arg Ser Asp Val Ile Leu Leu Glu Gly Glu Ile Gly
            20                  25                  30
Phe Glu Thr Asp Thr Gly Phe Ala Lys Phe Gly Asp Gly Gln Asn Thr
        35                  40                  45
Phe Ser Lys Leu Lys Tyr Leu Thr Gly Pro Lys Gly Pro Lys Gly Asp
    50                  55                  60
Thr Gly Leu Gln Gly Lys Thr Gly Gly Thr Gly Pro Arg Gly Pro Ala
65                  70                  75                  80
Gly Lys Pro Gly Thr Thr Asp Tyr Asp Gln Leu Gln Asn Lys Pro Asp
                85                  90                  95
Leu Gly Ala Phe Ala Gln Lys Glu Glu Thr Asn Ser Lys Ile Thr Lys
            100                 105                 110
Leu Glu Ser Ser Lys Ala Asp Lys Ser Ala Val Tyr Ser Lys Ala Glu
        115                 120                 125
Ser Lys Ile Glu Leu Asp Lys Lys Leu Ser Leu Thr Gly Gly Ile Val
    130                 135                 140
Thr Gly Gln Leu Gln Phe Lys Pro Asn Lys Ser Gly Ile Lys Pro Ser
145                 150                 155                 160
Ser Ser Val Gly Gly Ala Ile Asn Ile Asp Met Ser Lys Ser Glu Gly
                165                 170                 175
Ala Ala Met Val Met Tyr Thr Asn Lys Asp Thr Thr Asp Gly Pro Leu
            180                 185                 190
Met Ile Leu Arg Ser Asp Lys Asp Thr Phe Asp Gln Ser Ala Gln Phe
        195                 200                 205
Val Asp Tyr Ser Gly Lys Thr Asn Ala Val Asn Ile Val Met Arg Gln
    210                 215                 220
Pro Ser Ala Pro Asn Phe Ser Ser Ala Leu Asn Ile Thr Ser Ala Asn
225                 230                 235                 240
Glu Gly Gly Ser Ala Met Gln Ile Arg Gly Val Glu Lys Ala Leu Gly
                245                 250                 255
Thr Leu Lys Ile Thr His Glu Asn Pro Asn Val Glu Ala Lys Tyr Asp
```

260 265 270
Glu Asn Ala Ala Ala Leu Ser Ile Asp Ile Val Lys Lys Gln Lys Gly
275 280 285
Gly Lys Gly Thr Ala Ala Gln Gly Ile Tyr Ile Asn Ser Thr Ser Gly
290 295 300
Thr Ala Gly Lys Met Leu Arg Ile Arg Asn Lys Asn Glu Asp Lys Phe
305 310 315 320
Tyr Val Gly Pro Asp Gly Gly Phe His Ser Gly Ala Asn Ser Thr Val
325 330 335
Ala Gly Asn Leu Thr Val Lys Asp Pro Thr Ser Gly Lys His Ala Ala
340 345 350
Thr Lys Asp Tyr Val Asp Glu Lys Ile Ala Glu Leu Lys Lys Leu Ile
355 360 365
Leu Lys Lys
370

<210> 35
<211> 1628
<212> PRT
<213> Clostridium perfringens

<220>
<223> hyaluronidase

<400> 35
Met Asn Lys Asn Ile Arg Lys Ile Ile Thr Ser Thr Val Leu Ala Ala
1 5 10 15
Met Thr Ile Ser Val Leu Pro Ser Asn Leu Val Val Phe Ala Thr Asp
20 25 30
Gly Ile Thr Glu Asn Phe Tyr Glu Ile Tyr Pro Lys Pro Gln Glu Ile
35 40 45
Ser Tyr Ser Gly Gly Glu Phe Gln Ile Ser Asp Glu Ile Asn Ile Val
50 55 60
Tyr Asp Asp Gly Ile Asp Thr Tyr Thr Lys Lys Arg Val Asp Glu Val
65 70 75 80
Leu Glu Ala Ser Asn Leu Glu Ala Thr Val Ser Asn Glu Ile Val Pro
85 90 95
Gly Lys Thr Asn Phe Leu Val Gly Ile Asn Glu Ser Gly Gly Val Val
100 105 110
Asp Asn Tyr Phe Asn Lys Asn Ile Pro His Asp Glu Ser Phe Phe Asp
115 120 125
Glu Lys Met Asp Ala Asn Ile Val Ser Val Lys Asp Gly Val Ile Gly
130 135 140
Val Ile Gly Glu Asp Thr Asp Ser Ala Phe Tyr Gly Val Thr Thr Leu
145 150 155 160
Lys His Val Phe Asn Gln Leu Glu Glu Gly Asn Lys Ile Gln Ser Phe
165 170 175
Arg Ala Asp Asp Tyr Ala Glu Val Ala His Arg Gly Phe Ile Glu Gly
180 185 190
Tyr Tyr Gly Asn Pro Trp Ser Asn Glu Asp Arg Ala Glu Leu Met Lys
195 200 205
Phe Gly Gly Asp Tyr Lys Leu Asn Gln Tyr Val Phe Ala Pro Lys Asp
210 215 220
Asp Pro Tyr His Asn Ser Lys Trp Arg Asp Leu Tyr Pro Glu Glu Lys
225 230 235 240
Leu Ser Glu Ile Lys Lys Leu Ala Gln Val Gly Asn Glu Thr Lys Asn
245 250 255
Arg Tyr Val Tyr Ala Leu His Pro Phe Met Asn Asn Pro Val Arg Phe
260 265 270
Asp Thr Glu Glu Asn Tyr Gln Asn Asp Leu Gly Val Ile Lys Ala Lys
275 280 285
Phe Thr Gln Leu Leu Glu Asn Asp Val Arg Gln Phe Ala Ile Leu Ala
290 295 300
Asp Asp Ala Ser Ala Pro Ala Gln Gly Ala Ser Met Tyr Val Lys Leu
305 310 315 320
Leu Thr Asp Leu Thr Arg Trp Leu Glu Glu Gln Gln Ser Thr Tyr Pro
325 330 335

```
Asp Leu Lys Thr Asp Leu Met Phe Cys Pro Ser Asp Tyr Tyr Gly Asn
            340                 345                 350
Gly Ser Ser Ala Gln Leu Lys Glu Leu Asn Lys Ala Glu Asp Asn Val
        355                 360                 365
Ser Ile Val Met Thr Gly Gly Arg Ile Trp Gly Glu Val Asp Glu Asn
    370                 375                 380
Phe Ala Asn Asn Phe Met Asn Asn Ile Ser Thr Glu Gly His Pro Gly
385                 390                 395                 400
Arg Ala Pro Phe Phe Trp Ile Asn Trp Pro Cys Ser Asp Asn Ser Lys
                405                 410                 415
Gln His Leu Ile Met Gly Gly Asn Asp Thr Phe Leu His Pro Gly Val
            420                 425                 430
Asp Pro Ser Lys Ile Asp Gly Ile Val Leu Asn Pro Met Gln Gln Ala
        435                 440                 445
Glu Ala Asn Lys Ser Ala Leu Phe Ala Ile Ala Asp Tyr Ala Trp Asn
    450                 455                 460
Ile Trp Asp Asn Lys Glu Glu Ala Asp Glu Asn Trp Asn Asp Ser Phe
465                 470                 475                 480
Lys Tyr Met Asp His Gly Thr Ala Glu Glu Thr Asn Ser Ser Leu Ala
                485                 490                 495
Leu Arg Glu Ile Ser Lys His Met Ile Asn Gln Asn Met Asp Gly Arg
            500                 505                 510
Val Arg Pro Leu Gln Glu Ser Val Glu Leu Ala Pro Lys Leu Glu Ala
        515                 520                 525
Phe Lys Gln Lys Tyr Asp Ser Gly Ala Ser Ile Lys Glu Asp Ala Leu
    530                 535                 540
Glu Leu Ile Ala Glu Phe Thr Asn Leu Gln Lys Ala Ala Asp Tyr Tyr
545                 550                 555                 560
Lys Asn Asn Pro Gly Asn Glu Arg Thr Arg Asp Gln Ile Ile Tyr Trp
                565                 570                 575
Leu Asn Cys Trp Glu Asp Thr Met Asp Ala Ala Ile Gly Tyr Leu Lys
            580                 585                 590
Ser Ala Ile Ala Ile Glu Glu Gly Asp Asp Glu Ala Ala Trp Ala Asn
        595                 600                 605
Tyr Ser Glu Ala Gln Gly Ala Phe Glu Lys Ser Lys Thr Tyr Gly Phe
    610                 615                 620
His Tyr Val Asp His Thr Glu Tyr Ala Glu Val Gly Val Gln His Ile
625                 630                 635                 640
Val Pro Phe Ile Lys Ser Met Gly Gln Asn Leu Ser Val Val Ile Gly
                645                 650                 655
Ser Ile Val Asp Pro Asn Arg Ile Ile Ala Thr Tyr Ile Ser Asn Arg
            660                 665                 670
Gln Asp Ala Pro Thr Gly Asn Pro Asp Asn Ile Phe Asp Asn Asn Ala
        675                 680                 685
Ser Thr Glu Leu Val Tyr Lys Asn Pro Asn Arg Ile Asp Val Gly Thr
    690                 695                 700
Tyr Val Gly Val Lys Tyr Ser Asn Pro Ile Thr Leu Asn Asn Val Glu
705                 710                 715                 720
Phe Leu Met Gly Ala Asn Ser Asn Pro Asn Asp Thr Met Gln Lys Ala
                725                 730                 735
Lys Ile Gln Tyr Thr Val Asp Gly Arg Glu Trp Ile Asp Leu Glu Glu
            740                 745                 750
Gly Val Glu Tyr Thr Met Pro Gly Ala Ile Lys Val Glu Asn Leu Asp
        755                 760                 765
Leu Lys Val Arg Gly Val Arg Leu Ile Ala Thr Glu Ala Arg Glu Asn
    770                 775                 780
Thr Trp Leu Gly Val Arg Asp Ile Asn Val Asn Lys Lys Glu Asp Ser
785                 790                 795                 800
Asn Ser Gly Val Glu Phe Asn Pro Ser Leu Ile Arg Ser Glu Ser Trp
                805                 810                 815
Gln Val Tyr Glu Gly Asn Glu Ala Asn Leu Leu Asp Gly Asp Asp Asn
            820                 825                 830
Thr Gly Val Trp Tyr Lys Thr Leu Asn Gly Asp Thr Ser Leu Ala Gly
        835                 840                 845
Glu Phe Ile Gly Leu Asp Leu Gly Lys Glu Ile Lys Leu Asp Gly Ile
    850                 855                 860
Arg Phe Val Ile Gly Lys Asn Gly Gly Gly Ser Ser Asp Lys Trp Asn
865                 870                 875                 880
```

```
Lys Phe Lys Leu Glu Tyr Ser Leu Asp Asn Glu Ser Trp Thr Thr Ile
                885                 890                 895
Lys Glu Tyr Asp Lys Thr Gly Ala Pro Ala Gly Lys Asp Val Ile Glu
            900                 905                 910
Glu Ser Phe Glu Thr Pro Ile Ser Ala Lys Tyr Ile Arg Leu Thr Asn
        915                 920                 925
Met Glu Asn Ile Asn Lys Trp Leu Thr Phe Ser Glu Phe Ala Ile Ile
    930                 935                 940
Ser Asp Glu Leu Glu Asn Ala Gly Asn Lys Glu Asn Val Tyr Thr Asn
945                 950                 955                 960
Thr Glu Leu Asp Leu Leu Ser Leu Ala Lys Glu Asp Val Thr Lys Leu
                965                 970                 975
Ile Pro Thr Asp Asp Ile Ser Leu Asn His Gly Glu Tyr Ile Gly Val
            980                 985                 990
Lys Leu Asn Arg Ile Lys Asp Leu Ser Asn Ile Asn Leu Glu Ile Ser
        995                 1000                1005
Asn Asp Thr Gly Leu Lys Leu Gln Ser Ser Met Asn Gly Val Glu Trp
    1010                1015                1020
Thr Glu Ile Thr Asp Lys Asn Thr Leu Glu Asp Gly Arg Tyr Val Arg
1025                1030                1035                1040
Leu Ile Asn Thr Ser Asn Glu Ala Val Asn Phe Asn Leu Thr Lys Phe
                1045                1050                1055
Glu Val Asn Ser Asn Glu Val Tyr Glu Pro Ser Leu Val Asp Ala Tyr
            1060                1065                1070
Val Gly Asp Asp Gly Ala Lys Lys Ala Val Asp Gly Asp Leu Lys Thr
        1075                1080                1085
Arg Val Lys Phe Leu Gly Ala Pro Ser Thr Gly Asp Thr Ile Val Tyr
    1090                1095                1100
Asp Leu Gly Gln Glu Ile Leu Val Asp Asn Leu Lys Tyr Val Val Leu
1105                1110                1115                1120
Asp Thr Glu Val Asp His Val Arg Asp Gly Lys Ile Gln Leu Ser Leu
                1125                1130                1135
Asp Gly Glu Thr Trp Thr Asp Ala Ile Thr Ile Gly Asp Gly Val Glu
            1140                1145                1150
Asn Gly Val Asp Asp Met Phe Ser Thr Pro Leu Lys Asn Gly Tyr Lys
        1155                1160                1165
His Gly Asn Gln Ser Gly Gly Ile Val Pro Ile Asp Ser Ala Tyr Val
    1170                1175                1180
Glu Gly Asp Asn Leu Asn Gln Lys Ala Arg Tyr Val Arg Ile Leu Phe
1185                1190                1195                1200
Thr Ala Pro Tyr Arg His Arg Trp Thr Val Ile Asn Glu Leu Met Ile
                1205                1210                1215
Asn Asn Gly Glu Tyr Ile Ser Thr Val Asn Asp Pro Thr Tyr Ile Ser
            1220                1225                1230
Asn Pro Ile Glu Glu Arg Gly Phe Ala Pro Ser Asn Leu Arg Asp Gly
        1235                1240                1245
Asn Leu Thr Thr Ser Tyr Lys Pro Asn Thr Asn Asn Gly Glu Ile Ser
    1250                1255                1260
Glu Gly Ser Ile Thr Tyr Arg Leu Ser Glu Lys Thr Asp Val Arg Lys
1265                1270                1275                1280
Val Thr Ile Val Gln Ser Gly Ser Ser Ile Ser Asn Ala Lys Val Met
                1285                1290                1295
Ala Arg Val Gly Asp Gly Ser Glu Asn Val Thr Asp Gln Trp Val Gln
            1300                1305                1310
Leu Gly Thr Leu Ser Asn Ser Leu Asn Glu Phe Ile Asn Arg Asp Tyr
        1315                1320                1325
Asn Asn Ile Tyr Glu Ile Lys Ile Glu Trp Thr Asp Val Ala Pro Asn
    1330                1335                1340
Ile Tyr Glu Ile Ile Thr Leu Asn Gln Glu Phe Glu Phe Pro Val Asn
1345                1350                1355                1360
Asp Ser Leu Lys Ala Lys Tyr Asp Glu Leu Ile Asn Leu Ser Gly Asp
                1365                1370                1375
Glu Tyr Thr Leu Ser Ser Phe Glu Thr Leu Lys Glu Ala Leu Asn Glu
            1380                1385                1390
Ala Lys Ser Ile Leu Asp Asp Ser Asn Ser Ser Gln Lys Lys Ile Asp
        1395                1400                1405
Lys Ala Leu Glu Lys Leu Asn Lys Ala Glu Glu Arg Leu Asp Leu Arg
    1410                1415                1420
```

Ala Thr Asp Phe Glu Asp Phe Asn Lys Val Leu Thr Leu Gly Asn Ser
1425 1430 1435 1440
Leu Val Glu Glu Glu Tyr Thr Ala Glu Ser Trp Ala Leu Phe Ser Glu
1445 1450 1455
Val Leu Glu Ala Ala Asn Glu Ala Asn Lys Asn Lys Ala Asp Tyr Thr
1460 1465 1470
Gln Asp Gln Ile Asn Gln Ile Val Ile Asp Leu Asp Ala Ser Ile Lys
1475 1480 1485
Ala Leu Val Lys Glu Thr Pro Glu Val Asp Lys Thr Asn Leu Gly Glu
1490 1495 1500
Leu Ile Asn Gln Gly Lys Ser Leu Leu Asp Glu Ser Val Glu Gly Phe
1505 1510 1515 1520
Asn Val Gly Glu Tyr His Lys Gly Ala Lys Asp Gly Leu Thr Val Glu
1525 1530 1535
Ile Asn Lys Ala Glu Glu Val Phe Asn Lys Glu Asp Ala Thr Glu Glu
1540 1545 1550
Glu Ile Asn Leu Ala Lys Glu Ser Leu Glu Gly Ala Ile Ala Arg Phe
1555 1560 1565
Asn Ser Leu Leu Ile Glu Glu Ser Thr Gly Asp Phe Asn Gly Asn Gly
1570 1575 1580
Lys Ile Asp Ile Gly Asp Leu Ala Met Val Ser Lys Asn Ile Gly Ser
1585 1590 1595 1600
Thr Thr Asn Thr Ser Leu Asp Leu Asn Lys Asp Gly Ser Ile Asp Glu
1605 1610 1615
Tyr Glu Ile Ser Phe Ile Asn His Arg Ile Leu Asn
1620 1625

<210> 36
<211> 435
<212> PRT
<213> Homo sapiens

<220>
<223> Hyaluronidase-1 [Precursor]

<400> 36
Met Ala Ala His Leu Leu Pro Ile Cys Ala Leu Phe Leu Thr Leu Leu
1 5 10 15
Asp Met Ala Gln Gly Phe Arg Gly Pro Leu Leu Pro Asn Arg Pro Phe
20 25 30
Thr Thr Val Trp Asn Ala Asn Thr Gln Trp Cys Leu Glu Arg His Gly
35 40 45
Val Asp Val Asp Val Ser Val Phe Asp Val Val Ala Asn Pro Gly Gln
50 55 60
Thr Phe Arg Gly Pro Asp Met Thr Ile Phe Tyr Ser Ser Gln Leu Gly
65 70 75 80
Thr Tyr Pro Tyr Tyr Thr Pro Thr Gly Glu Pro Val Phe Gly Gly Leu
85 90 95
Pro Gln Asn Ala Ser Leu Ile Ala His Leu Ala Arg Thr Phe Gln Asp
100 105 110
Ile Leu Ala Ala Ile Pro Ala Pro Asp Phe Ser Gly Leu Ala Val Ile
115 120 125
Asp Trp Glu Ala Trp Arg Pro Arg Trp Ala Phe Asn Trp Asp Thr Lys
130 135 140
Asp Ile Tyr Arg Gln Arg Ser Arg Ala Leu Val Gln Ala Gln His Pro
145 150 155 160
Asp Trp Pro Ala Pro Gln Val Glu Ala Val Ala Gln Asp Gln Phe Gln
165 170 175
Gly Ala Ala Arg Ala Trp Met Ala Gly Thr Leu Gln Leu Gly Arg Ala
180 185 190
Leu Arg Pro Arg Gly Leu Trp Gly Phe Tyr Gly Phe Pro Asp Cys Tyr
195 200 205
Asn Tyr Asp Phe Leu Ser Pro Asn Tyr Thr Gly Gln Cys Pro Ser Gly
210 215 220
Ile Arg Ala Gln Asn Asp Gln Leu Gly Trp Leu Trp Gly Gln Ser Arg
225 230 235 240
Ala Leu Tyr Pro Ser Ile Tyr Met Pro Ala Val Leu Glu Gly Thr Gly

```
                245                 250                 255
Lys Ser Gln Met Tyr Val Gln His Arg Val Ala Glu Ala Phe Arg Val
            260                 265                 270
Ala Val Ala Ala Gly Asp Pro Asn Leu Pro Val Leu Pro Tyr Val Gln
        275                 280                 285
Ile Phe Tyr Asp Thr Thr Asn His Phe Leu Pro Leu Asp Glu Leu Glu
    290                 295                 300
His Ser Leu Gly Glu Ser Ala Ala Gln Gly Ala Ala Gly Val Val Leu
305                 310                 315                 320
Trp Val Ser Trp Glu Asn Thr Arg Thr Lys Glu Ser Cys Gln Ala Ile
                325                 330                 335
Lys Glu Tyr Met Asp Thr Thr Leu Gly Pro Phe Ile Leu Asn Val Thr
            340                 345                 350
Ser Gly Ala Leu Leu Cys Ser Gln Ala Leu Cys Ser Gly His Gly Arg
        355                 360                 365
Cys Val Arg Arg Thr Ser His Pro Lys Ala Leu Leu Leu Leu Asn Pro
    370                 375                 380
Ala Ser Phe Ser Ile Gln Leu Thr Pro Gly Gly Gly Pro Leu Ser Leu
385                 390                 395                 400
Arg Gly Ala Leu Ser Leu Glu Asp Gln Ala Gln Met Ala Val Glu Phe
                405                 410                 415
Lys Cys Arg Cys Tyr Pro Gly Trp Gln Ala Pro Trp Cys Glu Arg Lys
            420                 425                 430
Ser Met Trp
        435
```

<210> 37
<211> 473
<212> PRT
<213> Homo sapiens

<220>
<223> Hyaluronidase-2 [Precursor]

<400> 37

```
Met Arg Ala Gly Pro Gly Pro Thr Val Thr Leu Ala Leu Val Leu Ala
 1               5                  10                  15
Val Ala Trp Ala Met Glu Leu Lys Pro Thr Ala Pro Pro Ile Phe Thr
            20                  25                  30
Gly Arg Pro Phe Val Val Ala Trp Asp Val Pro Thr Gln Asp Cys Gly
        35                  40                  45
Pro Arg Leu Lys Val Pro Leu Asp Leu Asn Ala Phe Asp Val Gln Ala
    50                  55                  60
Ser Pro Asn Glu Gly Phe Val Asn Gln Asn Ile Thr Ile Phe Tyr Arg
65                  70                  75                  80
Asp Arg Leu Gly Leu Tyr Pro Arg Phe Asp Ser Ala Gly Arg Ser Val
                85                  90                  95
His Gly Gly Val Pro Gln Asn Val Ser Leu Trp Ala His Arg Lys Met
            100                 105                 110
Leu Gln Lys Arg Val Glu His Tyr Ile Arg Thr Gln Glu Ser Ala Gly
        115                 120                 125
Leu Ala Val Ile Asp Trp Glu Asp Trp Arg Pro Val Trp Val Arg Asn
    130                 135                 140
Trp Gln Asp Lys Asp Val Tyr Arg Arg Leu Ser Arg Gln Leu Val Ala
145                 150                 155                 160
Ser Arg His Pro Asp Trp Pro Pro Asp Arg Ile Val Lys Gln Ala Gln
                165                 170                 175
Tyr Glu Phe Glu Phe Ala Ala Gln Gln Phe Met Leu Glu Thr Leu Arg
            180                 185                 190
Tyr Val Lys Ala Val Arg Pro Arg His Leu Trp Gly Phe Tyr Leu Phe
        195                 200                 205
Pro Asp Cys Tyr Asn His Asp Tyr Val Gln Asn Trp Glu Ser Tyr Thr
    210                 215                 220
Gly Arg Cys Pro Asp Val Glu Val Ala Arg Asn Asp Gln Leu Ala Trp
225                 230                 235                 240
Leu Trp Ala Glu Ser Thr Ala Leu Phe Pro Ser Val Tyr Leu Asp Glu
                245                 250                 255
```

```
Thr Leu Ala Ser Ser Arg His Gly Arg Asn Phe Val Ser Phe Arg Val
            260                 265                 270
Gln Glu Ala Leu Arg Val Ala Arg Thr His His Ala Asn His Ala Leu
        275                 280                 285
Pro Val Tyr Val Phe Thr Arg Pro Thr Tyr Ser Arg Arg Leu Thr Gly
    290                 295                 300
Leu Ser Glu Met Asp Leu Ile Ser Thr Ile Gly Glu Ser Ala Ala Leu
305                 310                 315                 320
Gly Ala Ala Gly Val Ile Leu Trp Gly Asp Ala Gly Tyr Thr Thr Ser
                325                 330                 335
Thr Glu Thr Cys Gln Tyr Leu Lys Asp Tyr Leu Thr Arg Leu Leu Val
            340                 345                 350
Pro Tyr Val Val Asn Val Ser Trp Ala Thr Gln Tyr Cys Ser Arg Ala
        355                 360                 365
Gln Cys His Gly His Gly Arg Cys Val Arg Arg Asn Pro Ser Ala Ser
    370                 375                 380
Thr Phe Leu His Leu Ser Thr Asn Ser Phe Arg Leu Val Pro Gly His
385                 390                 395                 400
Ala Pro Gly Glu Pro Gln Leu Arg Pro Val Gly Glu Leu Ser Trp Ala
                405                 410                 415
Asp Ile Asp His Leu Gln Thr His Phe Arg Cys Gln Cys Tyr Leu Gly
            420                 425                 430
Trp Ser Gly Glu Gln Cys Gln Trp Asp His Arg Gln Ala Ala Gly Gly
        435                 440                 445
Ala Ser Glu Ala Trp Ala Gly Ser His Leu Thr Ser Leu Leu Ala Leu
    450                 455                 460
Ala Ala Leu Ala Phe Thr Trp Thr Leu
465                 470


<210> 38
<211> 417
<212> PRT
<213> Homo sapiens

<220>
<223> Hyaluronidase-3 [Precursor]

<400> 38
Met Thr Thr Gln Leu Gly Pro Ala Leu Val Leu Gly Val Ala Leu Cys
 1                5                  10                  15
Leu Gly Cys Gly Gln Pro Leu Pro Gln Val Pro Glu Arg Pro Phe Ser
            20                  25                  30
Val Leu Trp Asn Val Pro Ser Ala His Cys Glu Ala Arg Phe Gly Val
        35                  40                  45
His Leu Pro Leu Asn Ala Leu Gly Ile Ile Ala Asn Arg Gly Gln His
    50                  55                  60
Phe His Gly Gln Asn Met Thr Ile Phe Tyr Lys Asn Gln Leu Gly Leu
65                  70                  75                  80
Tyr Pro Tyr Phe Gly Pro Arg Gly Thr Ala His Asn Gly Gly Ile Pro
                85                  90                  95
Gln Ala Leu Pro Leu Asp Arg His Leu Ala Leu Ala Ala Tyr Gln Ile
            100                 105                 110
His His Ser Leu Arg Pro Gly Phe Ala Gly Pro Ala Val Leu Asp Trp
        115                 120                 125
Glu Glu Trp Cys Pro Leu Trp Ala Gly Asn Trp Gly Arg Arg Arg Ala
    130                 135                 140
Tyr Gln Ala Ala Ser Trp Ala Trp Ala Gln Gln Val Phe Pro Asp Leu
145                 150                 155                 160
Asp Pro Gln Glu Gln Leu Tyr Lys Ala Tyr Thr Gly Phe Glu Gln Ala
                165                 170                 175
Ala Arg Ala Leu Met Glu Asp Thr Leu Arg Val Ala Gln Ala Leu Arg
            180                 185                 190
Pro His Gly Leu Trp Gly Phe Tyr His Tyr Pro Ala Cys Gly Asn Gly
        195                 200                 205
Trp His Ser Met Ala Ser Asn Tyr Thr Gly Arg Cys His Ala Ala Thr
    210                 215                 220
Leu Ala Arg Asn Thr Gln Leu His Trp Leu Trp Ala Ala Ser Ser Ala
```

225 230 235 240
Leu Phe Pro Ser Ile Tyr Leu Pro Pro Arg Leu Pro Pro Ala His His
245 250 255
Gln Ala Phe Val Arg His Arg Leu Glu Glu Ala Phe Arg Val Ala Leu
260 265 270
Val Gly His Arg His Pro Leu Pro Val Leu Ala Tyr Val Arg Leu Thr
275 280 285
His Arg Arg Ser Gly Arg Phe Leu Ser Gln Asp Asp Leu Val Gln Ser
290 295 300
Ile Gly Val Ser Ala Ala Leu Gly Ala Ala Gly Val Val Leu Trp Gly
305 310 315 320
Asp Leu Ser Leu Ser Ser Ser Glu Glu Glu Cys Trp His Leu His Asp
325 330 335
Tyr Leu Val Asp Thr Leu Gly Pro Tyr Val Ile Asn Val Thr Arg Ala
340 345 350
Ala Met Ala Cys Ser His Gln Arg Cys His Gly His Gly Arg Cys Ala
355 360 365
Arg Arg Asp Pro Gly Gln Met Glu Ala Phe Leu His Leu Trp Pro Asp
370 375 380
Gly Ser Leu Gly Asp Trp Lys Ser Phe Ser Cys His Cys Tyr Trp Gly
385 390 395 400
Trp Ala Gly Pro Thr Cys Gln Glu Pro Arg Pro Gly Pro Lys Glu Ala
405 410 415
Val

<210> 39
<211> 481
<212> PRT
<213> Homo sapiens

<220>
<223> Hyaluronidase-4

<400> 39
Met Lys Val Leu Ser Glu Gly Gln Leu Lys Leu Cys Val Val Gln Pro
1 5 10 15
Val His Leu Thr Ser Trp Leu Leu Ile Phe Phe Ile Leu Lys Ser Ile
20 25 30
Ser Cys Leu Lys Pro Ala Arg Leu Pro Ile Tyr Gln Arg Lys Pro Phe
35 40 45
Ile Ala Ala Trp Asn Ala Pro Thr Asp Gln Cys Leu Ile Lys Tyr Asn
50 55 60
Leu Arg Leu Asn Leu Lys Met Phe Pro Val Ile Gly Ser Pro Leu Ala
65 70 75 80
Lys Ala Arg Gly Gln Asn Val Thr Ile Phe Tyr Val Asn Arg Leu Gly
85 90 95
Tyr Tyr Pro Trp Tyr Thr Ser Gln Gly Val Pro Ile Asn Gly Gly Leu
100 105 110
Pro Gln Asn Ile Ser Leu Gln Val His Leu Glu Lys Ala Asp Gln Asp
115 120 125
Ile Asn Tyr Tyr Ile Pro Ala Glu Asp Phe Ser Gly Leu Ala Val Ile
130 135 140
Asp Trp Glu Tyr Trp Arg Pro Gln Trp Ala Arg Asn Trp Asn Ser Lys
145 150 155 160
Asp Val Tyr Arg Gln Lys Ser Arg Lys Leu Ile Ser Asp Met Gly Lys
165 170 175
Asn Val Ser Ala Thr Asp Ile Glu Tyr Leu Ala Lys Val Thr Phe Glu
180 185 190
Glu Ser Ala Lys Ala Phe Met Lys Glu Thr Ile Lys Leu Gly Ile Lys
195 200 205
Ser Arg Pro Lys Gly Leu Trp Gly Tyr Tyr Leu Tyr Pro Asp Cys His
210 215 220
Asn Tyr Asn Val Tyr Ala Pro Asn Tyr Ser Gly Ser Cys Pro Glu Asp
225 230 235 240
Glu Val Leu Arg Asn Asn Glu Leu Ser Trp Leu Trp Asn Ser Ser Ala
245 250 255
Ala Leu Tyr Pro Ser Ile Gly Val Trp Lys Ser Leu Gly Asp Ser Glu

```
            260                 265                 270
Asn Ile Leu Arg Phe Ser Lys Phe Arg Val His Glu Ser Met Arg Ile
        275                 280                 285
Ser Thr Met Thr Ser His Asp Tyr Ala Leu Pro Val Phe Val Tyr Thr
    290                 295                 300
Arg Leu Gly Tyr Arg Asp Glu Pro Leu Phe Phe Leu Ser Lys Gln Asp
305                 310                 315                 320
Leu Val Ser Thr Ile Gly Glu Ser Ala Ala Leu Gly Ala Ala Gly Ile
                325                 330                 335
Val Ile Trp Gly Asp Met Asn Leu Thr Ala Ser Lys Ala Asn Cys Thr
            340                 345                 350
Lys Val Lys Gln Phe Val Ser Ser Asp Leu Gly Ser Tyr Ile Ala Asn
        355                 360                 365
Val Thr Arg Ala Ala Glu Val Cys Ser Leu His Leu Cys Arg Asn Asn
    370                 375                 380
Gly Arg Cys Ile Arg Lys Met Trp Asn Ala Pro Ser Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Ala Ser Tyr His Ile Glu Ala Ser Glu Asp Gly Glu Phe Thr
                405                 410                 415
Val Lys Gly Lys Ala Ser Asp Thr Asp Leu Ala Val Met Ala Asp Thr
            420                 425                 430
Phe Ser Cys His Cys Tyr Gln Gly Tyr Glu Gly Ala Asp Cys Arg Glu
        435                 440                 445
Ile Lys Thr Ala Asp Gly Cys Ser Gly Val Ser Pro Ser Pro Gly Ser
    450                 455                 460
Leu Met Thr Leu Cys Leu Leu Leu Leu Ala Ser Tyr Arg Ser Ile Gln
465                 470                 475                 480
Leu
```

<210> 40
<211> 467
<212> PRT
<213> Homo sapiens

<220>
<223> sHuPH20 precursor 1-467

<400> 40

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
```

225 230 235 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
245 250 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
260 265 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
275 280 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
290 295 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305 310 315 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
325 330 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
340 345 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
355 360 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
370 375 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385 390 395 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
405 410 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
420 425 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
435 440 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
450 455 460
Ile Asp Ala
465

<210> 41
<211> 477
<212> PRT
<213> Homo sapiens

<220>
<223> sHuPH20 precursor 1-477

<400> 41
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
1 5 10 15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
20 25 30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
35 40 45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
50 55 60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65 70 75 80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
85 90 95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
100 105 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
115 120 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
130 135 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145 150 155 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
165 170 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
180 185 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
195 200 205

```
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu
465                 470                 475
```

<210> 42
<211> 478
<212> PRT
<213> Homo sapiens

<220>
<223> sHuPH20 precursor 1-478

<400> 42

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
```

```
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro
465                 470                 475
```

<210> 43
<211> 479
<212> PRT
<213> Homo sapiens

<220>
<223> sHuPH20 precursor 1-479

<400> 43

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1                5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
```

```
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln
465                 470                 475


<210> 44
<211> 480
<212> PRT
<213> Homo sapiens

<220>
<223> sHuPH20 precursor 1-480

<400> 44
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1                5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
```

```
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480


<210> 45
<211> 481
<212> PRT
<213> Homo sapiens

<220>
<223> sHuPH20 precursor 1-481

<400> 45
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
```

```
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe
```

<210> 46
<211> 483
<212> PRT
<213> Homo sapiens

<220>
<223> sHuPH20 precursor 1-483

<400> 46

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
```

```
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn
```

<210> 47
<211> 432
<212> PRT
<213> Homo sapiens

<220>
<223> sHuPH20 mature 36-467

<400> 47

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
```

```
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430


<210> 48
<211> 448
<212> PRT
<213> Homo sapiens

<220>
<223> sHuPH20 mature 36-483

<400> 48
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Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
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Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
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<210> 49
<211> 1446
<212> DNA
<213> Homo sapiens

<220>
<223> DNA encoding soluble rHuPH20 "precursor"

<400> 49

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cctcctgtta ttccaaatgt gcctttcctc tgggcctgga atgccccaag tgaattttgt 180
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ggaatggctg ttattgactg ggaagaatgg agacccactt gggcaagaaa ctggaaacct 480
aaagatgttt acaagaatag gtctattgaa ttggttcagc aacaaaatgt acaacttagt 540
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<211> 509
<212> PRT
<213> Homo sapiens

<220>
<223> PH20 variant P48A

<400> 50
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            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Ala
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
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Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser Ile Leu Phe Leu Ile Ile Ser Ser Val Ala Ser Leu
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<210> 51
<211> 509
<212> PRT
<213> Homo sapiens

<220>
<223> precursor PH20 variant L499W

<400> 51
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            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
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        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser Ile Trp Phe Leu Ile Ile Ser Ser Val Ala Ser Leu
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<210> 52
<211> 6630
<212> DNA
<213> Artificial Sequence

<220>
<223> HZ24 vector

<400> 52

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tatgagaaag cgccacgctt cccgaaggga gaaaggcgga caggtatccg gtaagcggca 6420
gggtcggaac aggagagcgc acgagggagc ttccaggggg aaacgcctgg tatctttata 6480
gtcctgtcgg gtttcgccac ctctgacttg agcgtcgatt tttgtgatgc tcgtcagggg 6540
ggcggagcct atggaaaaac gccagcaacg cggccttttt acggttcctg gccttttgct 6600
ggccttttgc tcacatggct cgacagatct 6630

<210> 53
<211> 186
<212> PRT
<213> Mus musculus

<220>
<223> dihydrofolate reductase

<400> 53
Val Arg Pro Leu Asn Cys Ile Val Ala Val Ser Gln Asn Met Gly Ile
 1   5   10   15
Gly Lys Asn Gly Asp Leu Pro Trp Pro Pro Leu Arg Asn Glu Phe Lys
            20   25   30
Tyr Phe Gln Arg Met Thr Thr Thr Ser Ser Val Glu Gly Lys Gln Asn
        35   40   45
Leu Val Ile Met Gly Arg Lys Thr Trp Phe Ser Ile Pro Glu Lys Asn
    50   55   60
Arg Pro Leu Lys Asp Arg Ile Asn Ile Val Leu Ser Arg Glu Leu Lys
65   70   75   80
Glu Pro Pro Arg Gly Ala His Phe Leu Ala Lys Ser Leu Asp Asp Ala
                85   90   95
Leu Arg Leu Ile Glu Gln Pro Glu Leu Ala Ser Lys Val Asp Met Val
            100   105   110
Trp Ile Val Gly Gly Ser Ser Val Tyr Gln Glu Ala Met Asn Gln Pro
        115   120   125
Gly His Leu Arg Leu Phe Val Thr Arg Ile Met Gln Glu Phe Glu Ser
    130   135   140
Asp Thr Phe Phe Pro Glu Ile Asp Leu Gly Lys Tyr Lys Leu Leu Pro
145   150   155   160
Glu Tyr Pro Gly Val Leu Ser Glu Val Gln Glu Glu Lys Gly Ile Lys
                165   170   175
Tyr Lys Phe Glu Val Tyr Glu Lys Lys Asp
            180   185

<210> 54
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> His tag

<400> 54
His His His His His His
 1   5

<210> 55
<211> 8
<212> PRT

<213> Artificial Sequence

<220>
<223> Flag tag

<400> 55
Asp Tyr Lys Asp Asp Asp Asp Lys
 1   5

<210> 56
<211> 1449
<212> DNA
<213> Homo sapiens

<220>
<223> Gen2 mRNA sequence

<400> 56
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tcccagatag ttttcacctt ccttctgatt ccatgttgct tgactctgaa tttcagagca 120
cctcctgtta ttccaaatgt gcctttcctc tgggcctgga atgccccaag tgaattttgt 180
cttggaaaat ttgatgagcc actagatatg agcctcttct ctttcatagg aagcccccga 240
ataaacgcca ccgggcaagg tgttacaata ttttatgttg atagacttgg ctactatcct 300
tacatagatt caatcacagg agtaactgtg aatggaggaa tcccccagaa gatttcctta 360
caagaccatc tggacaaagc taagaaagac attacatttt atatgccagt agacaatttg 420
ggaatggctg ttattgactg ggaagaatgg agacccactt gggcaagaaa ctggaaacct 480
aaagatgttt acaagaatag gtctattgaa ttggttcagc aacaaaatgt acaacttagt 540
ctcacagagg ccactgagaa agcaaaacaa gaatttgaaa aggcagggaa ggatttcctg 600
gtagagacta taaaattggg aaaattactt cggccaaatc acttgtgggg ttattatctt 660
tttccggatt gttacaacca tcactataag aaacccggtt acaatggaag ttgcttcaat 720
gtagaaataa aaagaaatga tgatctcagc tggttgtgga atgaaagcac tgctctttac 780
ccatccattt atttgaacac tcagcagtct cctgtagctg ctacactcta tgtgcgcaat 840
cgagttcggg aagccatcag agtttccaaa atacctgatg caaaaagtcc acttccggtt 900
tttgcatata cccgcatagt ttttactgat caagttttga aattcctttc tcaagatgaa 960
cttgtgtata catttggcga aactgttgct ctgggtgctt ctggaattgt aatatgggga 1020
accctcagta taatgcgaag tatgaaatct tgcttgctcc tagacaatta catggagact 1080
atactgaatc cttacataat caacgtcaca ctagcagcca aaatgtgtag tcaagtgctt 1140
tgccaggagc aaggagtgtg tataaggaaa aactggaatt caagtgacta tcttcacctc 1200
aacccagata attttgctat tcaacttgag aaaggtggaa agttcacagt acgtggaaaa 1260
ccgacacttg aagacctgga gcaatttttct gaaaaatttt attgcagctg ttatagcacc 1320
ttgagttgta aggagaaagc tgatgtaaaa gacactgatg ctgttgatgt gtgtattgct 1380
gatggtgtct gtatagatgc ttttctaaaa cctcccatgg agacagaaga acctcaaatt 1440
ttctactga   1449

<210> 57
<211> 17
<212> PRT
<213> Homo sapiens

<220>
<223> C-terminal peptide cleaved from soluble rHuPH20 aa
      431-447

<400> 57
Asp Ala Phe Lys Leu Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe
 1   5   10   15
Tyr

<210> 58
<211> 16
<212> PRT
<213> Homo sapiens

<220>

<223> C-terminal peptide cleaved from soluble rHuPH20 aa
431-446

<400> 58
Asp Ala Phe Lys Leu Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe
1 5 10 15

<210> 59
<211> 15
<212> PRT
<213> Homo sapiens

<220>
<223> C-terminal peptide cleaved from soluble rHuPH20 aa
431-445

<400> 59
Asp Ala Phe Lys Leu Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
1 5 10 15

<210> 60
<211> 14
<212> PRT
<213> Homo sapiens

<220>
<223> C-terminal peptide cleaved from soluble rHuPH20 aa
431-444

<400> 60
Asp Ala Phe Lys Leu Pro Pro Met Glu Thr Glu Glu Pro Gln
1 5 10

<210> 61
<211> 13
<212> PRT
<213> Homo sapiens

<220>
<223> C-terminal peptide cleaved from soluble rHuPH20 aa
431-443

<400> 61
Asp Ala Phe Lys Leu Pro Pro Met Glu Thr Glu Glu Pro
1 5 10

<210> 62
<211> 12
<212> PRT
<213> Homo sapiens

<220>
<223> C-terminal peptide cleaved from soluble rHuPH20 aa
431-442

<400> 62
Asp Ala Phe Lys Leu Pro Pro Met Glu Thr Glu Glu
1 5 10

<210> 63
<211> 520
<212> PRT

<213> Ovis aries

<400> 63

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Leu Asp Phe Pro Ala Pro Pro Leu Ile Ser Asn Thr Ser Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ala Glu Arg Cys Val Lys Ile Phe Lys Leu Pro
            20                  25                  30
Pro Asp Leu Arg Leu Phe Ser Val Lys Gly Ser Pro Gln Lys Ser Ala
        35                  40                  45
Thr Gly Gln Phe Ile Thr Leu Phe Tyr Ala Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro His Ile Asp Glu Lys Thr Gly Asn Thr Val Tyr Gly Gly Ile Pro
65                  70                  75                  80
Gln Leu Gly Asn Leu Lys Asn His Leu Glu Lys Ala Lys Lys Asp Ile
                85                  90                  95
Ala Tyr Tyr Ile Pro Asn Asp Ser Val Gly Leu Ala Val Ile Asp Trp
            100                 105                 110
Glu Asn Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Arg Asp Glu Ser Val Glu Leu Val Leu Gln Lys Asn Pro Gln Leu
    130                 135                 140
Ser Phe Pro Glu Ala Ser Lys Ile Ala Lys Val Asp Phe Glu Thr Ala
145                 150                 155                 160
Gly Lys Ser Phe Met Gln Glu Thr Leu Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
Asn Tyr Asn Gln Pro Thr Tyr Asn Gly Asn Cys Ser Asp Leu Glu Lys
        195                 200                 205
Arg Arg Asn Asp Asp Leu Asp Trp Leu Trp Lys Glu Ser Thr Ala Leu
    210                 215                 220
Phe Pro Ser Val Tyr Leu Asn Ile Lys Leu Lys Ser Thr Pro Lys Ala
225                 230                 235                 240
Ala Phe Tyr Val Arg Asn Arg Val Gln Glu Ala Ile Arg Leu Ser Lys
                245                 250                 255
Ile Ala Ser Val Glu Ser Pro Leu Pro Val Phe Val Tyr His Arg Pro
            260                 265                 270
Val Phe Thr Asp Gly Ser Ser Thr Tyr Leu Ser Gln Gly Asp Leu Val
        275                 280                 285
Asn Ser Val Gly Glu Ile Val Ala Leu Gly Ala Ser Gly Ile Ile Met
    290                 295                 300
Trp Gly Ser Leu Asn Leu Ser Leu Thr Met Gln Ser Cys Met Asn Leu
305                 310                 315                 320
Gly Asn Tyr Asn Thr Thr Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys His Asp Glu Gly Val Cys
            340                 345                 350
Thr Arg Lys Gln Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Ile
        355                 360                 365
Met Asn Phe Ala Ile Gln Thr Gly Lys Gly Gly Lys Tyr Thr Val Pro
    370                 375                 380
Gly Lys Val Thr Leu Glu Asp Leu Gln Thr Phe Ser Asp Lys Phe Tyr
385                 390                 395                 400
Cys Ser Cys Tyr Ala Asn Ile Asn Cys Lys Lys Arg Val Asp Ile Lys
                405                 410                 415
Asn Val His Ser Val Asn Val Cys Met Ala Glu Asp Ile Cys Ile Glu
            420                 425                 430
Gly Pro Val Lys Leu Gln Pro Ser Asp His Ser Ser Ser Gln Asn Glu
        435                 440                 445
Ala Ser Thr Thr Thr Val Ser Ser Ile Ser Pro Ser Thr Thr Ala Thr
    450                 455                 460
Thr Val Val Ser Pro Cys Thr Pro Glu Lys Gln Ser Pro Glu Cys Leu
465                 470                 475                 480
Lys Val Arg Cys Leu Glu Ala Ile Ala Asn Val Thr Gln Thr Gly Cys
                485                 490                 495
Gln Gly Val Lys Trp Lys Asn Thr Ser Ser Gln Ser Gln Ser Ser Ile
            500                 505                 510
Gln Asn Ile Lys Asn Gln Thr Thr
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515 520

<210> 64
<211> 474
<212> PRT
<213> Bos taurus

<220>
<223> PH20

<400> 64
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20 25 30
Cys Leu Ala Leu Asp Phe Arg Ala Pro Pro Leu Ile Ser Asn Thr Ser
35 40 45
Phe Leu Trp Ala Trp Asn Ala Pro Val Glu Arg Cys Val Asn Arg Arg
50 55 60
Phe Gln Leu Pro Pro Asp Leu Arg Leu Phe Ser Val Lys Gly Ser Pro
65 70 75 80
Gln Lys Ser Ala Thr Gly Gln Phe Ile Thr Leu Phe Tyr Ala Asp Arg
85 90 95
Leu Gly Tyr Tyr Pro His Ile Asp Glu Lys Thr Gly Lys Thr Val Phe
100 105 110
Gly Gly Ile Pro Gln Leu Gly Asn Leu Lys Ser His Leu Glu Lys Ala
115 120 125
Lys Asn Asp Ile Ala Tyr Tyr Ile Pro Asn Asp Ser Val Gly Leu Ala
130 135 140
Val Ile Asp Trp Glu Asn Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys
145 150 155 160
Pro Lys Asp Val Tyr Arg Asp Glu Ser Val Glu Leu Val Leu Gln Lys
165 170 175
Asn Pro Gln Leu Ser Phe Pro Glu Ala Ser Lys Ile Ala Lys Val Asp
180 185 190
Phe Glu Thr Ala Gly Lys Ser Phe Met Gln Glu Thr Leu Lys Leu Gly
195 200 205
Lys Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp
210 215 220
Cys Tyr Asn His Asn His Asn Gln Pro Thr Tyr Asn Gly Asn Cys Pro
225 230 235 240
Asp Val Glu Lys Arg Arg Asn Asp Asp Leu Glu Trp Leu Trp Lys Glu
245 250 255
Ser Thr Ala Leu Phe Pro Ser Val Tyr Leu Asn Ile Arg Leu Lys Ser
260 265 270
Thr Gln Asn Ala Ala Leu Tyr Val Arg Asn Arg Val Gln Glu Ala Ile
275 280 285
Arg Leu Ser Lys Ile Ala Ser Val Glu Ser Pro Leu Pro Val Phe Val
290 295 300
Tyr Ala Arg Pro Val Phe Thr Asp Gly Ser Ser Thr Tyr Leu Ser Gln
305 310 315 320
Gly Asp Leu Val Asn Ser Val Gly Glu Ile Val Ser Leu Gly Ala Ser
325 330 335
Gly Ile Ile Met Trp Gly Ser Leu Asn Leu Ser Leu Ser Val Gln Ser
340 345 350
Cys Met Asn Leu Gly Thr Tyr Leu Asn Thr Thr Leu Asn Pro Tyr Ile
355 360 365
Ile Asn Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys His
370 375 380
Asp Gly Gly Val Cys Thr Arg Lys His Trp Asn Ser Ser Asp Tyr Leu
385 390 395 400
His Leu Asn Pro Met Asn Phe Ala Ile Gln Thr Gly Glu Gly Gly Lys
405 410 415
Tyr Thr Val Pro Gly Thr Leu Thr Leu Glu Asp Leu Gln Lys Phe Ser
420 425 430
Asp Thr Phe Tyr Cys Ser Cys Tyr Ser Asn Leu Ser Cys Lys Lys Arg
435 440 445

Val Asp Ile Lys Asn Val His Ser Val Asp Val Cys Met Ala Glu Asp
450 455 460
Val Cys Ile Asp Ala Phe Leu Lys Pro Pro
465 470

<210> 65
<211> 517
<212> PRT
<213> Ovis aries

<400> 65
Asp Phe Arg Ala Pro Pro Leu Ile Ser Asn Thr Ser Phe Leu Trp Ala
1 5 10 15
Trp Asn Ala Pro Ala Glu Arg Cys Ile Lys Ile Phe Lys Leu Pro Pro
20 25 30
Asp Leu Arg Leu Phe Ser Val Lys Gly Ser Pro Gln Lys Ser Ala Thr
35 40 45
Gly Gln Phe Ile Thr Leu Phe Tyr Ala Asp Arg Leu Gly Tyr Tyr Pro
50 55 60
His Ile Asp Glu Lys Thr Gly Asn Thr Val Tyr Gly Gly Ile Pro Gln
65 70 75 80
Leu Gly Asn Leu Lys Asn His Leu Glu Lys Ala Lys Lys Asp Ile Ala
85 90 95
Tyr Tyr Ile Pro Asn Asp Ser Val Gly Leu Ala Val Ile Asp Trp Glu
100 105 110
Asn Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val Tyr
115 120 125
Arg Asp Glu Ser Val Glu Leu Val Leu Gln Lys Asn Pro Gln Leu Ser
130 135 140
Phe Pro Glu Ala Ser Lys Ile Ala Lys Val Asp Phe Glu Thr Ala Gly
145 150 155 160
Lys Ser Phe Met Gln Glu Thr Leu Lys Leu Gly Lys Leu Leu Arg Pro
165 170 175
Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His Asn
180 185 190
Tyr Asn Gln Pro Thr Tyr Asn Gly Asn Cys Ser Asp Leu Glu Lys Arg
195 200 205
Arg Asn Asp Asp Leu Asp Trp Leu Trp Lys Glu Ser Thr Ala Leu Phe
210 215 220
Pro Ser Val Tyr Leu Asn Ile Lys Leu Lys Ser Thr Pro Lys Ala Ala
225 230 235 240
Phe Tyr Val Arg Asn Arg Val Gln Glu Ala Ile Arg Leu Ser Lys Ile
245 250 255
Ala Ser Val Glu Ser Pro Leu Pro Val Phe Val Tyr His Arg Pro Val
260 265 270
Phe Thr Asp Gly Ser Ser Thr Tyr Leu Ser Gln Gly Asp Leu Val Asn
275 280 285
Ser Val Gly Glu Ile Val Ala Leu Gly Ala Ser Gly Ile Ile Met Trp
290 295 300
Gly Ser Leu Asn Leu Ser Leu Thr Met Gln Ser Cys Met Asn Leu Gly
305 310 315 320
Asn Tyr Leu Asn Thr Thr Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
325 330 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys His Asp Glu Gly Val Cys
340 345 350
Thr Arg Lys Gln Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Met
355 360 365
Asn Phe Ala Ile Gln Thr Gly Lys Gly Gly Lys Tyr Thr Val Pro Gly
370 375 380
Lys Val Thr Leu Glu Asp Leu Gln Thr Phe Ser Asp Lys Phe Tyr Cys
385 390 395 400
Ser Cys Tyr Ala Asn Ile Asn Cys Lys Lys Arg Val Asp Ile Lys Asn
405 410 415
Val His Ser Val Asn Val Cys Met Ala Glu Asp Ile Cys Ile Glu Gly
420 425 430
Pro Val Lys Leu Gln Pro Ser Asp His Ser Ser Ser Gln Asn Glu Ala
435 440 445

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Ser Thr Thr Thr Val Ser Ser Ile Ser Pro Ser Thr Thr Ala Thr Thr
    450                 455                 460
Val Ser Pro Cys Thr Pro Glu Lys Gln Ser Pro Glu Cys Leu Lys Val
465                 470                 475                 480
Arg Cys Leu Glu Ala Ile Ala Asn Val Thr Gln Thr Gly Cys Gln Gly
                485                 490                 495
Val Lys Trp Lys Asn Thr Ser Ser Gln Ser Ser Ile Gln Asn Ile Lys
            500                 505                 510
Asn Gln Thr Thr Tyr
        515
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<210> 66
<211> 1620
<212> DNA
<213> Ovis aries

<400> 66

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cctctgggcc tggaatgccc cagctgaacg ttgtattaaa atctttaaac tacctccaga 120
tctgagactc ttctctgtaa aaggaagccc ccaaaaaagt gctacgggac aatttattac 180
attattttat gctgatagac ttggctacta tcctcatata gatgaaaaaa caggcaacac 240
tgtatatgga ggaattcccc agttgggaaa cttaaaaaat catttggaaa aagccaaaaa 300
agacattgcc tattacatac caaatgacag cgtgggcttg gcggtcattg actgggaaaa 360
ctggaggcct acctgggcaa gaaactggaa acctaaagat gtttacaggg atgagtctgt 420
tgagttggtt ctgcaaaaaa atccacaact cagtttccca gaggcttcca agattgcaaa 480
agtggatttt gagacagcag gaaagagttt catgcaagag actttaaaac tgggaaaatt 540
acttcggcca aatcacttat ggggttatta tctttttcct gattgttaca atcataatta 600
taaccagcct acttacaatg gaaattgctc tgatttagaa aaaaggagaa atgatgatct 660
cgactggttg tggaaggaaa gcactgccct tttcccttct gtttatttga atatcaagtt 720
aaaatctact ccaaaagctg ccttctatgt tcgtaatcgt gtccaggaag ccattcggtt 780
gtctaaaata gcgagtgttg aaagtccact tcccgttttt gtatatcacc gtccagtttt 840
tactgatggg tcttcaacat acctttctca gggtgacctt gtgaattcgg ttggtgagat 900
cgttgctcta ggtgcctctg ggattataat gtggggcagt ctcaatctaa gcctaactat 960
gcaatcttgc atgaacctag gcaattactt gaacactaca ctgaatcctt acataatcaa 1020
cgtcacccta gcagccaaaa tgtgcagcca agtgctttgc cacgatgaag gagtgtgtac 1080
aaggaaacaa tggaattcaa gcgactatct tcacctgaac ccaatgaatt ttgctattca 1140
aactgggaaa ggtggaaaat acacagtacc tgggaaagtc acacttgaag acctgcaaac 1200
gttttctgat aaattttatt gcagttgtta tgccaacatc aactgtaaga agagagttga 1260
tataaaaaat gttcatagtg ttaatgtatg tatggcagaa gacatttgta tagagggccc 1320
tgtgaagtta caacccagtg atcattcctc tagccagaat gaggcatcta ctaccaccgt 1380
cagcagtatc tcaccctcta ctacagccac cacagtatct ccatgtactc ctgagaaaca 1440
gtcccctgag tgcctcaaag tcaggtgttt ggaagccatc gccaacgtca cccaaacggg 1500
gtgtcaaggt gttaaatgga agaacacttc cagtcagtca agtattcaaa atattaaaaa 1560
tcaaacaacc tattaaaata taaattcagt gcttataaaa aaaaaaaaaa aaaaaaaaaa 1620
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<210> 67
<211> 793
<212> PRT
<213> Arthrobacter sp. (strain FB24)

<220>
<223> hyaluronan lyase

<400> 67

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Met Thr Arg Glu Phe Ser Arg Arg Thr Ala Leu Lys Gly Ala Ala Leu
 1               5                  10                  15
Ser Gly Leu Leu Leu Ala Met Val His Gly Pro Ala His Ala Ala Ala
            20                  25                  30
Thr Ala Asn Ala Thr Leu Thr Pro Ala Asp Phe Ala Gly Leu Arg Gln
        35                  40                  45
Arg Trp Val Asp Gln Ile Thr Gly Arg Lys Val Leu Val Ala Gly Asp
    50                  55                  60
Asn Asp Phe Val Thr Ala Leu Ala Ala Leu Asp Lys Lys Ala Arg Thr
65                  70                  75                  80
Ala Ile Asp Leu Leu Glu Arg Ser Ala Gly Arg Leu Thr Val Phe Ser
                85                  90                  95
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Asp Leu Ser Leu Ala Lys Asp Thr Asp Leu Val Thr Thr His Thr Arg
            100                 105                 110
Leu Ala Thr Met Ala Thr Ala Trp Ala Thr Pro Gly Ser Glu His Phe
        115                 120                 125
Ala Asp Ala Gly Leu Leu Ala Ala Ile Arg Ala Gly Leu Ala Asp Ala
    130                 135                 140
Asn Ser Leu Cys Tyr Asn Ala Ser Lys Glu Glu Gln Gly Asn Trp Trp
145                 150                 155                 160
Ser Trp Glu Ile Gly Thr Pro Lys Ala Leu Ala Asp Thr Met Val Leu
                165                 170                 175
Leu His Ala Glu Leu Thr Ala Ala Glu Arg Ala Ala Tyr Cys Ala Ala
            180                 185                 190
Ile Asp His Phe Val Pro Asp Pro Trp Gln Gln Phe Pro Pro Lys Arg
        195                 200                 205
Gly Lys Ile Thr Ser Val Gly Ala Asn Arg Val Asp Leu Cys Gln Ala
    210                 215                 220
Val Thr Ile Arg Ser Leu Val Asp Glu Asp Ala Glu Lys Leu Thr His
225                 230                 235                 240
Ala Val Ala Gly Leu Ser Glu Val Trp Gln Tyr Val Ser Ala Gly Asn
                245                 250                 255
Gly Phe Phe Thr Asp Gly Ser Phe Ile Gln His Ser Thr Thr Pro Tyr
            260                 265                 270
Thr Gly Ser Tyr Gly Val Val Leu Leu Thr Gly Leu Ser Lys Leu Phe
        275                 280                 285
Ala Leu Leu Gly Gly Thr Gly Ala Glu Val Ser Asp Pro Ser Arg Asp
    290                 295                 300
Ile Leu Phe Lys Thr Val Glu Gly Ser Phe Ala Pro Phe Met Val Ala
305                 310                 315                 320
Gly Ala Met Ala Asp Ser Val Arg Gly Arg Ser Ile Ser Arg Glu Ser
                325                 330                 335
Asn Thr Gly Phe Asp Leu Gly Ala Ser Thr Ile Glu Ser Ile Leu Leu
            340                 345                 350
Leu Ala Arg Ala Val Asp Pro Val Thr Ala Arg Arg Trp Arg Ser Leu
        355                 360                 365
Cys Leu Ala Trp Ile Asn Gln Asn Arg Lys Ala Pro Ile Leu Ala Asp
    370                 375                 380
Ala Gly Val Gly Arg Thr Ala Leu Val Lys Glu Leu Leu Ala Met Gly
385                 390                 395                 400
Leu Thr Glu Thr Asp Leu Pro Gly Gly His Tyr Leu Phe Pro Ala Met
                405                 410                 415
Asp Arg Thr Met His His Ser Gln Gly Trp Thr Leu Ser Thr Ala Met
            420                 425                 430
Ala Ser Ser Arg Ile Ala Trp Tyr Glu Cys Gly Asn Gly Glu Asn Asn
        435                 440                 445
Arg Gly Tyr His Thr Gly Ser Gly Met Thr Tyr Val Tyr Asp Gly Asp
    450                 455                 460
Leu Gly Gln Tyr Asp Asp Ala Phe Trp Ala Thr Ala Asn His Cys Arg
465                 470                 475                 480
Leu Pro Gly Ile Thr Val Asp Thr Ser Ser Leu Pro Asp Lys Val Glu
                485                 490                 495
Gly Glu Trp Gly Ala Ala Thr Pro Ala Asn Glu Trp Thr Gly Ser Thr
            500                 505                 510
Ala Tyr Gly Asp Val Ala Ala Val Gly Gln His Leu Ile Gly Pro Gly
        515                 520                 525
Gly Thr Gly Leu Thr Ala Arg Lys Ser Trp Phe Val Ser Lys Asp Val
    530                 535                 540
Ile Val Cys Leu Gly Ala Asp Ile Arg Thr Gly Ser Gly Ser Arg Ile
545                 550                 555                 560
Glu Thr Val Val Asp His Arg Asn Leu His Ala Gly Phe Asn Ala Met
                565                 570                 575
Gly Thr Ala Ala Gly Thr Val Ala Ala Thr Pro Gly His Pro Glu Val
            580                 585                 590
Leu Thr Val Asp Arg Trp Val His Leu Glu Gly Phe Gly Gly Tyr Val
        595                 600                 605
Val Leu Asp Ala Ala Pro Leu Gln Val Leu Arg Glu Gln Arg Glu Gly
    610                 615                 620
Ser Trp Ser Glu Val Asn Val Lys Gly Ser Ala Ala Arg Gln Thr Arg
625                 630                 635                 640
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Asn Tyr Ala Thr Leu Tyr Phe Asp His Gly His Glu Pro Glu Ala Ala
                645                 650                 655
Ser Tyr Ala Tyr Leu Val Ala Pro Gly Ala Ser Ala Ser Met Thr Ser
            660                 665                 670
Ser Leu Ser Gly Gln Ser Phe His Thr Val Leu Arg Asn Asp Glu Val
        675                 680                 685
Ala Gln Ala Val Lys Phe Lys Lys Glu Lys Thr Thr Ala Ala Thr Phe
    690                 695                 700
Trp Arg Pro Gly Thr Val Gly Asp Leu Ala Leu Ser Gly Pro Ala Cys
705                 710                 715                 720
Val Val Val Lys Glu Val Gly Asp Arg Leu Ser Ile Ala Val Ser Asp
                725                 730                 735
Pro Thr Gln Asn Ala Ser Thr Leu Thr Leu Arg Leu Lys Thr Lys Arg
            740                 745                 750
Phe Phe Arg Ile Ile Glu Gly Gln Gly Ala Ser Leu Ser His Gly Ala
        755                 760                 765
Asp Gly Phe Thr Val Leu Glu Val Asp Ile Ala Asn His Ala Gly Arg
    770                 775                 780
Thr Lys Gln Ile Glu Leu Ser Ala Glu
785                 790


<210> 68
<211> 557
<212> PRT
<213> Bdellovibrio bacteriovorus

<220>
<223> hyaluronan lyase

<400> 68
Met Thr Lys Phe Phe Phe Leu Leu Thr Leu Ile Ser Ala Thr Ala Phe
 1                5                  10                  15
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            20                  25                  30
Gly Arg Ser Asn Ile Tyr Ser Trp Asn Asp Phe Asp Phe Gln Ala Thr
        35                  40                  45
Leu Asn Lys Gly Lys Ile His Ala Gln Val Tyr Pro Val Thr Val Thr
    50                  55                  60
Gly Met Leu Pro Pro Tyr Glu Pro Val Arg Arg Leu Ile Glu Glu Lys
65                  70                  75                  80
Asn Ser Asn Pro Leu Arg Lys Trp Ile Gln Ser Leu Met Lys Gly Leu
                85                  90                  95
Ser Gly Phe Arg Ser Phe Glu Asp Val Leu Lys Asn Leu Gly Leu His
            100                 105                 110
Lys Tyr Pro Leu Glu Asn Glu Arg Gly Val Tyr Ala Val Pro Tyr Pro
        115                 120                 125
Asn Glu Ile Arg Pro Asp Thr Leu Met Gly Phe Gly Leu Ile Glu Arg
    130                 135                 140
Asn Gly Ala Glu Gly Phe Thr Phe Ser Cys Ala Ala Cys His Ser Ser
145                 150                 155                 160
Asn Leu Phe Gly Lys Thr Val Leu Gly Met Thr Asn Arg Phe Pro Arg
                165                 170                 175
Ala Asn Glu Phe Phe Ile Lys Ala Lys Lys Val Met Pro Leu Met Asp
            180                 185                 190
Pro His Ile Phe Gln Ala Tyr Thr Arg Ala Thr Asp Ala Glu Thr Ala
        195                 200                 205
Leu Leu Val Glu Ser Lys Glu Arg Leu Lys Ser Val Ala Leu Lys Gln
    210                 215                 220
Pro Ile Ala Leu Gly Leu Asp Thr Ser Leu Ala Gln Val Ser Leu Ser
225                 230                 235                 240
Leu Asn Arg Arg Ala Lys Asp Gly Tyr Ala Asn Tyr Ser Asp Lys Ala
                245                 250                 255
Ala Arg Ser Pro Arg Ala Asp Ala Tyr Leu Asp Asn Lys Pro Ala Asp
            260                 265                 270
Ser Lys Pro Ala Val Trp Trp Asn Val Lys Tyr Lys Asn Arg Trp Leu
        275                 280                 285
Ser Asp Gly Ser Val Leu Ser Gly Asn Pro Ile Phe Thr Asn Leu Ile
```

```
    290                 295                 300
Trp Asn Glu Ile Gly Arg Gly Ala Asp Leu His Glu Leu Glu Gln Trp
305                 310                 315                 320
Leu Ala Asp Asn Asp His Ile Ile Lys Glu Leu Thr Thr Ala Val Phe
                325                 330                 335
Ala Ser Glu Ala Pro His Ile Thr Asp Phe Tyr Pro Ala Glu Lys Ile
            340                 345                 350
Asp Leu Gly Arg Ala Lys Ala Gly Glu Gln Ile Phe Lys Asn Thr Cys
        355                 360                 365
Ala Lys Cys His Gly His Tyr Glu Lys Ala Trp Asn Leu Pro Gln Ala
    370                 375                 380
Leu Val Leu Ser Ala Ala Glu Arg Leu Lys Thr Val Glu Val Arg Tyr
385                 390                 395                 400
Lys Glu Lys Thr Pro Val Val Asn Val Gly Thr Asp Pro Phe Arg Arg
                405                 410                 415
Gln Gly Met Lys Ser Leu Glu Gln Leu Asn Asp Leu Glu Ile Ser Lys
            420                 425                 430
Lys Asn Gly Ile Val Ile Lys Ala Gln Glu Gly Tyr Val Pro Pro Pro
        435                 440                 445
Leu Val Gly Ile Trp Ala Arg Trp Pro Tyr Met His Asn Asn Ser Ile
    450                 455                 460
Pro Asn Leu Cys Val Leu Leu Thr Pro Ala Lys Lys Arg Pro Ser Ile
465                 470                 475                 480
Tyr Tyr Ser Gly Glu Ala Leu Asn Lys Asp Thr Asp Tyr Asp Phe Ser
                485                 490                 495
Cys Gly Gly Tyr Pro Ile Gly Asp Lys Thr Pro Lys Ala Trp Lys Thr
            500                 505                 510
Arg Glu His Leu Tyr Asp Thr Arg Asn Pro Gly Met Gly Asn Met Gly
        515                 520                 525
His Asp Glu Gly Ile Phe Ile Lys Asp Gly Lys Glu Ile Leu Ser Ala
    530                 535                 540
Glu Asp Lys Tyr Asn Leu Ile Gln Phe Leu Gln Thr Leu
545                 550                 555


<210> 69
<211> 813
<212> PRT
<213> Propionibacterium acnes

<220>
<223> hyaluronan lyase

<400> 69
Met Phe Gly Thr Pro Ser Arg Arg Thr Phe Leu Thr Ala Ser Ala Leu
 1               5                  10                  15
Ser Ala Met Ala Leu Ala Ala Ser Pro Thr Val Thr Asp Ala Ile Ala
            20                  25                  30
Ala Pro Gly Pro Asp Ser Trp Ser Ala Leu Cys Glu Arg Trp Ile Asp
        35                  40                  45
Ile Ile Thr Gly Arg Arg Ala Ala Arg Thr Ser Asp Pro Arg Ala Arg
    50                  55                  60
Ala Ile Ile Ala Lys Thr Asp Arg Lys Val Ala Glu Ile Leu Thr Asp
65                  70                  75                  80
Leu Val Ser Gly Ser Ser Arg Gln Thr Val Leu Ile Ser Ala Asp Leu
                85                  90                  95
Arg Lys Glu Gln Ser Pro Phe Ile Thr Lys Thr Ala Arg Ala Ile Glu
            100                 105                 110
Ser Met Ala Cys Ala Trp Ala Thr Pro Gly Ser Ser Tyr His Lys Asp
        115                 120                 125
Pro Glu Ile Leu Ser Ala Cys Ile Glu Gly Leu Arg Asp Phe Cys Arg
    130                 135                 140
Leu Arg Tyr Asn Pro Ser Gln Asp Glu Tyr Gly Asn Trp Trp Asp Trp
145                 150                 155                 160
Glu Asp Gly Ala Ser Arg Ala Val Ala Asp Val Met Cys Ile Leu His
                165                 170                 175
Asp Val Leu Pro Pro Glu Val Met Ser Ala Ala Ala Ala Gly Ile Asp
            180                 185                 190
```

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His Phe Ile Pro Asp Pro Trp Phe Gln Gln Pro Ala Ser Val Lys Pro
        195                 200                 205
Thr Ala Asn Pro Val Gln Pro Val Val Ser Thr Gly Ala Asn Arg Met
    210                 215                 220
Asp Leu Thr Arg Ala Val Met Cys Arg Ser Ile Ala Thr Gly Asp Glu
225                 230                 235                 240
Lys Arg Leu Arg His Ala Val Asp Gly Leu Pro Asp Ala Trp Arg Val
                245                 250                 255
Thr Thr Glu Gly Asp Gly Phe Arg Ala Asp Gly Gly Phe Ile Gln His
            260                 265                 270
Ser His Ile Pro Tyr Thr Gly Gly Tyr Gly Asp Val Leu Phe Ser Gly
        275                 280                 285
Leu Ala Met Leu Phe Pro Leu Val Ser Gly Met Arg Phe Asp Ile Val
    290                 295                 300
Glu Ser Ala Arg Lys Ala Phe His Asp Gln Val Glu Arg Gly Phe Ile
305                 310                 315                 320
Pro Val Met Tyr Asn Gly Gln Ile Leu Asp Asp Val Arg Gly Arg Ser
                325                 330                 335
Ile Ser Arg Ile Asn Glu Ser Ala Ala Met His Gly Ile Ser Ile Ala
            340                 345                 350
Arg Ala Met Leu Met Met Ala Asp Ala Leu Pro Thr His Arg Ala Glu
        355                 360                 365
Gln Trp Arg Gly Ile Val His Gly Trp Met Ala Arg Asn Thr Phe Asp
    370                 375                 380
His Leu Ser Glu Pro Ser Thr Leu Val Asp Ile Ser Leu Phe Asp Ala
385                 390                 395                 400
Ala Ala Lys Ala Arg Pro Val Pro Glu Ser Ser Thr Pro Ser Tyr Phe
                405                 410                 415
Ala Ser Met Asp Arg Leu Val His Arg Thr Ala Asp Trp Leu Ile Thr
            420                 425                 430
Val Ser Asn Cys Ser Asp Arg Ile Ala Trp Tyr Glu Tyr Gly Asn Gly
        435                 440                 445
Glu Asn Glu Trp Ala Ser Arg Thr Ser Gln Gly Met Arg Tyr Leu Leu
    450                 455                 460
Leu Pro Gly Asp Met Gly Gln Tyr Glu Asp Gly Tyr Trp Ala Thr Val
465                 470                 475                 480
Asp Tyr Ser Ala Pro Thr Gly Thr Thr Val Asp Ser Thr Pro Leu Lys
                485                 490                 495
Arg Ala Val Gly Ala Ser Trp Ala Ala Lys Thr Pro Thr Asn Glu Trp
            500                 505                 510
Ser Gly Gly Leu Ala Ser Gly Ser Trp Ser Ala Ala Ala Ser His Ile
        515                 520                 525
Thr Ser Gln Asp Ser Ala Leu Lys Ala Arg Arg Leu Trp Val Gly Leu
    530                 535                 540
Lys Asp Ala Met Val Glu Leu Thr Thr Asp Val Thr Thr Asp Ala Ser
545                 550                 555                 560
Arg Ala Ile Thr Val Val Glu His Arg Lys Val Ala Ser Ser Ser Thr
                565                 570                 575
Lys Leu Leu Val Asp Gly Asn Arg Val Ser Ser Ala Thr Ser Phe Gln
            580                 585                 590
Asn Pro Arg Trp Ala His Leu Asp Gly Val Gly Gly Tyr Val Phe Ala
        595                 600                 605
Thr Asp Thr Asp Leu Ser Ala Asp Val Ala Thr Arg Lys Gly Thr Trp
    610                 615                 620
Ile Asp Val Asn Pro Ser Arg Lys Val Lys Gly Ala Asp Glu Val Ile
625                 630                 635                 640
Glu Arg Ala Tyr Ala Ser Leu His Val Thr His His Asp Arg Pro Val
                645                 650                 655
Ala Trp Ala Leu Leu Pro Thr Ala Ser Arg Ser His Thr Met Ala Leu
            660                 665                 670
Ala Thr Arg Pro Gly Val Glu Pro Phe Thr Val Leu Arg Asn Asp Ala
        675                 680                 685
Thr Val Gln Ala Val Arg Ser Ala Gly Ala Leu Leu Thr Lys Asp Pro
    690                 695                 700
Thr Val Val Thr Thr Leu Ala Phe Trp Lys Pro Ala Thr Cys Gly Gly
705                 710                 715                 720
Val Ala Val Asn Arg Pro Ala Leu Val Gln Thr Arg Glu Ser Ala Asn
                725                 730                 735
```

Gln Met Glu Val Val Ile Val Glu Pro Thr Gln Lys Arg Gly Ser Leu
            740   745   750
Thr Val Thr Ile Glu Gly Ser Trp Lys Val Lys Thr Ala Asp Ser His
        755   760   765
Val Asp Val Ser Cys Glu Asn Ala Ala Gly Thr Leu His Val Asp Thr
    770   775   780
Ala Gly Leu Gly Gly Gln Ser Val Arg Val Thr Leu Ala Arg Gln Val
785   790   795   800
Thr Gln Thr Pro Ser Gly Gly Gly Arg His Asp Arg Ala
                805   810

<210> 70
<211> 1072
<212> PRT
<213> Streptococcus agalactiae

<220>
<223> hyaluronan lyase

<400> 70
Met Glu Ile Lys Lys Lys Tyr Arg Ile Met Leu Tyr Ser Ala Leu Ile
 1   5   10   15
Leu Gly Thr Ile Leu Val Asn Asn Ser Tyr Gln Ala Lys Ala Glu Glu
            20   25   30
Leu Thr Lys Thr Thr Ser Thr Ser Gln Ile Arg Asp Thr Gln Thr Asn
        35   40   45
Asn Ile Glu Val Leu Gln Thr Glu Ser Thr Thr Val Lys Glu Thr Ser
    50   55   60
Thr Thr Thr Thr Gln Gln Asp Leu Ser Asn Pro Thr Ala Ser Thr Ala
65   70   75   80
Thr Ala Thr Ala Thr His Ser Thr Met Lys Gln Val Val Asp Asn Gln
                85   90   95
Thr Gln Asn Lys Glu Leu Val Lys Asn Gly Asp Phe Asn Gln Thr Asn
            100   105   110
Pro Val Ser Gly Ser Trp Ser His Thr Ser Ala Arg Glu Trp Ser Ala
        115   120   125
Trp Ile Asp Lys Glu Asn Thr Ala Asp Lys Ser Pro Ile Ile Gln Arg
    130   135   140
Thr Glu Gln Gly Gln Val Ser Leu Ser Ser Asp Lys Gly Phe Arg Gly
145   150   155   160
Ala Val Thr Gln Lys Val Asn Ile Asp Pro Thr Lys Lys Tyr Glu Val
                165   170   175
Lys Phe Asp Ile Glu Thr Ser Asn Lys Ala Gly Gln Ala Phe Leu Arg
            180   185   190
Ile Met Glu Lys Lys Asp Asn Asn Thr Arg Leu Trp Leu Ser Glu Met
        195   200   205
Thr Ser Gly Thr Thr Asn Lys His Thr Leu Thr Lys Ile Tyr Asn Pro
    210   215   220
Lys Leu Asn Val Ser Glu Val Thr Leu Glu Leu Tyr Tyr Glu Lys Gly
225   230   235   240
Thr Gly Ser Ala Thr Phe Asp Asn Ile Ser Met Lys Ala Lys Gly Pro
                245   250   255
Lys Asp Ser Glu His Pro Gln Pro Val Thr Thr Gln Ile Glu Glu Ser
            260   265   270
Val Asn Thr Ala Leu Asn Lys Asn Tyr Val Phe Asn Lys Ala Asp Tyr
        275   280   285
Gln Tyr Thr Leu Thr Asn Pro Ser Leu Gly Lys Ile Val Gly Gly Ile
    290   295   300
Leu Tyr Pro Asn Ala Thr Gly Ser Thr Thr Val Lys Ile Ser Asp Lys
305   310   315   320
Ser Gly Lys Ile Ile Lys Glu Val Pro Leu Ser Val Thr Ala Ser Thr
                325   330   335
Glu Asp Lys Phe Thr Lys Leu Leu Asp Lys Trp Asn Asp Val Thr Ile
            340   345   350
Gly Asn His Val Tyr Asp Thr Asn Asp Ser Asn Met Gln Lys Ile Asn
        355   360   365
Gln Lys Leu Asp Glu Thr Asn Ala Lys Asn Ile Lys Thr Ile Lys Leu

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    370                 375                 380
Asp Ser Asn His Thr Phe Leu Trp Lys Asp Leu Asp Asn Leu Asn Asn
385                 390                 395                 400
Ser Ala Gln Leu Thr Ala Thr Tyr Arg Arg Leu Glu Asp Leu Ala Lys
                405                 410                 415
Gln Ile Thr Asn Pro His Ser Thr Ile Tyr Lys Asn Glu Lys Ala Ile
            420                 425                 430
Arg Thr Val Lys Glu Ser Leu Ala Trp Leu His Gln Asn Phe Tyr Asn
        435                 440                 445
Val Asn Lys Asp Ile Glu Gly Ser Ala Asn Trp Trp Asp Phe Glu Ile
    450                 455                 460
Gly Val Pro Arg Ser Ile Thr Ala Thr Leu Ala Leu Met Asn Asn Tyr
465                 470                 475                 480
Phe Thr Asp Ala Glu Ile Lys Thr Tyr Thr Asp Pro Ile Glu His Phe
                485                 490                 495
Val Pro Asp Ala Gly Tyr Phe Arg Lys Thr Leu Asp Asn Pro Phe Lys
            500                 505                 510
Ala Leu Gly Gly Asn Leu Val Asp Met Gly Arg Val Lys Ile Ile Glu
        515                 520                 525
Gly Leu Leu Arg Lys Asp Asn Thr Ile Ile Glu Lys Thr Ser His Ser
    530                 535                 540
Leu Lys Asn Leu Phe Thr Thr Ala Thr Lys Ala Glu Gly Phe Tyr Ala
545                 550                 555                 560
Asp Gly Ser Tyr Ile Asp His Thr Asn Val Ala Tyr Thr Gly Ala Tyr
                565                 570                 575
Gly Asn Val Leu Ile Asp Gly Leu Thr Gln Leu Leu Pro Ile Ile Gln
            580                 585                 590
Glu Thr Asp Tyr Lys Ile Ser Asn Gln Glu Leu Asp Met Val Tyr Lys
        595                 600                 605
Trp Ile Asn Gln Ser Phe Leu Pro Leu Ile Val Lys Gly Glu Leu Met
    610                 615                 620
Asp Met Ser Arg Gly Arg Ser Ile Ser Arg Glu Ala Ala Ser Ser His
625                 630                 635                 640
Ala Ala Ala Val Glu Val Leu Arg Gly Phe Leu Arg Leu Ala Asn Met
                645                 650                 655
Ser Asn Glu Glu Arg Asn Leu Asp Leu Lys Ser Thr Ile Lys Thr Ile
            660                 665                 670
Ile Thr Ser Asn Lys Phe Tyr Asn Val Phe Asn Asn Leu Lys Ser Tyr
        675                 680                 685
Ser Asp Ile Ala Asn Met Asn Lys Met Leu Asn Asp Ser Thr Val Ala
    690                 695                 700
Thr Lys Pro Leu Lys Ser Asn Leu Ser Thr Phe Asn Ser Met Asp Arg
705                 710                 715                 720
Leu Ala Tyr Tyr Asn Ala Glu Lys Asp Phe Gly Phe Ala Leu Ser Leu
                725                 730                 735
His Ser Lys Arg Thr Leu Asn Tyr Glu Gly Met Asn Asp Glu Asn Thr
            740                 745                 750
Arg Gly Trp Tyr Thr Gly Asp Gly Met Phe Tyr Leu Tyr Asn Ser Asp
        755                 760                 765
Gln Ser His Tyr Ser Asn His Phe Trp Pro Thr Val Asn Pro Tyr Lys
    770                 775                 780
Met Ala Gly Thr Thr Glu Lys Asp Ala Lys Arg Glu Asp Thr Thr Lys
785                 790                 795                 800
Glu Phe Met Ser Lys His Ser Lys Asp Ala Lys Glu Lys Thr Gly Gln
                805                 810                 815
Val Thr Gly Thr Ser Asp Phe Val Gly Ser Val Lys Leu Asn Asp His
            820                 825                 830
Phe Ala Leu Ala Ala Met Asp Phe Thr Asn Trp Asp Arg Thr Leu Thr
        835                 840                 845
Ala Gln Lys Gly Trp Val Ile Leu Asn Asp Lys Ile Val Phe Leu Gly
    850                 855                 860
Ser Asn Ile Lys Asn Thr Asn Gly Ile Gly Asn Val Ser Thr Thr Ile
865                 870                 875                 880
Asp Gln Arg Lys Asp Asp Ser Lys Thr Pro Tyr Thr Thr Tyr Val Asn
                885                 890                 895
Gly Lys Thr Ile Asp Leu Lys Gln Ala Ser Ser Gln Gln Phe Thr Asp
            900                 905                 910
Thr Lys Ser Val Phe Leu Glu Ser Lys Glu Pro Gly Arg Asn Ile Gly
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915 920 925
Tyr Ile Phe Phe Lys Asn Ser Thr Ile Asp Ile Glu Arg Lys Glu Gln
930 935 940
Thr Gly Thr Trp Asn Ser Ile Asn Arg Thr Ser Lys Asn Thr Ser Ile
945 950 955 960
Val Ser Asn Pro Phe Ile Thr Ile Ser Gln Lys His Asp Asn Lys Gly
965 970 975
Asp Ser Tyr Gly Tyr Met Met Val Pro Asn Ile Asp Arg Thr Ser Phe
980 985 990
Asp Lys Leu Ala Asn Ser Lys Glu Val Glu Leu Leu Glu Asn Ser Ser
995 1000 1005
Lys Gln Gln Val Ile Tyr Asp Lys Asn Ser Gln Thr Trp Ala Val Ile
1010 1015 1020
Lys His Asp Asn Gln Glu Ser Leu Ile Asn Asn Gln Phe Lys Met Asn
1025 1030 1035 1040
Lys Ala Gly Leu Tyr Leu Val Gln Lys Val Gly Asn Asp Tyr Gln Asn
1045 1050 1055
Val Tyr Tyr Gln Pro Gln Thr Met Thr Lys Thr Asp Gln Leu Ala Ile
1060 1065 1070

<210> 71
<211> 1072
<212> PRT
<213> Streptococcus agalactiae 18RS21

<220>
<223> hyaluronan lyase

<400> 71
Met Glu Ile Lys Lys Lys His Arg Ile Met Leu Tyr Ser Ala Leu Ile
1 5 10 15
Leu Gly Thr Ile Leu Val Asn Asn Ser Tyr Gln Ala Lys Ala Glu Glu
20 25 30
Leu Thr Lys Thr Thr Ser Thr Ser Gln Ile Arg Asp Thr Gln Thr Asn
35 40 45
Asn Ile Glu Val Leu Gln Thr Glu Ser Thr Thr Val Lys Glu Thr Ser
50 55 60
Thr Thr Thr Thr Gln Gln Asp Leu Ser Asn Pro Thr Ala Ser Thr Ala
65 70 75 80
Thr Ala Thr Ala Thr His Ser Thr Met Lys Gln Val Val Asp Asn Gln
85 90 95
Thr Gln Asn Lys Glu Leu Val Lys Asn Gly Asp Phe Asn Gln Thr Asn
100 105 110
Pro Val Ser Gly Ser Trp Ser His Thr Ser Ala Arg Glu Trp Ser Ala
115 120 125
Trp Ile Asp Lys Glu Asn Thr Ala Asp Lys Ser Pro Ile Ile Gln Arg
130 135 140
Thr Glu Gln Gly Gln Val Ser Leu Ser Ser Asp Lys Gly Phe Arg Gly
145 150 155 160
Ala Val Thr Gln Lys Val Asn Ile Asp Pro Thr Lys Lys Tyr Glu Val
165 170 175
Lys Phe Asp Ile Glu Thr Ser Asn Lys Ala Gly Gln Ala Phe Leu Arg
180 185 190
Ile Met Glu Lys Lys Asp Asn Asn Thr Arg Leu Trp Leu Ser Glu Met
195 200 205
Thr Ser Gly Thr Thr Asn Lys His Thr Leu Thr Lys Ile Tyr Asn Pro
210 215 220
Lys Leu Asn Val Ser Glu Val Thr Leu Glu Leu Tyr Tyr Glu Lys Gly
225 230 235 240
Thr Gly Ser Ala Thr Phe Asp Asn Ile Ser Met Lys Ala Lys Gly Pro
245 250 255
Lys Asp Ser Glu His Pro Gln Pro Val Thr Thr Gln Ile Glu Glu Ser
260 265 270
Val Asn Thr Ala Leu Asn Lys Asn Tyr Val Phe Asn Lys Ala Asp Tyr
275 280 285
Gln Tyr Thr Leu Thr Asn Pro Ser Leu Gly Lys Ile Val Gly Gly Ile
290 295 300

```
Leu Tyr Pro Asn Ala Thr Gly Ser Thr Thr Val Lys Ile Ser Asp Lys
305                 310                 315                 320
Ser Gly Lys Ile Ile Lys Glu Val Pro Leu Ser Val Thr Ala Ser Thr
                325                 330                 335
Glu Asp Lys Phe Thr Lys Leu Leu Asp Lys Trp Asn Asp Val Thr Ile
            340                 345                 350
Gly Asn His Val Tyr Asp Thr Asn Asp Ser Asn Met Gln Lys Ile Asn
        355                 360                 365
Gln Lys Leu Asp Glu Thr Asn Ala Lys Asn Ile Lys Thr Ile Lys Leu
    370                 375                 380
Asp Ser Asn His Thr Phe Leu Trp Lys Asp Leu Asp Asn Leu Asn Asn
385                 390                 395                 400
Ser Ala Gln Leu Thr Ala Thr Tyr Arg Arg Leu Glu Asp Leu Ala Lys
                405                 410                 415
Gln Ile Thr Asn Pro His Ser Thr Ile Tyr Lys Asn Glu Lys Ala Ile
            420                 425                 430
Arg Thr Val Lys Glu Ser Leu Ala Trp Leu His Gln Asn Phe Tyr Asn
        435                 440                 445
Val Asn Lys Asp Ile Glu Gly Ser Ala Asn Trp Trp Asp Phe Glu Ile
    450                 455                 460
Gly Val Pro Arg Ser Ile Thr Ala Thr Leu Ala Leu Met Asn Asn Tyr
465                 470                 475                 480
Phe Thr Asp Ala Glu Ile Lys Thr Tyr Thr Asp Pro Ile Glu His Phe
                485                 490                 495
Val Pro Asp Ala Gly Tyr Phe Arg Lys Thr Leu Asp Asn Pro Phe Lys
            500                 505                 510
Ala Leu Gly Gly Asn Leu Val Asp Met Gly Arg Val Lys Ile Ile Glu
        515                 520                 525
Gly Leu Leu Arg Lys Asp Asn Thr Ile Ile Glu Lys Thr Ser His Ser
    530                 535                 540
Leu Lys Asn Leu Phe Thr Thr Ala Thr Lys Ala Glu Gly Phe Tyr Ala
545                 550                 555                 560
Asp Gly Ser Tyr Ile Asp His Thr Asn Val Ala Tyr Thr Gly Ala Tyr
                565                 570                 575
Gly Asn Val Leu Ile Asp Gly Leu Thr Gln Leu Leu Pro Ile Ile Gln
            580                 585                 590
Glu Thr Asp Tyr Lys Ile Ser Asn Gln Glu Leu Asp Met Val Tyr Lys
        595                 600                 605
Trp Ile Asn Gln Ser Phe Leu Pro Leu Ile Val Lys Gly Glu Leu Met
    610                 615                 620
Asp Met Ser Arg Gly Arg Ser Ile Ser Arg Glu Ala Ala Ser Ser His
625                 630                 635                 640
Ala Ala Ala Val Glu Val Leu Arg Gly Phe Leu Arg Leu Ala Asn Met
                645                 650                 655
Ser Asn Glu Glu Arg Asn Leu Asp Leu Ile Ser Thr Ile Lys Thr Ile
            660                 665                 670
Ile Thr Ser Asn Lys Phe Tyr Asn Val Phe Asn Asn Leu Lys Ser Tyr
        675                 680                 685
Ser Asp Ile Ala Asn Met Asn Lys Met Leu Asn Asp Ser Thr Val Ala
    690                 695                 700
Thr Lys Pro Leu Lys Ser Asn Leu Ser Thr Phe Asn Ser Met Asp Arg
705                 710                 715                 720
Leu Ala Tyr Tyr Asn Ala Glu Lys Asp Phe Gly Phe Ala Leu Ser Leu
                725                 730                 735
His Ser Lys Arg Thr Leu Asn Tyr Glu Gly Met Asn Asp Glu Asn Thr
            740                 745                 750
Arg Gly Trp Tyr Thr Gly Asp Gly Met Phe Tyr Leu Tyr Asn Ser Asp
        755                 760                 765
Gln Ser His Tyr Ser Asn His Phe Trp Pro Thr Val Asn Pro Tyr Lys
    770                 775                 780
Met Ala Gly Thr Thr Glu Lys Asp Ala Lys Arg Glu Asp Thr Thr Lys
785                 790                 795                 800
Glu Phe Met Ser Lys His Ser Lys Asp Ala Lys Glu Lys Thr Gly Gln
                805                 810                 815
Val Thr Gly Thr Ser Asp Phe Val Gly Ser Val Lys Leu Asn Asp His
            820                 825                 830
Phe Ala Leu Ala Ala Met Asp Phe Thr Asn Trp Asp Arg Thr Leu Thr
        835                 840                 845
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Ala Gln Lys Gly Trp Val Ile Leu Asn Asp Lys Ile Val Phe Leu Gly
    850                 855                 860
Ser Asn Ile Lys Asn Thr Asn Gly Ile Gly Asn Val Ser Thr Thr Ile
865                 870                 875                 880
Asp Gln Arg Lys Asp Asp Ser Lys Thr Pro Tyr Thr Thr Tyr Val Asn
                885                 890                 895
Gly Lys Thr Ile Asp Leu Lys Gln Ala Ser Ser Gln Gln Phe Thr Asp
            900                 905                 910
Thr Lys Ser Val Phe Leu Glu Ser Lys Glu Pro Gly Arg Asn Ile Gly
        915                 920                 925
Tyr Ile Phe Phe Lys Asn Ser Thr Ile Asp Ile Glu Arg Lys Glu Gln
    930                 935                 940
Thr Gly Thr Trp Asn Ser Ile Asn Arg Thr Ser Lys Asn Thr Ser Ile
945                 950                 955                 960
Val Ser Asn Pro Phe Ile Thr Ile Ser Gln Lys His Asp Asn Lys Gly
                965                 970                 975
Asp Ser Tyr Gly Tyr Met Met Val Pro Asn Ile Asp Arg Thr Ser Phe
            980                 985                 990
Asp Lys Leu Ala Asn Ser Lys Glu Val Glu Leu Leu Glu Asn Ser Ser
        995                 1000                1005
Lys Gln Gln Val Ile Tyr Asp Lys Asn Ser Gln Thr Trp Ala Val Ile
    1010                1015                1020
Lys His Asp Asn Gln Glu Ser Leu Ile Asn Asn Gln Phe Lys Met Asn
1025                1030                1035                1040
Lys Ala Gly Leu Tyr Leu Val Gln Lys Val Gly Asn Asp Tyr Gln Asn
                1045                1050                1055
Val Tyr Tyr Gln Pro Gln Thr Met Thr Lys Thr Asp Gln Leu Ala Ile
            1060                1065                1070
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<210> 72
<211> 1081
<212> PRT
<213> Streptococcus agalactiae serotype Ia

<220>
<223> hyaluronan lyase

<400> 72

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Met Glu Ile Lys Lys Lys His Arg Ile Met Leu Tyr Ser Ala Leu Ile
 1                5                  10                  15
Leu Gly Thr Ile Leu Val Asn Asn Ser Tyr Gln Ala Lys Ala Glu Glu
            20                  25                  30
Phe Thr Lys Thr Thr Ser Thr Ser Gln Ile Arg Asp Thr Gln Thr Asn
        35                  40                  45
Asn Val Glu Val Pro Gln Thr Glu Ser Thr Thr Val Lys Gly Thr Ser
    50                  55                  60
Thr Thr Thr Thr Gln Gln Asp Leu Ser Asn Ser Thr Ala Ser Thr Ala
65                  70                  75                  80
Thr Ala Thr Ala Thr His Ser Thr Met Lys Gln Val Val Asp Asn Gln
                85                  90                  95
Thr Gln Asn Lys Glu Leu Val Lys Asn Gly Asp Phe Lys Glu Lys Ile
            100                 105                 110
Ile Asp Lys Lys Ile Asp Lys Lys Ser Gln Trp Thr Asn Leu Tyr Gly
        115                 120                 125
Ala Lys Asp Trp Asn Thr Tyr Ile Asp Gln Thr Lys Ser Val Asn Lys
    130                 135                 140
Ser Pro Ile Ile Gln Arg Thr Glu Gln Gly Gln Val Ser Leu Ser Ser
145                 150                 155                 160
Asp Lys Gly Phe Arg Gly Ala Val Thr Gln Lys Val Asn Ile Asp Pro
                165                 170                 175
Thr Lys Lys Tyr Glu Val Lys Phe Asp Ile Glu Thr Ser Asn Lys Val
            180                 185                 190
Gly Gln Ala Phe Leu Arg Ile Met Lys Lys Lys Asp Lys Asn Thr Arg
        195                 200                 205
Leu Trp Leu Ser Glu Met Thr Ser Gly Thr Thr Asn Lys His Thr Leu
    210                 215                 220
Thr Lys Ile Tyr Asn Pro Lys Leu Asn Val Ser Glu Val Thr Leu Glu
```

225 230 235 240
Leu Tyr Tyr Glu Lys Gly Thr Gly Ser Val Thr Phe Asp Asn Ile Ser
245 250 255
Met Lys Ala Lys Gly Pro Lys Asp Ser Glu His Pro Gln Pro Val Thr
260 265 270
Thr Gln Ile Glu Glu Ser Val Asn Thr Ala Leu Asn Lys Asn Tyr Val
275 280 285
Phe Asn Lys Ala Asp Tyr Gln Tyr Thr Leu Thr Asn Pro Ser Leu Gly
290 295 300
Lys Ile Val Gly Gly Ile Leu Tyr Pro Ser Ala Thr Gly Ser Thr Thr
305 310 315 320
Val Lys Ile Ser Asp Lys Ser Gly Lys Ile Ile Lys Glu Val Pro Leu
325 330 335
Ser Val Thr Ala Ser Thr Glu Asp Asn Phe Thr Lys Leu Leu Asp Lys
340 345 350
Trp Asn Asp Val Thr Ile Gly Asn His Val Tyr Asp Thr Asn Asp Ser
355 360 365
Asn Met Gln Lys Leu Asn Gln Lys Leu Asp Glu Thr Asn Ala Lys Asn
370 375 380
Ile Lys Asp Ile Lys Leu Asp Ser Asn Arg Thr Phe Leu Trp Glu Asp
385 390 395 400
Leu Lys Gly Leu Asn Asn Ser Ala Gln Leu Thr Ala Thr Tyr Arg Arg
405 410 415
Leu Glu Asp Leu Ala Lys Gln Ile Thr Asn Pro His Ser Thr Ile Tyr
420 425 430
Lys Asn Glu Lys Ala Ile Arg Thr Val Lys Glu Ser Leu Ala Trp Leu
435 440 445
His Gln Asn Phe Tyr Asn Val Asn Lys Asp Ile Glu Gly Ser Ala Asn
450 455 460
Trp Trp Asp Phe Glu Ile Gly Val Pro Arg Ser Ile Thr Ala Thr Leu
465 470 475 480
Ala Leu Met Asn Asn Tyr Phe Thr Asp Ala Glu Ile Lys Thr Tyr Thr
485 490 495
Asp Pro Ile Glu His Phe Val Pro Asp Ala Gly Tyr Phe Arg Lys Thr
500 505 510
Leu Val Asn Pro Phe Lys Ala Leu Gly Gly Asn Leu Val Asp Met Gly
515 520 525
Arg Val Lys Ile Ile Glu Gly Leu Leu Arg Lys Asp Asn Thr Ile Ile
530 535 540
Lys Lys Thr Ser His Ser Leu Lys Asn Leu Phe Thr Thr Ala Thr Lys
545 550 555 560
Ala Glu Gly Phe Tyr Ala Asp Gly Ser Tyr Ile Asp His Thr Asn Val
565 570 575
Ala Tyr Thr Gly Ala Tyr Gly Asn Val Leu Ile Asp Gly Leu Thr Gln
580 585 590
Leu Leu Pro Ile Ile Gln Glu Thr Asp Tyr Lys Ile Ser Asn Gln Glu
595 600 605
Leu Asp Met Val Tyr Lys Trp Ile Asn Gln Ser Phe Leu Pro Leu Ile
610 615 620
Val Lys Gly Glu Leu Met Asp Met Ser Arg Gly Arg Ser Ile Ser Arg
625 630 635 640
Glu Ala Ala Ser Ser His Ala Ala Ala Val Glu Val Leu Arg Gly Phe
645 650 655
Leu Arg Leu Ala Asn Met Ser Asn Glu Glu Arg Asn Leu Asp Leu Lys
660 665 670
Ser Thr Ile Lys Thr Ile Ile Thr Ser Asn Lys Phe Tyr Asn Val Phe
675 680 685
Asn Asn Leu Lys Ser Tyr Ser Asp Ile Ala Asn Met Asn Lys Leu Leu
690 695 700
Asn Asp Ser Thr Val Ala Thr Lys Pro Leu Lys Ser Asn Leu Ser Thr
705 710 715 720
Phe Asn Ser Met Asp Arg Leu Ala Tyr Tyr Asn Ala Glu Lys Asp Phe
725 730 735
Gly Phe Ala Leu Ser Leu His Ser Lys Arg Thr Leu Asn Tyr Glu Gly
740 745 750
Met Asn Asp Glu Asn Thr Arg Gly Trp Tyr Thr Gly Asp Gly Met Phe
755 760 765
Tyr Leu Tyr Asn Ser Asp Gln Ser His Tyr Ser Asn His Phe Trp Pro

770 775 780
Thr Val Asn Pro Tyr Lys Met Ala Gly Thr Thr Glu Lys Asp Thr Gly
785 790 795 800
Arg Glu Asp Thr Ile Lys Lys Leu Met Asn Arg Tyr Asp Lys Thr Asn
805 810 815
Lys Asn Ser Lys Val Met Thr Gly Gln Val Thr Gly Thr Ser Asp Phe
820 825 830
Val Gly Ser Val Lys Leu Asn Asp His Phe Ala Leu Ala Ala Met Asp
835 840 845
Phe Thr Asn Trp Asp Arg Thr Leu Thr Ala Gln Lys Gly Trp Val Ile
850 855 860
Leu Asn Asp Lys Ile Val Phe Leu Gly Ser Asn Ile Lys Asn Thr Asn
865 870 875 880
Gly Val Gly Asn Val Ser Thr Thr Ile Asp Gln Arg Lys Asp Asp Ser
885 890 895
Lys Thr Pro Tyr Thr Thr Tyr Val Asn Gly Lys Thr Val Asp Leu Lys
900 905 910
Gln Ala Ser Ser Gln Gln Phe Thr Asp Thr Lys Ser Val Phe Leu Glu
915 920 925
Ser Lys Glu Pro Gly Arg Asn Ile Gly Tyr Ile Phe Phe Lys Asn Ser
930 935 940
Thr Ile Asp Ile Glu Arg Lys Glu Gln Thr Gly Thr Trp Asn Ser Ile
945 950 955 960
Asn Arg Thr Ser Lys Asn Thr Ser Ile Val Ser Asn Pro Phe Ile Thr
965 970 975
Ile Ser Gln Lys His Asp Asn Lys Gly Asp Ser Tyr Gly Tyr Met Met
980 985 990
Val Pro Asn Ile Asp Arg Thr Ser Phe Asp Lys Leu Ala Asn Ser Lys
995 1000 1005
Glu Val Glu Leu Leu Glu Asn Ser Ser Lys Gln Gln Val Ile Tyr Asp
1010 1015 1020
Lys Asn Ser Gln Thr Trp Ala Val Ile Lys His Asp Asn Gln Glu Ser
1025 1030 1035 1040
Leu Ile Asn Asn Gln Phe Lys Met Asn Lys Ala Gly Leu Tyr Leu Val
1045 1050 1055
Gln Lys Val Gly Asn Asp Tyr Gln Asn Val Tyr Tyr Gln Pro Gln Thr
1060 1065 1070
Met Thr Lys Thr Asp Gln Leu Ala Ile
1075 1080

<210> 73
<211> 984
<212> PRT
<213> Streptococcus agalactiae serotype III

<220>
<223> hyaluronan lyase

<400> 73
Met Lys Gln Val Val Asp Asn Gln Thr Gln Asn Lys Glu Leu Val Lys
1 5 10 15
Asn Gly Asp Phe Asn Gln Thr Asn Pro Val Ser Gly Ser Trp Ser His
20 25 30
Thr Ser Ala Arg Glu Trp Ser Ala Trp Ile Asp Lys Glu Asn Thr Ala
35 40 45
Asp Lys Ser Pro Ile Ile Gln Arg Thr Glu Gln Gly Gln Val Ser Leu
50 55 60
Ser Ser Asp Lys Gly Phe Arg Gly Ala Val Thr Gln Lys Val Asn Ile
65 70 75 80
Asp Pro Thr Lys Lys Tyr Glu Val Lys Phe Asp Ile Glu Thr Ser Asn
85 90 95
Lys Ala Gly Gln Ala Phe Leu Arg Ile Met Glu Lys Lys Asp Asn Asn
100 105 110
Thr Arg Leu Trp Leu Ser Glu Met Thr Ser Gly Thr Thr Asn Lys His
115 120 125
Thr Leu Thr Lys Ile Tyr Asn Pro Lys Leu Asn Val Ser Glu Val Thr
130 135 140

```
Leu Glu Leu Tyr Tyr Glu Lys Gly Thr Gly Ser Ala Thr Phe Asp Asn
145                 150                 155                 160
Ile Ser Met Lys Ala Lys Gly Pro Lys Asp Ser Glu His Pro Gln Pro
                165                 170                 175
Val Thr Thr Gln Ile Glu Glu Ser Val Asn Thr Ala Leu Asn Lys Asn
            180                 185                 190
Tyr Val Phe Asn Lys Ala Asp Tyr Gln Tyr Thr Leu Thr Asn Pro Ser
        195                 200                 205
Leu Gly Lys Ile Val Gly Gly Ile Leu Tyr Pro Asn Ala Thr Gly Ser
    210                 215                 220
Thr Thr Val Lys Ile Ser Asp Lys Ser Gly Lys Ile Ile Lys Glu Val
225                 230                 235                 240
Pro Leu Ser Val Thr Ala Ser Thr Glu Asp Lys Phe Thr Lys Leu Leu
                245                 250                 255
Asp Lys Trp Asn Asp Val Thr Ile Gly Asn His Val Tyr Asp Thr Asn
            260                 265                 270
Asp Ser Asn Met Gln Lys Ile Asn Gln Lys Leu Asp Glu Thr Asn Ala
        275                 280                 285
Lys Asn Ile Lys Thr Ile Lys Leu Asp Ser Asn His Thr Phe Leu Trp
    290                 295                 300
Lys Asp Leu Asp Asn Leu Asn Asn Ser Ala Gln Leu Thr Ala Thr Tyr
305                 310                 315                 320
Arg Arg Leu Glu Asp Leu Ala Lys Gln Ile Thr Asn Pro His Ser Thr
                325                 330                 335
Ile Tyr Lys Asn Glu Lys Ala Ile Arg Thr Val Lys Glu Ser Leu Ala
            340                 345                 350
Trp Leu His Gln Asn Phe Tyr Asn Val Asn Lys Asp Ile Glu Gly Ser
        355                 360                 365
Ala Asn Trp Trp Asp Phe Glu Ile Gly Val Pro Arg Ser Ile Thr Ala
    370                 375                 380
Thr Leu Ala Leu Met Asn Asn Tyr Phe Thr Asp Ala Glu Ile Lys Thr
385                 390                 395                 400
Tyr Thr Asp Pro Ile Glu His Phe Val Pro Asp Ala Gly Tyr Phe Arg
                405                 410                 415
Lys Thr Leu Asp Asn Pro Phe Lys Ala Leu Gly Gly Asn Leu Val Asp
            420                 425                 430
Met Gly Arg Val Lys Ile Ile Glu Gly Leu Leu Arg Lys Asp Asn Thr
        435                 440                 445
Ile Ile Glu Lys Thr Ser His Ser Leu Lys Asn Leu Phe Thr Thr Ala
    450                 455                 460
Thr Lys Ala Glu Gly Phe Tyr Ala Asp Gly Ser Tyr Ile Asp His Thr
465                 470                 475                 480
Asn Val Ala Tyr Thr Gly Ala Tyr Gly Asn Val Leu Ile Asp Gly Leu
                485                 490                 495
Thr Gln Leu Leu Pro Ile Ile Gln Glu Thr Asp Tyr Lys Ile Ser Asn
            500                 505                 510
Gln Glu Leu Asp Met Val Tyr Lys Trp Ile Asn Gln Ser Phe Leu Pro
        515                 520                 525
Leu Ile Val Lys Gly Glu Leu Met Asp Met Ser Arg Gly Arg Ser Ile
    530                 535                 540
Ser Arg Glu Ala Ala Ser Ser His Ala Ala Ala Val Glu Val Leu Arg
545                 550                 555                 560
Gly Phe Leu Arg Leu Ala Asn Met Ser Asn Glu Glu Arg Asn Leu Asp
                565                 570                 575
Leu Lys Ser Thr Ile Lys Thr Ile Ile Thr Ser Asn Lys Phe Tyr Asn
            580                 585                 590
Val Phe Asn Asn Leu Lys Ser Tyr Ser Asp Ile Ala Asn Met Asn Lys
        595                 600                 605
Met Leu Asn Asp Ser Thr Val Ala Thr Lys Pro Leu Lys Ser Asn Leu
    610                 615                 620
Ser Thr Phe Asn Ser Met Asp Arg Leu Ala Tyr Tyr Asn Ala Glu Lys
625                 630                 635                 640
Asp Phe Gly Phe Ala Leu Ser Leu His Ser Lys Arg Thr Leu Asn Tyr
                645                 650                 655
Glu Gly Met Asn Asp Glu Asn Thr Arg Asp Trp Tyr Thr Gly Asp Gly
            660                 665                 670
Met Phe Tyr Leu Tyr Asn Ser Asp Gln Ser His Tyr Ser Asn His Phe
        675                 680                 685
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Trp Pro Thr Val Asn Pro Tyr Lys Met Ala Gly Thr Thr Glu Lys Asp
    690                 695                 700
Ala Lys Arg Glu Asp Thr Thr Lys Glu Phe Met Ser Lys His Ser Lys
705                 710                 715                 720
Asp Ala Lys Glu Lys Thr Gly Gln Val Thr Gly Thr Ser Asp Phe Val
                725                 730                 735
Gly Ser Val Lys Leu Asn Asp His Phe Ala Leu Ala Ala Met Asp Phe
            740                 745                 750
Thr Asn Trp Asp Arg Thr Leu Thr Ala Gln Lys Gly Trp Val Ile Leu
        755                 760                 765
Asn Asp Lys Ile Val Phe Leu Gly Ser Asn Ile Lys Asn Thr Asn Gly
    770                 775                 780
Ile Gly Asn Val Ser Thr Thr Ile Asp Gln Arg Lys Asp Asp Ser Lys
785                 790                 795                 800
Thr Pro Tyr Thr Thr Tyr Val Asn Gly Lys Thr Ile Asp Leu Lys Gln
                805                 810                 815
Ala Ser Ser Gln Gln Phe Thr Asp Thr Lys Ser Val Phe Leu Glu Ser
            820                 825                 830
Lys Glu Pro Gly Arg Asn Ile Gly Tyr Ile Phe Phe Lys Asn Ser Thr
        835                 840                 845
Ile Asp Ile Glu Arg Lys Glu Gln Thr Gly Thr Trp Asn Ser Ile Asn
    850                 855                 860
Arg Thr Ser Lys Asn Thr Ser Ile Val Ser Asn Pro Phe Ile Thr Ile
865                 870                 875                 880
Ser Gln Lys His Asp Asn Lys Gly Asp Ser Tyr Gly Tyr Met Met Val
                885                 890                 895
Pro Asn Ile Asp Arg Thr Ser Phe Asp Lys Leu Ala Asn Ser Lys Glu
            900                 905                 910
Val Glu Leu Leu Glu Asn Ser Ser Lys Gln Gln Val Ile Tyr Asp Lys
        915                 920                 925
Asn Ser Gln Thr Trp Ala Val Ile Lys His Asp Asn Gln Glu Ser Leu
    930                 935                 940
Ile Asn Asn Gln Phe Lys Met Asn Lys Ala Gly Leu Tyr Leu Val Gln
945                 950                 955                 960
Lys Val Gly Asn Asp Tyr Gln Asn Val Tyr Tyr Gln Pro Gln Thr Met
                965                 970                 975
Thr Lys Thr Asp Gln Leu Ala Ile
            980
```

<210> 74
<211> 807
<212> PRT
<213> Staphylococcus aureus (strain COL)

<220>
<223> hyaluronan lyase

<400> 74

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Met Thr Tyr Arg Ile Lys Lys Trp Gln Lys Leu Ser Thr Ile Thr Leu
 1               5                  10                  15
Leu Met Ala Gly Val Ile Thr Leu Asn Gly Gly Glu Phe Arg Ser Val
            20                  25                  30
Asp Lys His Gln Ile Ala Val Ala Asp Thr Asn Val Gln Thr Pro Asp
        35                  40                  45
Tyr Glu Lys Leu Arg Asn Thr Trp Leu Asp Val Asn Tyr Gly Tyr Asp
    50                  55                  60
Lys Tyr Asp Glu Asn Asn Pro Asp Met Lys Lys Lys Phe Asp Ala Thr
65                  70                  75                  80
Glu Lys Glu Ala Thr Asn Leu Leu Lys Glu Met Lys Thr Glu Ser Gly
                85                  90                  95
Arg Lys Tyr Leu Trp Ser Gly Ala Glu Thr Leu Glu Thr Asn Ser Ser
            100                 105                 110
His Met Thr Arg Thr Tyr Arg Asn Ile Glu Lys Ile Ala Glu Ala Met
        115                 120                 125
Arg Asn Pro Lys Thr Thr Leu Asn Thr Asp Glu Asn Lys Lys Lys Val
    130                 135                 140
Lys Asp Ala Leu Glu Trp Leu His Lys Asn Ala Tyr Gly Lys Glu Pro
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145                 150                 155                 160
Asp Lys Lys Val Lys Glu Leu Ser Glu Asn Phe Thr Lys Thr Thr Gly
                165                 170                 175
Lys Asn Thr Asn Leu Asn Trp Trp Asp Tyr Glu Ile Gly Thr Pro Lys
            180                 185                 190
Ser Leu Thr Asn Thr Leu Ile Leu Leu Asn Asp Gln Phe Ser Asn Glu
        195                 200                 205
Glu Lys Lys Lys Phe Thr Ala Pro Ile Lys Thr Phe Ala Pro Asp Ser
    210                 215                 220
Asp Lys Ile Leu Ser Ser Val Gly Lys Ala Glu Leu Ala Lys Gly Gly
225                 230                 235                 240
Asn Leu Val Asp Ile Ser Lys Val Lys Leu Leu Glu Cys Ile Ile Glu
                245                 250                 255
Glu Asp Lys Asp Met Met Lys Lys Ser Ile Asp Ser Phe Asn Lys Val
            260                 265                 270
Phe Thr Tyr Val Gln Asp Ser Ala Thr Gly Lys Glu Arg Asn Gly Phe
        275                 280                 285
Tyr Lys Asp Gly Ser Tyr Ile Asp His Gln Asp Val Pro Tyr Thr Gly
    290                 295                 300
Ala Tyr Gly Val Val Leu Leu Glu Gly Ile Ser Gln Met Met Pro Met
305                 310                 315                 320
Ile Lys Glu Thr Pro Phe Asn Asp Lys Thr Gln Asn Asp Thr Thr Leu
                325                 330                 335
Lys Ser Trp Ile Asp Asp Gly Phe Met Pro Leu Ile Tyr Lys Gly Glu
            340                 345                 350
Met Met Asp Leu Ser Arg Gly Arg Ala Ile Ser Arg Glu Asn Glu Thr
        355                 360                 365
Ser His Ser Ala Ser Ala Thr Val Met Lys Ser Leu Leu Arg Leu Ser
    370                 375                 380
Asp Ala Met Asp Asp Ser Thr Lys Ala Lys Tyr Lys Lys Ile Val Lys
385                 390                 395                 400
Ser Ser Val Glu Ser Asp Ser Ser Tyr Lys Gln Asn Asp Tyr Leu Asn
                405                 410                 415
Ser Tyr Ser Asp Ile Asp Lys Met Lys Ser Leu Met Thr Asp Asn Ser
            420                 425                 430
Ile Ser Lys Asn Gly Leu Thr Gln Gln Leu Lys Ile Tyr Asn Asp Met
        435                 440                 445
Asp Arg Val Thr Tyr His Asn Lys Asp Leu Asp Phe Ala Phe Gly Leu
    450                 455                 460
Ser Met Thr Ser Lys Asn Val Ala Arg Tyr Glu Ser Ile Asn Gly Glu
465                 470                 475                 480
Asn Leu Lys Gly Trp His Thr Gly Ala Gly Met Ser Tyr Leu Tyr Asn
                485                 490                 495
Ser Asp Val Lys His Tyr His Asp Asn Phe Trp Val Thr Ala Asp Met
            500                 505                 510
Lys Arg Leu Ser Gly Thr Thr Thr Leu Asp Asn Glu Ile Leu Lys Asp
        515                 520                 525
Thr Asp Asp Lys Lys Ser Ser Lys Thr Phe Val Gly Gly Thr Lys Val
    530                 535                 540
Asp Asp Gln His Ala Ser Ile Gly Met Asp Phe Glu Asn Gln Asp Lys
545                 550                 555                 560
Thr Leu Thr Ala Lys Lys Ser Tyr Phe Ile Leu Asn Asp Lys Ile Val
                565                 570                 575
Phe Leu Gly Thr Gly Ile Lys Ser Thr Asp Ser Ser Lys Asn Pro Val
            580                 585                 590
Thr Thr Ile Glu Asn Arg Lys Ala Asn Gly Tyr Thr Leu Tyr Thr Asp
        595                 600                 605
Asp Lys Gln Thr Thr Asn Ser Asp Asn Gln Glu Asn Asn Ser Val Phe
    610                 615                 620
Leu Glu Ser Thr Asp Thr Lys Lys Asn Ile Gly Tyr His Phe Leu Asn
625                 630                 635                 640
Lys Pro Lys Ile Thr Val Lys Lys Glu Ser His Thr Gly Lys Trp Lys
                645                 650                 655
Glu Ile Asn Lys Ser Gln Lys Asp Thr Gln Lys Thr Asp Glu Tyr Tyr
            660                 665                 670
Glu Val Thr Gln Lys His Ser Asn Ser Asp Asn Lys Tyr Gly Tyr Val
        675                 680                 685
Leu Tyr Pro Gly Leu Ser Lys Asp Val Phe Lys Thr Lys Lys Asp Glu
```

690 695 700
Val Thr Val Val Lys Gln Glu Asp Asp Phe His Val Val Lys Asp Asn
705 710 715 720
Glu Ser Val Trp Ala Gly Val Asn Tyr Ser Asn Ser Thr Gln Thr Phe
725 730 735
Asp Ile Asn Asn Thr Lys Val Glu Val Lys Ala Lys Gly Met Phe Ile
740 745 750
Leu Lys Lys Lys Asp Asp Asn Thr Tyr Glu Cys Ser Phe Tyr Asn Pro
755 760 765
Glu Ser Thr Asn Ser Ala Ser Asp Ile Glu Ser Lys Ile Ser Met Thr
770 775 780
Gly Tyr Ser Ile Thr Asn Lys Asn Thr Ser Thr Ser Asn Glu Ser Gly
785 790 795 800
Val His Phe Glu Leu Thr Lys
805

<210> 75
<211> 420
<212> PRT
<213> Staphylococcus aureus (strain MRSA252)

<220>
<223> hyaluronan lyase

<400> 75
Met Thr Tyr Arg Met Lys Lys Trp Gln Lys Leu Ser Thr Ile Thr Leu
1 5 10 15
Leu Met Ala Gly Val Ile Thr Leu Asn Gly Gly Glu Phe Arg Ser Ile
20 25 30
Asp Lys His Gln Ile Ala Val Ala Asp Thr Asn Val Gln Thr Thr Asp
35 40 45
Tyr Glu Lys Leu Arg Asn Ile Trp Leu Asp Val Asn Tyr Gly Tyr Asp
50 55 60
Lys Tyr Asp Glu Asn Asn Pro Asp Met Lys Lys Lys Phe Glu Ala Thr
65 70 75 80
Glu Asn Glu Ala Glu Lys Leu Leu Lys Glu Met Lys Thr Glu Ser Asp
85 90 95
Arg Lys Tyr Leu Trp Glu Ser Ser Lys Asp Leu Asp Thr Lys Ser Ala
100 105 110
Asp Met Thr Arg Thr Tyr Arg Asn Ile Glu Lys Ile Ser Glu Ala Met
115 120 125
Lys His Lys Asn Thr Lys Leu Lys Thr Asp Glu Asn Lys Thr Lys Val
130 135 140
Lys Asp Ala Leu Glu Trp Leu His Lys Asn Ala Tyr Gly Lys Glu Pro
145 150 155 160
Asp Lys Lys Val Ala Asp Leu Thr Ser Asn Phe Lys Asn Lys Thr Ser
165 170 175
Arg Asn Thr Asn Leu Asn Trp Trp Asp Tyr Glu Ile Gly Thr Pro Arg
180 185 190
Ala Leu Thr Asn Thr Leu Ile Leu Leu Gln Glu Asp Phe Thr Asp Glu
195 200 205
Glu Lys Lys Lys Tyr Thr Ala Pro Ile Lys Thr Phe Ala Pro Asp Ser
210 215 220
Asp Lys Ile Leu Ser Ser Val Gly Lys Ser Glu Pro Ala Lys Gly Gly
225 230 235 240
Asn Leu Val Asp Ile Ser Lys Val Lys Leu Leu Glu Ser Ile Ile Glu
245 250 255
Glu Asp Lys Asp Met Met Lys Lys Ser Ile Asp Ser Phe Asn Thr Val
260 265 270
Phe Thr Tyr Ala Gln Asn Ser Ala Thr Gly Lys Glu Arg Asn Gly Phe
275 280 285
Tyr Lys Asp Gly Ser Tyr Ile Asp His Gln Asp Val Pro Tyr Thr Gly
290 295 300
Ala Tyr Gly Val Val Leu Leu Glu Gly Ile Ser Gln Met Met Pro Met
305 310 315 320
Ile Lys Glu Thr Pro Phe Asn Asp Ser Asn Gln Asn Asp Thr Thr Leu
325 330 335

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Lys Ser Trp Ile Asp Asp Gly Phe Met Pro Leu Ile Tyr Lys Gly Glu
            340                 345                 350
Met Met Asp Leu Ser Arg Gly Arg Ala Ile Ser Arg Glu Asn Glu Thr
        355                 360                 365
Ser His Ser Ala Ser Ala Thr Val Met Lys Ser Leu Leu Arg Leu Ser
    370                 375                 380
Asp Thr Met Asp Lys Ser Thr Lys Ala Lys Tyr Lys Lys Ile Val Lys
385                 390                 395                 400
Thr Ser Val Glu Ser Asp Ser Ser Tyr Lys Gln Thr Asp Tyr Leu Ser
                405                 410                 415
Ser Tyr Ser Asp
            420


<210> 76
<211> 806
<212> PRT
<213> Staphylococcus aureus (strain MRSA252)

<220>
<223> hyaluronan lyase

<400> 76
Met Thr Asn Lys Met Lys Lys Trp Gln Lys Leu Ser Thr Ile Thr Leu
 1                  5                  10                  15
Leu Met Thr Gly Val Ile Ala Leu Asn Asn Gly Glu Phe Arg Asn Val
            20                  25                  30
Asp Lys His Gln Ile Ala Val Ala Asp Thr Asn Val Gln Thr Pro Asp
        35                  40                  45
Tyr Glu Lys Leu Lys Lys Thr Trp Leu Asp Val Asn Tyr Gly Tyr Asp
    50                  55                  60
Gln Tyr Asp Glu Asn Asn Gln Asp Met Lys Lys Lys Phe Asp Ala Lys
65                  70                  75                  80
Glu Lys Glu Ala Lys Lys Leu Leu Asp Asp Met Lys Thr Asp Thr Asn
                85                  90                  95
Arg Thr Tyr Leu Trp Ser Gly Ala Glu Asn Leu Glu Thr Asn Ser Ser
            100                 105                 110
His Met Thr Lys Thr Tyr Arg Asn Ile Glu Lys Ile Ala Glu Ser Met
        115                 120                 125
Gln His Lys Asn Thr Val Leu Lys Thr Val Glu Asn Lys Leu Lys Ile
    130                 135                 140
Lys Glu Ala Leu Asp Trp Met His Lys Asn Val Tyr Gly Lys Asn Pro
145                 150                 155                 160
Ser Gln Lys Val Glu Asp Leu Thr Lys Asn Arg Lys Gly Gln Thr Thr
                165                 170                 175
Pro Lys Asn Asn Ser Leu Asn Trp Trp Asp Tyr Glu Ile Gly Thr Pro
            180                 185                 190
Arg Ala Leu Thr Asn Thr Leu Leu Leu Met Asp Asp Met Leu Thr Lys
        195                 200                 205
Asp Glu Met Lys Asn Tyr Ser Lys Pro Ile Ser Thr Tyr Ala Pro Ser
    210                 215                 220
Ser Asp Lys Ile Leu Ser Ser Val Gly Glu Ser Glu Asp Ala Lys Gly
225                 230                 235                 240
Gly Asn Leu Val Asp Ile Ser Lys Val Lys Leu Leu Glu Ser Val Ile
                245                 250                 255
Glu Glu Asp Val Asp Met Leu Lys Lys Ser Ile Asp Ser Phe Asn Lys
            260                 265                 270
Val Phe Thr Tyr Val Gln Asp Ser Ala Thr Gly Lys Gly Arg Asn Gly
        275                 280                 285
Phe Tyr Lys Asp Gly Ser Tyr Ile Asp His Gln Asp Val Pro Tyr Thr
    290                 295                 300
Gly Ala Tyr Gly Val Val Leu Leu Glu Gly Ile Ser Gln Met Met Pro
305                 310                 315                 320
Met Ile Lys Glu Ser Pro Phe Lys Thr Thr Gln Asp Asn Ala Thr Leu
                325                 330                 335
Ser Asn Trp Ile Asp Glu Gly Phe Met Pro Leu Ile Tyr Lys Gly Glu
            340                 345                 350
Met Met Asp Leu Ser Arg Gly Arg Ala Ile Ser Arg Glu Asn Glu Thr
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        355                 360                 365
Ser His Thr Ala Ser Ala Thr Val Met Lys Ser Leu Leu Arg Leu Asn
    370                 375                 380
Asp Thr Met Asp Asp Ser Thr Lys Thr Arg Tyr Lys Gln Ile Val Lys
385                 390                 395                 400
Thr Ser Val Asn Ser Asp Ser Ser Tyr Asn Gln Asn Asn Tyr Leu Asn
                405                 410                 415
Ser Tyr Ser Asp Ile Ala Lys Met Lys Lys Leu Met Asn Asp Ser Thr
            420                 425                 430
Ile Ser Lys Asn Asp Leu Thr Gln Gln Leu Lys Ile Tyr Asn Asp Met
        435                 440                 445
Asp Arg Val Thr Tyr His Asn Lys Asp Leu Asp Phe Ala Phe Gly Leu
    450                 455                 460
Ser Met Thr Ser Lys Asn Ile Ala Arg Tyr Glu Asn Ile Asn Gly Glu
465                 470                 475                 480
Asn Leu Lys Gly Trp His Thr Gly Ala Gly Met Ser Tyr Leu Tyr Asn
                485                 490                 495
Ser Asp Val Lys His Tyr Arg Asp Asn Phe Trp Ala Thr Ala Asp Met
            500                 505                 510
Thr Cys Leu Pro Gly Thr Thr Thr Leu Asn Asp Met Pro Ser Thr Asn
        515                 520                 525
Thr Lys Asn Asp Lys Ser Phe Val Gly Gly Thr Lys Leu Asn Asn Lys
    530                 535                 540
Tyr Ala Ser Ile Gly Met Asp Phe Glu Asn Gln Asp Lys Thr Leu Thr
545                 550                 555                 560
Ala Lys Lys Ser Tyr Phe Ile Leu Asn Asp Lys Ile Val Phe Leu Gly
                565                 570                 575
Thr Gly Ile Lys Ser Thr Asp Ser Ser Lys Asn Pro Val Thr Ser Val
            580                 585                 590
Glu Asn Arg Lys Ala Asn Gly Tyr Lys Leu Phe Lys Asp Asp Ile Glu
        595                 600                 605
Ile Thr Thr Ser Asp Val Asn Ala Gln Glu Thr His Ser Val Phe Leu
    610                 615                 620
Glu Ser Asn Asp Thr Lys Lys Asn Ile Gly Tyr His Phe Leu Asp Lys
625                 630                 635                 640
Pro Lys Ile Thr Val Lys Lys Glu Ser His Thr Gly Lys Trp Ser Glu
                645                 650                 655
Ile Asn Lys Ser Gln Lys Lys Asp Asp Lys Lys Asp Glu Tyr Tyr Glu
            660                 665                 670
Val Thr Gln Thr His Asn Thr Ser Asp Ser Lys Tyr Ala Tyr Val Leu
        675                 680                 685
Tyr Pro Gly Leu Ser Lys Ser Asp Phe Lys Ser Lys Asn Asn Asn Val
    690                 695                 700
Ser Ile Val Lys Gln Asp Glu Asp Phe His Val Ile Lys Asp Asn Asp
705                 710                 715                 720
Gly Val Phe Ala Gly Val Asn Tyr Ser Asp Asn Thr Lys Ser Phe Asp
                725                 730                 735
Ile Asn Gly Ile Thr Val Glu Leu Lys Glu Lys Gly Met Phe Val Ile
            740                 745                 750
Lys Lys Lys Asp Asp Lys Ala Tyr Lys Cys Ser Phe Tyr Asn Pro Glu
        755                 760                 765
Thr Thr Asn Thr Ala Ser Asn Ile Glu Ser Lys Ile Phe Ile Lys Gly
    770                 775                 780
Tyr Thr Ile Thr Asn Lys Ser Val Ile Asn Ser Asn Asp Ala Gly Val
785                 790                 795                 800
Asn Phe Glu Leu Thr Lys
                805
```

<210> 77
<211> 815
<212> PRT
<213> Staphylococcus aureus (strain MSSA476)

<220>
<223> hyaluronan lyase

<400> 77

```
Met Thr Tyr Arg Ile Lys Lys Trp Gln Lys Leu Ser Thr Ile Thr Leu
 1               5                  10                  15
Leu Met Ala Gly Val Ile Thr Leu Asn Gly Gly Glu Phe Arg Ser Ile
            20                  25                  30
Asp Lys Tyr Gln Ile Ala Val Ala Asp Thr Asn Val Gln Thr Pro Asp
        35                  40                  45
Tyr Glu Lys Leu Arg Asn Thr Trp Leu Asp Val Asn Tyr Gly Tyr Asp
    50                  55                  60
Lys Tyr Asp Glu Lys Asn Asp Ala Met Lys Lys Lys Phe Glu Ala Thr
65                  70                  75                  80
Glu Asn Glu Ala Lys Lys Leu Leu Ser Glu Met Lys Thr Glu Ser Asp
                85                  90                  95
Arg Lys Tyr Leu Trp Glu Asn Ser Lys Asp Leu Asp Thr Lys Ser Ala
            100                 105                 110
Asp Met Thr Arg Thr Tyr Arg Asn Ile Glu Lys Ile Ala Glu Ala Met
        115                 120                 125
Lys His Lys Asp Thr Lys Leu Lys Ile Asp Glu Asn Lys Lys Lys Val
    130                 135                 140
Lys Asp Ala Leu Glu Trp Leu His Lys Asn Ala Tyr Gly Lys Glu Pro
145                 150                 155                 160
Asp Lys Lys Val Ala Asp Leu Thr Ser Asn Phe Lys Asn Lys Thr Ser
                165                 170                 175
Arg Asn Thr Asn Leu Asn Trp Trp Asp Tyr Glu Ile Gly Thr Pro Arg
            180                 185                 190
Ala Leu Thr Asn Thr Leu Ile Leu Leu Asn Asp Gln Phe Ser Asn Asp
        195                 200                 205
Glu Lys Lys Lys Tyr Thr Ala Pro Ile Lys Thr Phe Ala Pro Glu Ser
    210                 215                 220
Asp Lys Ile Leu Ser Ser Val Gly Gln Pro Glu Gln Ala Lys Gly Gly
225                 230                 235                 240
Asn Leu Val Asp Ile Ala Lys Val Lys Leu Leu Glu Ser Ile Ile Glu
                245                 250                 255
Glu Asp Lys Asp Ile Thr Lys Asn Ser Ile Asp Ala Phe Asn Lys Val
            260                 265                 270
Phe Thr Tyr Val Gln Ser Asn Ala Thr Gly Lys Glu Arg Asn Gly Phe
        275                 280                 285
Tyr Lys Asp Gly Ser Tyr Ile Asp His Gln Asp Val Pro Tyr Thr Gly
    290                 295                 300
Ala Tyr Gly Val Val Leu Leu Glu Gly Ile Ser Gln Met Met Pro Met
305                 310                 315                 320
Ile Lys Glu Thr Pro Phe Asn Asp Lys Thr Gln Asn Asp Thr Thr Leu
                325                 330                 335
Lys Ser Trp Ile Asp Asp Gly Phe Met Pro Leu Ile Tyr Lys Gly Glu
            340                 345                 350
Met Met Asp Leu Ser Arg Gly Arg Ala Ile Ser Arg Glu Asn Glu Thr
        355                 360                 365
Ser His Thr Ala Ser Ala Thr Val Met Lys Ser Leu Leu Arg Leu Ser
    370                 375                 380
Asp Ala Met Asp Asp Ser Thr Lys Ala Lys Tyr Lys Gln Ile Val Lys
385                 390                 395                 400
Thr Ser Val Lys Ser Asp Ser Ser Tyr Gly Gln Asn Asp Thr Leu Ser
                405                 410                 415
Ser Tyr Ser Asp Ile Ser Lys Met Lys Ser Leu Met Glu Asp Ser Thr
            420                 425                 430
Ile Ser Thr Asn Gly Leu Thr Gln Gln Leu Lys Ile Tyr Asn Asp Met
        435                 440                 445
Asp Arg Val Thr Tyr His Asn Lys Asp Leu Asp Phe Ala Phe Gly Leu
    450                 455                 460
Ser Met Thr Ser Lys Asn Val Ala Arg Tyr Glu Ser Ile Asn Gly Glu
465                 470                 475                 480
Asn Leu Lys Gly Trp His Thr Gly Ala Gly Met Ser Tyr Leu Tyr Asn
                485                 490                 495
Ser Asp Val Lys His Tyr Arg Asp Asn Phe Trp Ala Thr Ala Asp Met
            500                 505                 510
Lys Arg Leu Ala Gly Thr Thr Thr Leu Glu Asn Glu Glu Pro Lys Gly
        515                 520                 525
Thr Asp Val Lys Lys Ser Ser Lys Thr Phe Val Gly Gly Thr Lys Phe
    530                 535                 540
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Asp Asp Gln His Ala Ser Ile Gly Met Asp Phe Glu Asn Gln Asp Lys
545                 550                 555                 560
Thr Leu Thr Ala Lys Lys Ser Tyr Phe Ile Leu Asn Asp Lys Ile Val
                565                 570                 575
Phe Leu Gly Thr Gly Ile Lys Ser Thr Asp Ser Ser Lys Asn Pro Val
            580                 585                 590
Thr Thr Ile Glu Asn Arg Lys Ala Asn Gly Tyr Thr Leu Tyr Thr Asp
        595                 600                 605
Asp Lys Gln Thr Thr Ala Ser Asp Asn Gln Gly Thr Asn Ser Val Phe
    610                 615                 620
Leu Glu Ser Thr Asn Lys Pro Lys Asn Asn Ile Gly Tyr His Phe Leu
625                 630                 635                 640
Asn Glu Ser Lys Ile Thr Val Lys Lys Glu Ser His Thr Gly Lys Trp
                645                 650                 655
Ser Asp Ile Asn Lys Ser Gln Lys Gln Asp Ser Lys Thr Asn Gln Tyr
            660                 665                 670
Tyr Glu Val Thr Gln Lys His Ser Asn Thr Asp Ser Lys Tyr Ala Tyr
        675                 680                 685
Val Leu Tyr Pro Gly Leu Ser Lys Asp Asp Phe Asn Thr Lys Lys Asp
    690                 695                 700
Lys Val Thr Val Val Lys Gln Asp Asp Asp Phe His Val Val Lys Asp
705                 710                 715                 720
Asn Glu Ser Val Trp Ala Gly Val Asn Tyr Ser Asp Ser Thr Gln Thr
                725                 730                 735
Phe Ile Ile Asn Asn Thr Lys Val Glu Val Lys Ala Lys Gly Met Phe
            740                 745                 750
Ile Leu Lys Lys Lys Asp Asp Lys Thr Tyr Glu Cys Ser Phe Tyr Asn
        755                 760                 765
Pro Glu Ser Thr Asn Thr Ala Ser Asp Ile Glu Ser Lys Ile Ser Met
    770                 775                 780
Thr Gly Tyr Ser Ile Thr Asn Lys Asn Thr Ser Thr Ser Asn Glu Ser
785                 790                 795                 800
Gly Val Arg Phe Glu Leu Gln Gln Thr Leu Asn Lys Asp Asp Asn
                805                 810                 815


<210> 78
<211> 807
<212> PRT
<213> Staphylococcus aureus (strain NCTC 8325)

<220>
<223> hyaluronan lyase

<400> 78
Met Thr Tyr Arg Ile Lys Lys Trp Gln Lys Leu Ser Thr Ile Thr Leu
 1                5                  10                  15
Leu Met Ala Gly Val Ile Thr Leu Asn Gly Gly Glu Phe Arg Ser Val
            20                  25                  30
Asp Lys His Gln Ile Ala Val Ala Asp Thr Asn Val Gln Thr Pro Asp
        35                  40                  45
Tyr Glu Lys Leu Arg Asn Thr Trp Leu Asp Val Asn Tyr Gly Tyr Asp
    50                  55                  60
Lys Tyr Asp Glu Asn Asn Pro Asp Met Lys Lys Lys Phe Asp Ala Thr
65                  70                  75                  80
Glu Lys Glu Ala Thr Asn Leu Leu Lys Glu Met Lys Thr Glu Ser Gly
                85                  90                  95
Arg Lys Tyr Leu Trp Ser Gly Ala Glu Thr Leu Glu Thr Asn Ser Ser
            100                 105                 110
His Met Thr Arg Thr Tyr Arg Asn Ile Glu Lys Ile Ala Glu Ala Met
        115                 120                 125
Arg Asn Pro Lys Thr Thr Leu Asn Thr Asp Glu Asn Lys Lys Lys Val
    130                 135                 140
Lys Asp Ala Leu Glu Trp Leu His Lys Asn Ala Tyr Gly Lys Glu Pro
145                 150                 155                 160
Asp Lys Lys Val Lys Glu Leu Ser Glu Asn Phe Thr Lys Thr Thr Gly
                165                 170                 175
Lys Asn Thr Asn Leu Asn Trp Trp Asp Tyr Glu Ile Gly Thr Pro Lys
```

```
            180                 185                 190
Ser Leu Thr Asn Thr Leu Ile Leu Leu Asn Asp Gln Phe Ser Asn Glu
        195                 200                 205
Glu Lys Lys Lys Phe Thr Ala Pro Ile Lys Thr Phe Ala Pro Asp Ser
    210                 215                 220
Asp Lys Ile Leu Ser Ser Val Gly Lys Ala Glu Leu Ala Lys Gly Gly
225                 230                 235                 240
Asn Leu Val Asp Ile Ser Lys Val Lys Leu Leu Glu Cys Ile Ile Glu
                245                 250                 255
Glu Asp Lys Asp Met Met Lys Lys Ser Ile Asp Ser Phe Asn Lys Val
            260                 265                 270
Phe Thr Tyr Val Gln Asp Ser Ala Thr Gly Lys Glu Arg Asn Gly Phe
        275                 280                 285
Tyr Lys Asp Gly Ser Tyr Ile Asp His Gln Asp Val Pro Tyr Thr Gly
    290                 295                 300
Ala Tyr Gly Val Val Leu Leu Glu Gly Ile Ser Gln Met Met Pro Met
305                 310                 315                 320
Ile Lys Glu Thr Pro Phe Asn Asp Lys Thr Gln Asn Asp Thr Thr Leu
                325                 330                 335
Lys Ser Trp Ile Asp Asp Gly Phe Met Pro Leu Ile Tyr Lys Gly Glu
            340                 345                 350
Met Met Asp Leu Ser Arg Gly Arg Ala Ile Ser Arg Glu Asn Glu Thr
        355                 360                 365
Ser His Ser Ala Ser Ala Thr Val Met Lys Ser Leu Leu Arg Leu Ser
    370                 375                 380
Asp Ala Met Asp Asp Ser Thr Lys Ala Lys Tyr Lys Lys Ile Val Lys
385                 390                 395                 400
Ser Ser Val Glu Ser Asp Ser Ser Tyr Lys Gln Asn Asp Tyr Leu Asn
                405                 410                 415
Ser Tyr Ser Asp Ile Asp Lys Met Lys Ser Leu Met Thr Asp Asn Ser
            420                 425                 430
Ile Ser Lys Asn Gly Leu Thr Gln Gln Leu Lys Ile Tyr Asn Asp Met
        435                 440                 445
Asp Arg Val Thr Tyr His Asn Lys Asp Leu Asp Phe Ala Phe Gly Leu
    450                 455                 460
Ser Met Thr Ser Lys Asn Val Ala Arg Tyr Glu Ser Ile Asn Gly Glu
465                 470                 475                 480
Asn Leu Lys Gly Trp His Thr Gly Ala Gly Met Ser Tyr Leu Tyr Asn
                485                 490                 495
Ser Asp Val Lys His Tyr His Asp Asn Phe Trp Val Thr Ala Asp Met
            500                 505                 510
Lys Arg Leu Ser Gly Thr Thr Thr Leu Asp Asn Glu Ile Leu Lys Asp
        515                 520                 525
Thr Asp Asp Lys Lys Ser Ser Lys Thr Phe Val Gly Gly Thr Lys Val
    530                 535                 540
Asp Asp Gln His Ala Ser Ile Gly Met Asp Phe Glu Asn Gln Asp Lys
545                 550                 555                 560
Thr Leu Thr Ala Lys Lys Ser Tyr Phe Ile Leu Asn Asp Lys Ile Val
                565                 570                 575
Phe Leu Gly Thr Gly Ile Lys Ser Thr Asp Ser Ser Lys Asn Pro Val
            580                 585                 590
Thr Thr Ile Glu Asn Arg Lys Ala Asn Gly Tyr Thr Leu Tyr Thr Asp
        595                 600                 605
Asp Lys Gln Thr Thr Asn Ser Asp Asn Gln Glu Asn Asn Ser Val Phe
    610                 615                 620
Leu Glu Ser Thr Asp Thr Lys Lys Asn Ile Gly Tyr His Phe Leu Asn
625                 630                 635                 640
Lys Pro Lys Ile Thr Val Lys Lys Glu Ser His Thr Gly Lys Trp Lys
                645                 650                 655
Glu Ile Asn Lys Ser Gln Lys Asp Thr Gln Lys Thr Asp Glu Tyr Tyr
            660                 665                 670
Glu Val Thr Gln Lys His Ser Asn Ser Asp Asn Lys Tyr Gly Tyr Val
        675                 680                 685
Leu Tyr Pro Gly Leu Ser Lys Asp Val Phe Lys Thr Lys Lys Asp Glu
    690                 695                 700
Val Thr Val Val Lys Gln Glu Asp Asp Phe His Val Val Lys Asp Asn
705                 710                 715                 720
Glu Ser Val Trp Ala Gly Val Asn Tyr Ser Asn Ser Thr Gln Thr Phe
```

725 730 735
Asp Ile Asn Asn Thr Lys Val Glu Val Lys Ala Lys Gly Met Phe Ile
740 745 750
Leu Lys Lys Lys Asp Asp Asn Thr Tyr Glu Cys Ser Phe Tyr Asn Pro
755 760 765
Glu Ser Thr Asn Ser Ala Ser Asp Ile Glu Ser Lys Ile Ser Met Thr
770 775 780
Gly Tyr Ser Ile Thr Asn Lys Asn Thr Ser Thr Ser Asn Glu Ser Gly
785 790 795 800
Val His Phe Glu Leu Thr Lys
805

<210> 79
<211> 809
<212> PRT
<213> Staphylococcus aureus (strain bovine RF122)

<220>
<223> hyaluronan lyase

<400> 79
Met Thr Tyr Arg Met Lys Lys Trp Gln Lys Leu Ser Thr Ile Thr Leu
1 5 10 15
Leu Met Ala Gly Gly Ile Thr Phe Asn Asp Ser Glu Phe Arg Ser Val
20 25 30
Asp Lys His Gln Ile Ala Val Ala Asp Thr Asn Val Gln Thr Pro Asn
35 40 45
Tyr Glu Lys Leu Lys Asn Thr Trp Leu Asp Val Asn Tyr Gly Tyr Asp
50 55 60
Lys Tyr Asp Glu Ser Asn Pro Asp Met Lys Lys Lys Phe Glu Ala Thr
65 70 75 80
Glu Lys Glu Ala Arg Lys Leu Leu Ser Glu Met Lys Thr Glu Ser Asp
85 90 95
Arg Lys Tyr Leu Trp Glu Asn Ser Lys Asp Leu Asp Thr Lys Ser Ala
100 105 110
Asp Met Thr Arg Thr Tyr Arg Asn Ile Glu Lys Ile Ala Glu Ala Met
115 120 125
Lys His Pro Lys Thr Thr Leu Lys Asn Asp Glu Asn Lys Lys Lys Val
130 135 140
Lys Asp Ala Leu Glu Trp Leu His Lys Asn Ala Tyr Gly Lys Glu Pro
145 150 155 160
Gly Lys Lys Val Ala Asp Leu Lys Thr Asn Phe Ser Lys Ser Ala Pro
165 170 175
Gln Lys Asn Thr Asn Leu Asn Trp Trp Asp Tyr Glu Ile Gly Thr Pro
180 185 190
Arg Ala Leu Thr Asn Thr Leu Ile Leu Leu Lys Glu Asp Phe Thr Asp
195 200 205
Glu Glu Lys Lys Lys Tyr Thr Ala Pro Ile Lys Thr Phe Ala Pro Lys
210 215 220
Ser Asp Glu Ile Leu Ser Ser Val Gly Lys Ala Glu Pro Ala Lys Gly
225 230 235 240
Gly Asn Leu Val Asp Ile Ser Lys Val Lys Leu Leu Glu Ser Ile Ile
245 250 255
Glu Glu Asp Lys Asp Met Met Lys Asn Ser Ile Asp Ser Phe Asn Lys
260 265 270
Val Phe Thr Tyr Val Gln Asp Ser Ala Thr Asp Lys Glu Arg Asn Gly
275 280 285
Phe Tyr Lys Asp Gly Ser Tyr Ile Asp His Lys Asp Val Pro Tyr Thr
290 295 300
Gly Ala Tyr Gly Val Val Leu Leu Glu Gly Ile Ser Gln Met Met Pro
305 310 315 320
Met Ile Lys Glu Thr Pro Phe Asn Asp Lys Thr Gln Asn Asn Thr Thr
325 330 335
Leu Thr Ser Trp Ile Asp Asp Gly Phe Met Pro Leu Ile Tyr Lys Gly
340 345 350
Glu Met Met Asp Leu Ser Arg Gly Arg Ala Ile Ser Arg Glu Asn Glu
355 360 365

```
Thr Ser His Ser Ala Ser Ala Thr Val Met Lys Ser Leu Leu Arg Leu
    370                 375                 380
Ser Asp Ala Met Asp Glu Ser Thr Lys Ala Lys Tyr Lys Gln Ile Val
385                 390                 395                 400
Lys Asn Ser Val Lys Ser Asp Ser Ser Tyr Gly Gln Asn Asp Thr Leu
                405                 410                 415
Ser Ser Tyr Ser Asp Ile Asp Lys Met Lys Ser Leu Met Thr Asp Ser
            420                 425                 430
Thr Ile Ser Thr Asn Gly Leu Thr Gln Gln Leu Lys Ile Tyr Asn Ala
        435                 440                 445
Met Asp Arg Val Thr Tyr His Asn Lys Asp Leu Asp Phe Ala Phe Gly
    450                 455                 460
Leu Ser Met Thr Ser Lys Asn Val Ala Arg Tyr Glu Asn Ile Asn Gly
465                 470                 475                 480
Glu Asn Leu Lys Gly Trp His Thr Gly Ala Gly Met Ser Tyr Leu Tyr
                485                 490                 495
Asn Ser Asp Val Arg His Tyr Arg Asp Asn Phe Trp Ala Thr Ala Asp
            500                 505                 510
Met Lys Arg Leu Ala Asp Thr Thr Thr Leu Glu Asn Glu Glu Pro Lys
        515                 520                 525
Gly Thr Asp Val Lys Lys Ser Ser Lys Thr Phe Val Gly Gly Thr Lys
    530                 535                 540
Phe Asp Asp Gln His Ala Ser Ile Gly Met Asp Phe Glu Asn Gln Asp
545                 550                 555                 560
Lys Thr Leu Thr Ala Lys Lys Ser Tyr Phe Ile Leu Asn Asp Lys Ile
                565                 570                 575
Val Phe Leu Gly Thr Gly Ile Lys Thr Thr Asp Ser Ser Lys Asn Pro
            580                 585                 590
Val Thr Thr Ile Glu Asn Arg Lys Ala His Gly Tyr Thr Leu Tyr Thr
        595                 600                 605
Asp Asp Lys Gln Thr Thr Asn Ser Asn Asn Gln Glu Thr Asn Ser Val
    610                 615                 620
Phe Leu Glu Ser Thr Asn Ser Thr Gln Asn Asn Ile Gly Tyr His Phe
625                 630                 635                 640
Leu Asn Lys Ser Lys Ile Thr Val Lys Lys Glu Ser His Thr Gly Lys
                645                 650                 655
Trp Ser Asp Ile Asn Lys Ser Gln Lys Asp Thr Gln Lys Thr Asp Glu
            660                 665                 670
Tyr Tyr Glu Val Thr Gln Lys His Ser Asn Thr Asp Asp Lys Tyr Ala
        675                 680                 685
Tyr Val Leu Tyr Pro Gly Ile Thr Lys Asp Asn Phe Lys Ser Lys Ala
    690                 695                 700
Ser Gln Val Thr Val Val Lys Gln Asp Asp Asp Phe His Val Val Lys
705                 710                 715                 720
Asp Asn Glu Ser Val Trp Ala Gly Val Asn Tyr Ser Asp Ser Thr Gln
                725                 730                 735
Thr Phe Asp Ile Asn Gly Thr Lys Val Glu Val Lys Ala Lys Gly Met
            740                 745                 750
Phe Ile Leu Lys Lys Lys Asp Asp Asn Thr Tyr Glu Cys Ser Phe Tyr
        755                 760                 765
Asn Pro Glu Ser Thr Asn Ser Ala Ser Asp Ile Glu Ser Lys Ile Ser
    770                 775                 780
Met Thr Gly Tyr Ser Ile Thr Asn Lys Asn Thr Ser Asn Thr Asn Glu
785                 790                 795                 800
Ser Gly Val Arg Phe Glu Leu Thr Lys
                805
```

<210> 80
<211> 807
<212> PRT
<213> Staphylococcus aureus (strain bovine RF122)

<220>
<223> hyaluronan lyase

<400> 80

```
Met Thr Tyr Lys Met Lys Lys Trp Gln Lys Leu Ser Thr Ile Thr Leu
```

```
 1               5                  10                  15
Leu Met Ala Gly Val Ile Thr Leu Asn Asn Gly Glu Phe Arg Asn Val
            20                  25                  30
Asp Lys His Gln Ile Ala Val Ala Asp Thr Asn Val Gln Thr Pro Asp
        35                  40                  45
Tyr Glu Lys Leu Lys Lys Thr Trp Leu Asp Val Asn Tyr Gly Asn Asp
    50                  55                  60
Gln Tyr Asp Glu Asn Asn Gln Asp Met Lys Lys Lys Phe Asp Ala Lys
65                  70                  75                  80
Glu Asn Glu Ala Lys Lys Leu Leu Glu Asp Met Lys Thr Asp Thr Asn
                85                  90                  95
Arg Thr Tyr Leu Trp Ser Gly Ala Glu Asn Leu Glu Thr Asn Ser Ser
            100                 105                 110
His Met Thr Lys Thr Tyr Arg Asn Ile Glu Lys Ile Ala Glu Ala Met
        115                 120                 125
Arg His Lys Asn Thr Ser Leu Lys Thr Asp Glu Asn Lys Leu Lys Ile
    130                 135                 140
Lys Asp Ala Ile Lys Trp Leu His His Asn Val Tyr Gly Lys Asp Pro
145                 150                 155                 160
Asp Lys Lys Val Ala Asp Leu Thr Thr Asn Arg Lys Glu Lys Asp Ser
                165                 170                 175
Ser Lys Lys Asn Asn Ser Leu Asn Trp Trp Asp Tyr Glu Ile Gly Thr
            180                 185                 190
Pro Arg Ala Leu Thr Asn Thr Leu Leu Leu Met Asp Asn Met Leu Thr
        195                 200                 205
Lys Asp Glu Met Lys Asn Tyr Ser Lys Pro Ile Ser Ile Tyr Ser Pro
    210                 215                 220
Ser Ser Tyr Lys Ile Leu Ser Ser Val Gly Glu Ser Glu Asp Ala Lys
225                 230                 235                 240
Gly Gly Asn Leu Val Asp Ile Ala Lys Val Lys Phe Leu Glu Ser Val
                245                 250                 255
Ile Glu Glu Asp Val Asp Met Met Lys Lys Ser Ile Asp Ser Phe Asn
            260                 265                 270
Lys Val Phe Thr Tyr Val Gln Asp Ser Ala Thr Gly Lys Ala Arg Asn
        275                 280                 285
Gly Phe Tyr Lys Asp Gly Ser Tyr Ile Asp His Gln Asp Val Pro Tyr
    290                 295                 300
Thr Gly Ala Tyr Gly Val Val Leu Leu Glu Gly Ile Ser Gln Met Met
305                 310                 315                 320
Pro Met Ile Lys Glu Ser Pro Phe Lys His Thr Gln Asp Lys Ala Thr
                325                 330                 335
Leu Ser Asn Trp Ile Asp Glu Gly Phe Met Pro Leu Ile Tyr Lys Gly
            340                 345                 350
Glu Met Met Asp Leu Ser Arg Gly Arg Ala Ile Ser Arg Glu Asn Glu
        355                 360                 365
Thr Ser His Thr Ala Ser Ala Thr Val Met Lys Ser Leu Leu Arg Leu
    370                 375                 380
Ser Asp Thr Met Asp Glu Ser Thr Lys Thr Lys Tyr Lys Gln Ile Val
385                 390                 395                 400
Lys Thr Ser Val Lys Ser Asp Ser Ser Tyr Asp Ser Asn Asp Thr Leu
                405                 410                 415
Asn Ser Tyr Ser Asp Ile Asp Lys Met Lys Lys Leu Met Asn Asp Ser
            420                 425                 430
Thr Ile Ser Lys Asn Asp Leu Thr Gln Gln Leu Lys Ile Tyr Asn Asp
        435                 440                 445
Met Asp Arg Val Thr Tyr His Asn Lys Glu Leu Asp Phe Ala Phe Gly
    450                 455                 460
Leu Ser Met Thr Ser Lys Asn Ile Ala Arg Tyr Glu Asn Ile Asn Gly
465                 470                 475                 480
Glu Asn Leu Lys Gly Trp His Thr Gly Ala Gly Met Ser Tyr Leu Tyr
                485                 490                 495
Asn Ser Asp Val Lys His Tyr Arg Asp Asn Phe Trp Ala Thr Ala Asp
            500                 505                 510
Met Thr Arg Leu Pro Gly Thr Thr Thr Leu Asn Asp Met Pro Ser Thr
        515                 520                 525
Asn Thr Lys Asn Asp Lys Ser Phe Val Gly Gly Thr Lys Leu Asn Asn
    530                 535                 540
Lys Tyr Ala Ser Ile Gly Met Asp Phe Glu Asn Gln Asp Lys Thr Leu
```

545 550 555 560
Thr Ala Lys Lys Ser Tyr Phe Ile Leu Asn Asp Lys Ile Val Phe Ile
565 570 575
Gly Thr Gly Ile Lys Ser Thr Asp Ser Ser Lys Asn Pro Val Thr Ser
580 585 590
Val Glu Asn Arg Lys Ala Asn Gly Tyr Lys Leu Phe Lys Gly Asp Ile
595 600 605
Glu Ile Thr Thr Ser Asp Val Asn Ala Gln Glu Thr His Ser Val Phe
610 615 620
Leu Glu Ser Asn Asp Thr Lys Lys Asn Ile Gly Tyr His Phe Leu Asp
625 630 635 640
Lys Pro Lys Ile Thr Val Lys Lys Glu Ser His Thr Gly Lys Trp Ser
645 650 655
Glu Ile Asn Lys Ser Gln Lys Thr Asp Asp Lys Lys Asp Glu Tyr Tyr
660 665 670
Glu Val Thr Gln Thr His Asn Thr Ser Asp Ser Lys Tyr Ala Tyr Val
675 680 685
Leu Tyr Pro Gly Leu Ser Lys Ser Asp Phe Lys Ser Lys Asn Asn Asn
690 695 700
Val Ser Ile Val Lys Gln Asp Glu Asp Phe His Val Ile Lys Asp Asn
705 710 715 720
Asp Gly Val Phe Ala Gly Val Asn Tyr Ser Asp Ser Thr Lys Ser Phe
725 730 735
Asp Ile Asn Gly Thr Ile Val Glu Leu Lys Glu Lys Gly Met Phe Val
740 745 750
Ile Lys Lys Lys Asp Asp Asn Thr Tyr Glu Cys Ser Phe Tyr Asn Pro
755 760 765
Thr Ser Thr Asn Ser Thr Ser Asn Lys Glu Ser Lys Ile Ser Val Thr
770 775 780
Gly Tyr Thr Ile Thr Asn Gln Ser Val Ser Asn Phe Lys Glu Ser Asp
785 790 795 800
Ile His Phe Glu Leu Thr Lys
805

<210> 81
<211> 807
<212> PRT
<213> Staphylococcus aureus (strain USA300)

<220>
<223> hyaluronan lyase

<400> 81
Met Thr Tyr Arg Ile Lys Lys Trp Gln Lys Leu Ser Thr Ile Thr Leu
1 5 10 15
Leu Met Ala Gly Val Ile Thr Leu Asn Gly Gly Glu Phe Arg Ser Val
20 25 30
Asp Lys His Gln Ile Ala Val Ala Asp Thr Asn Val Gln Thr Pro Asp
35 40 45
Tyr Glu Lys Leu Arg Asn Thr Trp Leu Asp Val Asn Tyr Gly Tyr Asp
50 55 60
Lys Tyr Asp Glu Asn Asn Pro Asp Met Lys Lys Lys Phe Asp Ala Thr
65 70 75 80
Glu Lys Glu Ala Thr Asn Leu Leu Lys Glu Met Lys Thr Glu Ser Gly
85 90 95
Arg Lys Tyr Leu Trp Ser Gly Ala Glu Thr Leu Glu Thr Asn Ser Ser
100 105 110
His Met Thr Arg Thr Tyr Arg Asn Ile Glu Lys Ile Ala Glu Ala Met
115 120 125
Arg Asn Pro Lys Thr Thr Leu Asn Thr Asp Glu Asn Lys Lys Lys Val
130 135 140
Lys Asp Ala Leu Glu Trp Leu His Lys Asn Ala Tyr Gly Lys Glu Pro
145 150 155 160
Asp Lys Lys Val Lys Glu Leu Ser Glu Asn Phe Thr Lys Thr Thr Gly
165 170 175
Lys Asn Thr Asn Leu Asn Trp Trp Asp Tyr Glu Ile Gly Thr Pro Lys
180 185 190

```
Ser Leu Thr Asn Thr Leu Ile Leu Leu Asn Asp Gln Phe Ser Asn Glu
        195                 200                 205
Glu Lys Lys Lys Phe Thr Ala Pro Ile Lys Thr Phe Ala Pro Asp Ser
    210                 215                 220
Asp Lys Ile Leu Ser Ser Val Gly Lys Ala Glu Leu Ala Lys Gly Gly
225                 230                 235                 240
Asn Leu Val Asp Ile Ser Lys Val Lys Leu Leu Glu Cys Ile Ile Glu
                245                 250                 255
Glu Asp Lys Asp Met Met Lys Lys Ser Ile Asp Ser Phe Asn Lys Val
            260                 265                 270
Phe Thr Tyr Val Gln Asp Ser Ala Thr Gly Lys Glu Arg Asn Gly Phe
        275                 280                 285
Tyr Lys Asp Gly Ser Tyr Ile Asp His Gln Asp Val Pro Tyr Thr Gly
    290                 295                 300
Ala Tyr Gly Val Val Leu Leu Glu Gly Ile Ser Gln Met Met Pro Met
305                 310                 315                 320
Ile Lys Glu Thr Pro Phe Asn Asp Lys Thr Gln Asn Asp Thr Thr Leu
                325                 330                 335
Lys Ser Trp Ile Asp Asp Gly Phe Met Pro Leu Ile Tyr Lys Gly Glu
            340                 345                 350
Met Met Asp Leu Ser Arg Gly Arg Ala Ile Ser Arg Glu Asn Glu Thr
        355                 360                 365
Ser His Ser Ala Ser Ala Thr Val Met Lys Ser Leu Leu Arg Leu Ser
    370                 375                 380
Asp Ala Met Asp Asp Ser Thr Lys Ala Lys Tyr Lys Lys Ile Val Lys
385                 390                 395                 400
Ser Ser Val Glu Ser Asp Ser Ser Tyr Lys Gln Asn Asp Tyr Leu Asn
                405                 410                 415
Ser Tyr Ser Asp Ile Asp Lys Met Lys Ser Leu Met Thr Asp Asn Ser
            420                 425                 430
Ile Ser Lys Asn Gly Leu Thr Gln Gln Leu Lys Ile Tyr Asn Asp Met
        435                 440                 445
Asp Arg Val Thr Tyr His Asn Lys Asp Leu Asp Phe Ala Phe Gly Leu
    450                 455                 460
Ser Met Thr Ser Lys Asn Val Ala Arg Tyr Glu Ser Ile Asn Gly Glu
465                 470                 475                 480
Asn Leu Lys Gly Trp His Thr Gly Ala Gly Met Ser Tyr Leu Tyr Asn
                485                 490                 495
Ser Asp Val Lys His Tyr His Asp Asn Phe Trp Val Thr Ala Asp Met
            500                 505                 510
Lys Arg Leu Ser Gly Thr Thr Thr Leu Asp Asn Glu Ile Leu Lys Asp
        515                 520                 525
Thr Asp Asp Lys Lys Ser Ser Lys Thr Phe Val Gly Gly Thr Lys Val
    530                 535                 540
Asp Asp Gln His Ala Ser Ile Gly Met Asp Phe Glu Asn Gln Asp Lys
545                 550                 555                 560
Thr Leu Thr Ala Lys Lys Ser Tyr Phe Ile Leu Asn Asp Lys Ile Val
                565                 570                 575
Phe Leu Gly Thr Gly Ile Lys Ser Thr Asp Ser Ser Lys Asn Pro Val
            580                 585                 590
Thr Thr Ile Glu Asn Arg Lys Ala Asn Gly Tyr Thr Leu Tyr Thr Asp
        595                 600                 605
Asp Lys Gln Thr Thr Asn Ser Asp Asn Gln Glu Asn Asn Ser Val Phe
    610                 615                 620
Leu Glu Ser Thr Asp Thr Lys Lys Asn Ile Gly Tyr His Phe Leu Asn
625                 630                 635                 640
Lys Pro Lys Ile Thr Val Lys Lys Glu Ser His Thr Gly Lys Trp Lys
                645                 650                 655
Glu Ile Asn Lys Ser Gln Lys Asp Thr Gln Lys Thr Asp Glu Tyr Tyr
            660                 665                 670
Glu Val Thr Gln Lys His Ser Asn Ser Asp Asn Lys Tyr Gly Tyr Val
        675                 680                 685
Leu Tyr Pro Gly Leu Ser Lys Asp Val Phe Lys Thr Lys Lys Asp Glu
    690                 695                 700
Val Thr Val Val Lys Gln Glu Asp Asp Phe His Val Val Lys Asp Asn
705                 710                 715                 720
Glu Ser Val Trp Ala Gly Val Asn Tyr Ser Asn Ser Thr Gln Thr Phe
                725                 730                 735
```

```
Asp Ile Asn Asn Thr Lys Val Glu Val Lys Ala Lys Gly Met Phe Ile
            740                 745                 750
Leu Lys Lys Lys Asp Asp Asn Thr Tyr Glu Cys Ser Phe Tyr Asn Pro
        755                 760                 765
Glu Ser Thr Asn Ser Ala Ser Asp Ile Glu Ser Lys Ile Ser Met Thr
    770                 775                 780
Gly Tyr Ser Ile Thr Asn Lys Asn Thr Ser Thr Ser Asn Glu Ser Gly
785                 790                 795                 800
Val His Phe Glu Leu Thr Lys
                805
```

<210> 82
<211> 1066
<212> PRT
<213> Streptococcus pneumoniae

<220>
<223> hyaluronan lyase

<400> 82

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Met Gln Thr Lys Thr Lys Lys Leu Ile Val Ser Leu Ser Ser Leu Val
 1               5                  10                  15
Leu Ser Gly Phe Leu Leu Asn His Tyr Met Thr Ile Gly Ala Glu Glu
            20                  25                  30
Thr Thr Thr Asn Thr Ile Gln Gln Ser Gln Lys Glu Val Gln Tyr Gln
        35                  40                  45
Gln Arg Asp Thr Lys Asn Leu Val Glu Asn Gly Asp Phe Gly Gln Thr
    50                  55                  60
Glu Asp Gly Ser Ser Pro Trp Thr Gly Ser Lys Ala Gln Gly Trp Ser
65                  70                  75                  80
Ala Trp Val Asp Gln Lys Asn Ser Ala Asp Ala Ser Thr Arg Val Ile
                85                  90                  95
Glu Ala Lys Asp Gly Ala Ile Thr Ile Ser Ser His Glu Lys Leu Arg
            100                 105                 110
Ala Ala Leu His Arg Met Val Pro Ile Glu Ala Lys Lys Lys Tyr Lys
        115                 120                 125
Leu Arg Phe Lys Ile Lys Thr Asp Asn Lys Ile Gly Ile Ala Lys Val
    130                 135                 140
Arg Ile Ile Glu Glu Ser Gly Lys Asp Lys Arg Leu Trp Asn Ser Ala
145                 150                 155                 160
Thr Thr Ser Gly Thr Lys Asp Trp Gln Thr Ile Glu Ala Asp Tyr Ser
                165                 170                 175
Pro Thr Leu Asp Val Asp Lys Ile Lys Leu Glu Leu Phe Tyr Glu Thr
            180                 185                 190
Gly Thr Gly Thr Val Ser Phe Lys Asp Ile Glu Leu Val Glu Val Ala
        195                 200                 205
Asp Gln Leu Ser Glu Asp Ser Gln Thr Asp Lys Gln Leu Glu Glu Lys
    210                 215                 220
Ile Asp Leu Pro Ile Gly Lys Lys His Val Phe Ser Leu Ala Asp Tyr
225                 230                 235                 240
Thr Tyr Lys Val Glu Asn Pro Asp Val Ala Ser Val Lys Asn Gly Ile
                245                 250                 255
Leu Glu Pro Leu Lys Glu Gly Thr Thr Asn Val Ile Val Ser Lys Asp
            260                 265                 270
Gly Lys Glu Val Lys Lys Ile Pro Leu Lys Ile Leu Ala Ser Val Lys
        275                 280                 285
Asp Ala Tyr Thr Asp Arg Leu Asp Asp Trp Asn Gly Ile Ile Ala Gly
    290                 295                 300
Asn Gln Tyr Tyr Asp Ser Lys Asn Glu Gln Met Ala Lys Leu Asn Gln
305                 310                 315                 320
Glu Leu Glu Gly Lys Val Ala Asp Ser Leu Ser Ser Ile Ser Ser Gln
                325                 330                 335
Ala Asp Arg Thr Tyr Leu Trp Glu Lys Phe Ser Asn Tyr Lys Thr Ser
            340                 345                 350
Ala Asn Leu Thr Ala Thr Tyr Arg Lys Leu Glu Glu Met Ala Lys Gln
        355                 360                 365
Val Thr Asn Pro Ser Ser Arg Tyr Tyr Gln Asp Glu Thr Val Val Arg
```

```
    370                 375                 380
Thr Val Arg Asp Ser Met Glu Trp Met His Lys His Val Tyr Asn Ser
385                 390                 395                 400
Glu Lys Ser Ile Val Gly Asn Trp Trp Asp Tyr Glu Ile Gly Thr Pro
                405                 410                 415
Arg Ala Ile Asn Asn Thr Leu Ser Leu Met Lys Glu Tyr Phe Ser Asp
            420                 425                 430
Glu Glu Ile Lys Lys Tyr Thr Asp Val Ile Glu Lys Phe Val Pro Asp
        435                 440                 445
Pro Glu His Phe Arg Lys Thr Thr Asp Asn Pro Phe Lys Ala Leu Gly
    450                 455                 460
Gly Asn Leu Val Asp Met Gly Arg Val Lys Val Ile Ala Gly Leu Leu
465                 470                 475                 480
Arg Lys Asp Asp Gln Glu Ile Ser Ser Thr Ile Arg Ser Ile Glu Gln
                485                 490                 495
Val Phe Lys Leu Val Asp Gln Gly Glu Gly Phe Tyr Gln Asp Gly Ser
            500                 505                 510
Tyr Ile Asp His Thr Asn Val Ala Tyr Thr Gly Ala Tyr Gly Asn Val
        515                 520                 525
Leu Ile Asp Gly Leu Ser Gln Leu Leu Pro Val Ile Gln Lys Thr Lys
    530                 535                 540
Asn Pro Ile Asp Lys Asp Lys Met Gln Thr Met Tyr His Trp Ile Asp
545                 550                 555                 560
Lys Ser Phe Ala Pro Leu Leu Val Asn Gly Glu Leu Met Asp Met Ser
                565                 570                 575
Arg Gly Arg Ser Ile Ser Arg Ala Asn Ser Glu Gly His Val Ala Ala
            580                 585                 590
Val Glu Val Leu Arg Gly Ile His Arg Ile Ala Asp Met Ser Glu Gly
        595                 600                 605
Glu Thr Lys Gln Cys Leu Gln Ser Leu Val Lys Thr Ile Val Gln Ser
    610                 615                 620
Asp Ser Tyr Tyr Asp Val Phe Lys Asn Leu Lys Thr Tyr Lys Asp Ile
625                 630                 635                 640
Ser Leu Met Gln Ser Leu Leu Ser Asp Ala Gly Val Ala Ser Val Pro
                645                 650                 655
Arg Pro Ser Tyr Leu Ser Ala Phe Asn Lys Met Asp Lys Thr Ala Met
            660                 665                 670
Tyr Asn Ala Glu Lys Gly Phe Gly Phe Gly Leu Ser Leu Phe Ser Ser
        675                 680                 685
Arg Thr Leu Asn Tyr Glu His Met Asn Lys Glu Asn Lys Arg Gly Trp
    690                 695                 700
Tyr Thr Ser Asp Gly Met Phe Tyr Leu Tyr Asn Gly Asp Leu Ser His
705                 710                 715                 720
Tyr Ser Asp Gly Tyr Trp Pro Thr Val Asn Pro Tyr Lys Met Pro Gly
                725                 730                 735
Thr Thr Glu Thr Asp Ala Lys Arg Ala Asp Ser Asp Thr Gly Lys Val
            740                 745                 750
Leu Pro Ser Ala Phe Val Gly Thr Ser Lys Leu Asp Asp Ala Asn Ala
        755                 760                 765
Thr Ala Thr Met Asp Phe Thr Asn Trp Asn Gln Thr Leu Thr Ala His
    770                 775                 780
Lys Ser Trp Phe Met Leu Lys Asp Lys Ile Ala Phe Leu Gly Ser Asn
785                 790                 795                 800
Ile Gln Asn Thr Ser Thr Asp Thr Ala Ala Thr Thr Ile Asp Gln Arg
                805                 810                 815
Lys Leu Glu Ser Gly Asn Pro Tyr Lys Val Tyr Val Asn Asp Lys Glu
            820                 825                 830
Ala Ser Leu Thr Glu Gln Glu Lys Asp Tyr Pro Glu Thr Gln Ser Val
        835                 840                 845
Phe Leu Glu Ser Phe Asp Ser Lys Lys Asn Ile Gly Tyr Phe Phe Phe
    850                 855                 860
Lys Lys Ser Ser Ile Ser Met Ser Lys Ala Leu Gln Lys Gly Ala Trp
865                 870                 875                 880
Lys Asp Ile Asn Glu Gly Gln Ser Asp Lys Glu Val Glu Asn Glu Phe
                885                 890                 895
Leu Thr Ile Ser Gln Ala His Lys Gln Asn Arg Asp Ser Tyr Gly Tyr
            900                 905                 910
Met Leu Ile Pro Asn Val Asp Arg Ala Thr Phe Asn Gln Met Ile Lys
```

915 920 925
Glu Leu Glu Ser Ser Leu Ile Glu Asn Asn Glu Thr Leu Gln Ser Val
930 935 940
Tyr Asp Ala Lys Gln Gly Val Trp Gly Ile Val Lys Tyr Asp Asp Ser
945 950 955 960
Val Ser Thr Ile Ser Asn Gln Phe Gln Val Leu Lys Arg Gly Val Tyr
965 970 975
Thr Ile Arg Lys Glu Gly Asp Glu Tyr Lys Ile Ala Tyr Tyr Asn Pro
980 985 990
Glu Thr Gln Glu Ser Ala Pro Asp Gln Glu Val Phe Lys Lys Leu Glu
995 1000 1005
Gln Ala Ala Gln Pro Gln Val Gln Asn Ser Lys Glu Lys Glu Lys Ser
1010 1015 1020
Glu Glu Glu Lys Asn His Ser Asp Gln Lys Asn Leu Pro Gln Thr Gly
1025 1030 1035 1040
Glu Gly Gln Ser Ile Leu Ala Ser Leu Gly Phe Leu Leu Leu Gly Ala
1045 1050 1055
Phe Tyr Leu Phe Arg Arg Gly Lys Asn Asn
1060 1065

<210> 83
<211> 1078
<212> PRT
<213> Streptococcus pneumoniae (strain ATCC BAA-255/R6)

<220>
<223> hyaluronan lyase

<400> 83
Met Ile Leu Gln Tyr Val Tyr Trp Ser Val Tyr Met Gln Thr Lys Thr
1 5 10 15
Lys Lys Leu Ile Val Ser Leu Ser Ser Leu Val Leu Ser Gly Phe Leu
20 25 30
Leu Asn His Tyr Met Thr Val Gly Ala Glu Glu Thr Thr Thr Asn Thr
35 40 45
Ile Gln Gln Ser Gln Lys Glu Val Gln Tyr Gln Gln Arg Asp Thr Lys
50 55 60
Asn Leu Val Glu Asn Gly Asp Phe Gly Gln Thr Glu Asp Gly Ser Ser
65 70 75 80
Pro Trp Thr Gly Ser Lys Ala Gln Gly Trp Ser Ala Trp Val Asp Gln
85 90 95
Lys Asn Ser Ser Ala Asp Ala Ser Thr Arg Val Ile Glu Ala Lys Asp
100 105 110
Gly Ala Ile Thr Ile Ser Ser Pro Glu Lys Leu Arg Ala Ala Val His
115 120 125
Arg Met Val Pro Ile Glu Ala Lys Lys Lys Tyr Lys Leu Arg Phe Lys
130 135 140
Ile Lys Thr Asp Asn Lys Val Gly Ile Ala Lys Val Arg Ile Ile Glu
145 150 155 160
Glu Ser Gly Lys Asp Lys Arg Leu Trp Asn Ser Ala Thr Thr Ser Gly
165 170 175
Thr Lys Asp Trp Gln Thr Ile Glu Ala Asp Tyr Ser Pro Thr Leu Asp
180 185 190
Val Asp Lys Ile Lys Leu Glu Leu Phe Tyr Glu Thr Gly Thr Gly Thr
195 200 205
Val Ser Phe Lys Asp Ile Glu Leu Val Glu Val Ala Asp Gln Pro Ser
210 215 220
Glu Asp Ser Gln Thr Asp Lys Gln Leu Glu Glu Lys Ile Asp Leu Pro
225 230 235 240
Ile Gly Lys Lys His Val Phe Ser Leu Ala Asp Tyr Thr Tyr Lys Val
245 250 255
Glu Asn Pro Asp Val Ala Ser Val Lys Asn Gly Ile Leu Glu Pro Leu
260 265 270
Lys Glu Gly Thr Thr Asn Val Ile Val Ser Lys Asp Gly Lys Glu Val
275 280 285
Lys Lys Ile Pro Leu Lys Ile Leu Ala Ser Val Lys Asp Thr Tyr Thr
290 295 300

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Asp Arg Leu Asp Asp Trp Asn Gly Ile Ile Ala Gly Asn Gln Tyr Tyr
305                 310                 315                 320
Asp Ser Lys Asn Glu Gln Met Ala Lys Leu Asn Gln Glu Leu Glu Gly
                325                 330                 335
Lys Val Ala Asp Ser Leu Ser Ser Ile Ser Ser Gln Ala Asp Arg Ile
            340                 345                 350
Tyr Leu Trp Glu Lys Phe Ser Asn Tyr Lys Thr Ser Ala Asn Leu Thr
        355                 360                 365
Ala Thr Tyr Arg Lys Leu Glu Glu Met Ala Lys Gln Val Thr Asn Pro
    370                 375                 380
Ser Ser Arg Tyr Tyr Gln Asp Glu Thr Val Val Arg Thr Val Arg Asp
385                 390                 395                 400
Ser Met Glu Trp Met His Lys His Val Tyr Asn Ser Glu Lys Ser Ile
                405                 410                 415
Val Gly Asn Trp Trp Asp Tyr Glu Ile Gly Thr Pro Arg Ala Ile Asn
            420                 425                 430
Asn Thr Leu Ser Leu Met Lys Glu Tyr Phe Ser Asp Glu Glu Ile Lys
        435                 440                 445
Lys Tyr Thr Asp Val Ile Glu Lys Phe Val Pro Asp Pro Glu His Phe
    450                 455                 460
Arg Lys Thr Thr Asp Asn Pro Phe Lys Ala Leu Gly Gly Asn Leu Val
465                 470                 475                 480
Asp Met Gly Arg Val Lys Val Ile Ala Gly Leu Leu Arg Lys Asp Asp
                485                 490                 495
Gln Glu Ile Ser Ser Thr Ile Arg Ser Ile Glu Gln Val Phe Lys Leu
            500                 505                 510
Val Asp Gln Gly Glu Gly Phe Tyr Gln Asp Gly Ser Tyr Ile Asp His
        515                 520                 525
Thr Asn Val Ala Tyr Thr Gly Ala Tyr Gly Asn Val Leu Ile Asp Gly
    530                 535                 540
Leu Ser Gln Leu Leu Pro Val Ile Gln Lys Thr Lys Asn Pro Ile Asp
545                 550                 555                 560
Lys Asp Lys Met Gln Thr Met Tyr His Trp Ile Asp Lys Ser Phe Ala
                565                 570                 575
Pro Leu Leu Val Asn Gly Glu Leu Met Asp Met Ser Arg Gly Arg Ser
            580                 585                 590
Ile Ser Arg Ala Asn Ser Glu Gly His Val Ala Ala Val Glu Val Leu
        595                 600                 605
Arg Gly Ile His Arg Ile Ala Asp Met Ser Glu Gly Glu Thr Lys Gln
    610                 615                 620
Arg Leu Gln Ser Leu Val Lys Thr Ile Val Gln Ser Asp Ser Tyr Tyr
625                 630                 635                 640
Asp Val Phe Lys Asn Leu Lys Thr Tyr Lys Asp Ile Ser Leu Met Gln
                645                 650                 655
Ser Leu Leu Ser Asp Ala Gly Val Ala Ser Val Pro Arg Thr Ser Tyr
            660                 665                 670
Leu Ser Ala Phe Asn Lys Met Asp Lys Thr Ala Met Tyr Asn Ala Glu
        675                 680                 685
Lys Gly Phe Gly Phe Gly Leu Ser Leu Phe Ser Ser Arg Thr Leu Asn
    690                 695                 700
Tyr Glu His Met Asn Lys Glu Asn Lys Arg Gly Trp Tyr Thr Ser Asp
705                 710                 715                 720
Gly Met Phe Tyr Leu Tyr Asn Gly Asp Leu Ser His Tyr Ser Asp Gly
                725                 730                 735
Tyr Trp Pro Thr Val Asn Pro Tyr Lys Met Pro Gly Thr Thr Glu Thr
            740                 745                 750
Asp Ala Lys Arg Ala Asp Ser Asp Thr Gly Lys Val Leu Pro Ser Ala
        755                 760                 765
Phe Val Gly Thr Ser Lys Leu Asp Asp Ala Asn Ala Thr Ala Thr Met
    770                 775                 780
Asp Phe Thr Asn Trp Asn Gln Thr Leu Thr Ala His Lys Ser Trp Phe
785                 790                 795                 800
Met Leu Lys Asp Lys Ile Ala Phe Leu Gly Ser Asn Ile Gln Asn Thr
                805                 810                 815
Ser Thr Asp Thr Ala Ala Thr Thr Ile Asp Gln Arg Lys Leu Glu Ser
            820                 825                 830
Ser Asn Pro Tyr Lys Val Tyr Val Asn Asp Lys Glu Ala Ser Leu Thr
        835                 840                 845
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Glu Gln Glu Lys Asp Tyr Pro Glu Thr Gln Ser Val Phe Leu Glu Ser
    850                 855                 860
Ser Asp Ser Lys Lys Asn Ile Gly Tyr Phe Phe Phe Lys Lys Ser Ser
865                 870                 875                 880
Ile Ser Met Ser Lys Ala Leu Gln Lys Gly Ala Trp Lys Asp Ile Asn
                885                 890                 895
Glu Gly Gln Ser Asp Lys Glu Val Glu Asn Glu Phe Leu Thr Ile Ser
            900                 905                 910
Gln Ala His Lys Gln Asn Gly Asp Ser Tyr Gly Tyr Met Leu Ile Pro
        915                 920                 925
Asn Val Asp Arg Ala Thr Phe Asn Gln Met Ile Lys Glu Leu Glu Ser
    930                 935                 940
Ser Leu Ile Glu Asn Asn Glu Thr Leu Gln Ser Val Tyr Asp Ala Lys
945                 950                 955                 960
Gln Gly Val Trp Gly Ile Val Lys Tyr Asp Asp Ser Val Ser Thr Ile
                965                 970                 975
Ser Asn Gln Phe Gln Val Leu Lys Arg Gly Val Tyr Thr Ile Arg Lys
            980                 985                 990
Glu Gly Asp Glu Tyr Lys Ile Ala Tyr Tyr Asn Pro Glu Thr Gln Glu
        995                 1000                1005
Ser Ala Pro Asp Gln Glu Val Phe Lys Lys Leu Glu Gln Ala Ala Gln
    1010                1015                1020
Pro Gln Val Gln Asn Ser Lys Glu Lys Glu Lys Ser Glu Glu Glu Lys
1025                1030                1035                1040
Asn His Ser Asp Gln Lys Asn Leu Pro Gln Thr Gly Glu Gly Gln Ser
                1045                1050                1055
Ile Leu Ala Ser Leu Gly Phe Leu Leu Leu Gly Ala Phe Tyr Leu Phe
            1060                1065                1070
Arg Arg Gly Lys Asn Asn
        1075


<210> 84
<211> 1067
<212> PRT
<213> Streptococcus pneumoniae serotype 2 (strain D39/NCTC 7466)

<220>
<223> hyaluronan lyase

<400> 84
Met Gln Thr Lys Thr Lys Lys Leu Ile Val Ser Leu Ser Ser Leu Val
 1                5                  10                  15
Leu Ser Gly Phe Leu Leu Asn His Tyr Met Thr Val Gly Ala Glu Glu
            20                  25                  30
Thr Thr Thr Asn Thr Ile Gln Gln Ser Gln Lys Glu Val Gln Tyr Gln
        35                  40                  45
Gln Arg Asp Thr Lys Asn Leu Val Glu Asn Gly Asp Phe Gly Gln Thr
    50                  55                  60
Glu Asp Gly Ser Ser Pro Trp Thr Gly Ser Lys Ala Gln Gly Trp Ser
65                  70                  75                  80
Ala Trp Val Asp Gln Lys Asn Ser Ser Ala Asp Ala Ser Thr Arg Val
                85                  90                  95
Ile Glu Ala Lys Asp Gly Ala Ile Thr Ile Ser Ser Pro Glu Lys Leu
            100                 105                 110
Arg Ala Ala Val His Arg Met Val Pro Ile Glu Ala Lys Lys Lys Tyr
        115                 120                 125
Lys Leu Arg Phe Lys Ile Lys Thr Asp Asn Lys Val Gly Ile Ala Lys
    130                 135                 140
Val Arg Ile Ile Glu Glu Ser Gly Lys Asp Lys Arg Leu Trp Asn Ser
145                 150                 155                 160
Ala Thr Thr Ser Gly Thr Lys Asp Trp Gln Thr Ile Glu Ala Asp Tyr
                165                 170                 175
Ser Pro Thr Leu Asp Val Asp Lys Ile Lys Leu Glu Leu Phe Tyr Glu
            180                 185                 190
Thr Gly Thr Gly Thr Val Ser Phe Lys Asp Ile Glu Leu Val Glu Val
        195                 200                 205
Ala Asp Gln Pro Ser Glu Asp Ser Gln Thr Asp Lys Gln Leu Glu Glu
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    210                 215                 220
Lys Ile Asp Leu Pro Ile Gly Lys Lys His Val Phe Ser Leu Ala Asp
225                 230                 235                 240
Tyr Thr Tyr Lys Val Glu Asn Pro Asp Val Ala Ser Val Lys Asn Gly
                245                 250                 255
Ile Leu Glu Pro Leu Lys Glu Gly Thr Thr Asn Val Ile Val Ser Lys
            260                 265                 270
Asp Gly Lys Glu Val Lys Lys Ile Pro Leu Lys Ile Leu Ala Ser Val
        275                 280                 285
Lys Asp Thr Tyr Thr Asp Arg Leu Asp Asp Trp Asn Gly Ile Ile Ala
    290                 295                 300
Gly Asn Gln Tyr Tyr Asp Ser Lys Asn Glu Gln Met Ala Lys Leu Asn
305                 310                 315                 320
Gln Glu Leu Glu Gly Lys Val Ala Asp Ser Leu Ser Ser Ile Ser Ser
                325                 330                 335
Gln Ala Asp Arg Ile Tyr Leu Trp Glu Lys Phe Ser Asn Tyr Lys Thr
            340                 345                 350
Ser Ala Asn Leu Thr Ala Thr Tyr Arg Lys Leu Glu Glu Met Ala Lys
        355                 360                 365
Gln Val Thr Asn Pro Ser Ser Arg Tyr Tyr Gln Asp Glu Thr Val Val
    370                 375                 380
Arg Thr Val Arg Asp Ser Met Glu Trp Met His Lys His Val Tyr Asn
385                 390                 395                 400
Ser Glu Lys Ser Ile Val Gly Asn Trp Trp Asp Tyr Glu Ile Gly Thr
                405                 410                 415
Pro Arg Ala Ile Asn Asn Thr Leu Ser Leu Met Lys Glu Tyr Phe Ser
            420                 425                 430
Asp Glu Glu Ile Lys Lys Tyr Thr Asp Val Ile Glu Lys Phe Val Pro
        435                 440                 445
Asp Pro Glu His Phe Arg Lys Thr Thr Asp Asn Pro Phe Lys Ala Leu
    450                 455                 460
Gly Gly Asn Leu Val Asp Met Gly Arg Val Lys Val Ile Ala Gly Leu
465                 470                 475                 480
Leu Arg Lys Asp Asp Gln Glu Ile Ser Ser Thr Ile Arg Ser Ile Glu
                485                 490                 495
Gln Val Phe Lys Leu Val Asp Gln Gly Glu Gly Phe Tyr Gln Asp Gly
            500                 505                 510
Ser Tyr Ile Asp His Thr Asn Val Ala Tyr Thr Gly Ala Tyr Gly Asn
        515                 520                 525
Val Leu Ile Asp Gly Leu Ser Gln Leu Leu Pro Val Ile Gln Lys Thr
    530                 535                 540
Lys Asn Pro Ile Asp Lys Asp Lys Met Gln Thr Met Tyr His Trp Ile
545                 550                 555                 560
Asp Lys Ser Phe Ala Pro Leu Leu Val Asn Gly Glu Leu Met Asp Met
                565                 570                 575
Ser Arg Gly Arg Ser Ile Ser Arg Ala Asn Ser Glu Gly His Val Ala
            580                 585                 590
Ala Val Glu Val Leu Arg Gly Ile His Arg Ile Ala Asp Met Ser Glu
        595                 600                 605
Gly Glu Thr Lys Gln Arg Leu Gln Ser Leu Val Lys Thr Ile Val Gln
    610                 615                 620
Ser Asp Ser Tyr Tyr Asp Val Phe Lys Asn Leu Lys Thr Tyr Lys Asp
625                 630                 635                 640
Ile Ser Leu Met Gln Ser Leu Leu Ser Asp Ala Gly Val Ala Ser Val
                645                 650                 655
Pro Arg Thr Ser Tyr Leu Ser Ala Phe Asn Lys Met Asp Lys Thr Ala
            660                 665                 670
Met Tyr Asn Ala Glu Lys Gly Phe Gly Phe Gly Leu Ser Leu Phe Ser
        675                 680                 685
Ser Arg Thr Leu Asn Tyr Glu His Met Asn Lys Glu Asn Lys Arg Gly
    690                 695                 700
Trp Tyr Thr Ser Asp Gly Met Phe Tyr Leu Tyr Asn Gly Asp Leu Ser
705                 710                 715                 720
His Tyr Ser Asp Gly Tyr Trp Pro Thr Val Asn Pro Tyr Lys Met Pro
                725                 730                 735
Gly Thr Thr Glu Thr Asp Ala Lys Arg Ala Asp Ser Asp Thr Gly Lys
            740                 745                 750
Val Leu Pro Ser Ala Phe Val Gly Thr Ser Lys Leu Asp Asp Ala Asn
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        755                 760                 765
Ala Thr Ala Thr Met Asp Phe Thr Asn Trp Asn Gln Thr Leu Thr Ala
    770                 775                 780
His Lys Ser Trp Phe Met Leu Lys Asp Lys Ile Ala Phe Leu Gly Ser
785                 790                 795                 800
Asn Ile Gln Asn Thr Ser Thr Asp Thr Ala Ala Thr Thr Ile Asp Gln
                805                 810                 815
Arg Lys Leu Glu Ser Ser Asn Pro Tyr Lys Val Tyr Val Asn Asp Lys
            820                 825                 830
Glu Ala Ser Leu Thr Glu Gln Glu Lys Asp Tyr Pro Glu Thr Gln Ser
        835                 840                 845
Val Phe Leu Glu Ser Ser Asp Ser Lys Lys Asn Ile Gly Tyr Phe Phe
    850                 855                 860
Phe Lys Lys Ser Ser Ile Ser Met Ser Lys Ala Leu Gln Lys Gly Ala
865                 870                 875                 880
Trp Lys Asp Ile Asn Glu Gly Gln Ser Asp Lys Glu Val Glu Asn Glu
                885                 890                 895
Phe Leu Thr Ile Ser Gln Ala His Lys Gln Asn Gly Asp Ser Tyr Gly
            900                 905                 910
Tyr Met Leu Ile Pro Asn Val Asp Arg Ala Thr Phe Asn Gln Met Ile
        915                 920                 925
Lys Glu Leu Glu Ser Ser Leu Ile Glu Asn Asn Glu Thr Leu Gln Ser
    930                 935                 940
Val Tyr Asp Ala Lys Gln Gly Val Trp Gly Ile Val Lys Tyr Asp Asp
945                 950                 955                 960
Ser Val Ser Thr Ile Ser Asn Gln Phe Gln Val Leu Lys Arg Gly Val
                965                 970                 975
Tyr Thr Ile Arg Lys Glu Gly Asp Glu Tyr Lys Ile Ala Tyr Tyr Asn
            980                 985                 990
Pro Glu Thr Gln Glu Ser Ala Pro Asp Gln Glu Val Phe Lys Lys Leu
        995                 1000                1005
Glu Gln Ala Ala Gln Pro Gln Val Gln Asn Ser Lys Glu Lys Glu Lys
    1010                1015                1020
Ser Glu Glu Glu Lys Asn His Ser Asp Gln Lys Asn Leu Pro Gln Thr
1025                1030                1035                1040
Gly Glu Gly Gln Ser Ile Leu Ala Ser Leu Gly Phe Leu Leu Leu Gly
                1045                1050                1055
Ala Phe Tyr Leu Phe Arg Arg Gly Lys Asn Asn
            1060                1065


<210> 85
<211> 805
<212> PRT
<213> Streptococcus pyogenes serotype M1

<220>
<223> hyaluronan lyase

<400> 85
Met Asn Thr Tyr Phe Cys Thr His His Lys Gln Leu Leu Leu Tyr Ser
 1               5                  10                  15
Asn Leu Phe Leu Ser Phe Ala Met Met Gly Gln Gly Thr Ala Ile Tyr
            20                  25                  30
Ala Asp Thr Leu Thr Ser Asn Ser Glu Pro Asn Asn Thr Tyr Phe Gln
        35                  40                  45
Thr Gln Thr Leu Thr Thr Thr Asp Ser Glu Lys Lys Val Val Gln Pro
    50                  55                  60
Gln Gln Lys Asp Tyr Tyr Thr Glu Leu Leu Asp Gln Trp Asn Ser Ile
65                  70                  75                  80
Ile Ala Gly Asn Asp Ala Tyr Asp Lys Thr Asn Pro Asp Met Val Thr
                85                  90                  95
Phe His Asn Lys Ala Glu Lys Asp Ala Gln Asn Ile Ile Lys Ser Tyr
            100                 105                 110
Gln Gly Pro Asp His Glu Asn Arg Thr Tyr Leu Trp Glu His Ala Lys
        115                 120                 125
Asp Tyr Ser Ala Ser Ala Asn Ile Thr Lys Thr Tyr Arg Asn Ile Glu
    130                 135                 140
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Lys Ile Ala Lys Gln Ile Thr Asn Pro Glu Ser Cys Tyr Tyr Gln Asp
145                 150                 155                 160
Ser Lys Ala Ile Ala Ile Val Lys Asp Gly Met Ala Phe Met Tyr Glu
                165                 170                 175
His Ala Tyr Asn Leu Asp Arg Glu Asn His Gln Thr Thr Gly Lys Glu
            180                 185                 190
Asn Lys Glu Asn Trp Trp Val Tyr Glu Ile Gly Thr Pro Arg Ala Ile
        195                 200                 205
Asn Asn Thr Leu Ser Leu Met Tyr Pro Tyr Phe Thr Gln Glu Glu Ile
    210                 215                 220
Leu Lys Tyr Thr Ala Pro Ile Glu Lys Phe Val Pro Asp Pro Thr Arg
225                 230                 235                 240
Phe Arg Val Arg Ala Ala Asn Phe Ser Pro Phe Glu Ala Asn Ser Gly
                245                 250                 255
Asn Leu Ile Asp Met Gly Arg Val Lys Leu Ile Ser Gly Ile Leu Arg
            260                 265                 270
Lys Asp Asp Leu Glu Ile Ser Asp Thr Ile Lys Ala Ile Glu Lys Val
        275                 280                 285
Phe Thr Leu Val Asp Glu Gly Asn Gly Phe Tyr Gln Asp Gly Ser Leu
    290                 295                 300
Ile Asp His Val Val Thr Asn Ala Gln Ser Pro Leu Tyr Lys Lys Gly
305                 310                 315                 320
Ile Ala Tyr Thr Gly Ala Tyr Gly Asn Val Leu Ile Asp Gly Leu Ser
                325                 330                 335
Gln Leu Ile Pro Ile Ile Gln Lys Thr Lys Ser Pro Ile Lys Ala Asp
            340                 345                 350
Lys Met Ala Thr Ile Tyr His Trp Ile Asn His Ser Phe Phe Pro Ile
        355                 360                 365
Ile Val Arg Gly Glu Met Met Asp Met Thr Arg Gly Arg Ser Ile Ser
    370                 375                 380
Arg Phe Asn Ala Gln Ser His Val Ala Gly Ile Glu Ala Leu Arg Ala
385                 390                 395                 400
Ile Leu Arg Ile Ala Asp Met Ser Glu Glu Pro His Arg Leu Ala Leu
                405                 410                 415
Lys Thr Arg Ile Lys Thr Leu Val Thr Gln Gly Asn Ala Phe Tyr Asn
            420                 425                 430
Val Tyr Asp Asn Leu Lys Thr Tyr His Asp Ile Lys Leu Met Lys Glu
        435                 440                 445
Leu Leu Ser Asp Thr Ser Val Pro Val Gln Lys Leu Asp Ser Tyr Val
    450                 455                 460
Ala Ser Phe Asn Ser Met Asp Lys Leu Ala Leu Tyr Asn Asn Lys His
465                 470                 475                 480
Asp Phe Ala Phe Gly Leu Ser Met Phe Ser Asn Arg Thr Gln Asn Tyr
                485                 490                 495
Glu Ala Met Asn Asn Glu Asn Leu His Gly Trp Phe Thr Ser Asp Gly
            500                 505                 510
Met Phe Tyr Leu Tyr Asn Asn Asp Leu Gly His Tyr Ser Glu Asn Tyr
        515                 520                 525
Trp Ala Thr Val Asn Pro Tyr Arg Leu Pro Gly Thr Thr Glu Thr Glu
    530                 535                 540
Gln Lys Pro Leu Glu Gly Thr Pro Glu Asn Ile Lys Thr Asn Tyr Gln
545                 550                 555                 560
Gln Val Gly Met Thr Gly Leu Ser Asp Asp Ala Phe Val Ala Ser Lys
                565                 570                 575
Lys Leu Asn Asn Thr Ser Ala Leu Ala Ala Met Thr Phe Thr Asn Trp
            580                 585                 590
Asn Lys Ser Leu Thr Leu Asn Lys Gly Trp Phe Ile Leu Gly Asn Lys
        595                 600                 605
Ile Ile Phe Val Gly Ser Asn Ile Lys Asn Gln Ser Ser His Lys Ala
    610                 615                 620
Tyr Thr Thr Ile Glu Gln Arg Lys Glu Asn Gln Lys Tyr Pro Tyr Cys
625                 630                 635                 640
Ser Tyr Val Asn Asn Gln Pro Val Asp Leu Asn Asn Gln Leu Val Asp
                645                 650                 655
Phe Thr Asn Thr Lys Ser Ile Phe Leu Glu Ser Asp Asp Pro Ala Gln
            660                 665                 670
Asn Ile Gly Tyr Tyr Phe Phe Lys Pro Thr Thr Leu Ser Ile Ser Lys
        675                 680                 685
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Ala Leu Gln Thr Gly Lys Trp Gln Asn Ile Lys Ala Asp Asp Lys Ser
    690                 695                 700
Pro Glu Ala Ile Lys Glu Val Ser Asn Thr Phe Ile Thr Ile Met Gln
705                 710                 715                 720
Asn His Thr Gln Asp Gly Asp Arg Tyr Ala Tyr Met Met Leu Pro Asn
                725                 730                 735
Met Thr Arg Gln Glu Phe Glu Thr Tyr Ile Ser Lys Leu Asp Ile Asp
            740                 745                 750
Leu Leu Glu Asn Asn Asp Lys Leu Ala Ala Val Tyr Asp His Asp Ser
        755                 760                 765
Gln Gln Met His Val Ile His Tyr Gly Lys Lys Ala Thr Met Phe Ser
    770                 775                 780
Asn His Asn Leu Ser His Gln Gly Phe Tyr Ser Phe Pro His Pro Val
785                 790                 795                 800
Arg Gln Asn Gln Gln
                805


<210> 86
<211> 780
<212> PRT
<213> Streptococcus pyogenes serotype M2 (strain MGAS10270)

<220>
<223> hyaluronan lyase

<400> 86
Met Val Tyr Phe Tyr Leu Val Asn Gln Ser Thr Phe Ile Ile Ser Phe
 1                5                  10                  15
Leu Tyr Trp Arg Asn Val Ser Val Asn Thr Tyr Phe Cys Thr His His
            20                  25                  30
Lys Gln Leu Leu Leu Tyr Ser Asn Leu Phe Leu Ser Phe Ala Met Ile
        35                  40                  45
Gly Gln Gly Thr Ala Ile Tyr Ala Asp Thr Leu Thr Ser Asn Ser Glu
    50                  55                  60
Pro Asn Asn Thr Tyr Phe Gln Thr Gln Thr Leu Thr Thr Thr Asp Ser
65                  70                  75                  80
Glu Lys Lys Val Val Gln Pro Gln Gln Lys Asp Tyr Tyr Thr Glu Leu
                85                  90                  95
Leu Asp Gln Trp Asn Ser Ile Ile Ala Gly Asn Asp Ala Tyr Asp Lys
            100                 105                 110
Thr Asn Pro Asp Met Val Thr Phe His Asn Lys Ala Glu Lys Asp Ala
        115                 120                 125

Gln Asn Ile Ile Lys Ser Tyr Gln Gly Pro Asp His Glu Asn Arg Thr
    130                 135                 140
Tyr Leu Trp Glu His Ala Lys Asp Tyr Ser Ala Ser Thr Asn Ile Thr
145                 150                 155                 160
Lys Thr Tyr Arg Asn Ile Glu Lys Ile Ala Lys Gln Ile Thr Asn Pro
                165                 170                 175
Glu Ser Cys Tyr Tyr Gln Asp Ser Lys Ala Ile Ala Ile Val Lys Asp
            180                 185                 190
Gly Met Ala Phe Met Tyr Glu His Ala Tyr Asn Leu Asn Arg Glu Asn
        195                 200                 205
His Gln Thr Thr Gly Lys Glu Asn Lys Glu Asn Trp Trp Val Tyr Glu
    210                 215                 220
Ile Gly Thr Pro Arg Ala Ile Asn Asn Thr Leu Ser Leu Met Tyr Pro
225                 230                 235                 240
Tyr Phe Thr Gln Glu Glu Ile Leu Lys Tyr Thr Ala Pro Ile Glu Lys
                245                 250                 255
Phe Val Pro Asp Pro Thr Arg Phe Arg Val Arg Ala Ala Asn Phe Ser
            260                 265                 270
Pro Phe Glu Ala Asn Ser Gly Asn Leu Ile Asp Met Gly Arg Val Lys
        275                 280                 285
Leu Ile Ser Gly Ile Leu Arg Lys Asp Asp Leu Glu Ile Ser Asp Thr
    290                 295                 300
Ile Lys Ala Ile Glu Lys Val Phe Thr Leu Val Asp Glu Gly Asn Gly
305                 310                 315                 320
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Phe Tyr Gln Asp Gly Ser Leu Ile Asp His Val Val Thr Asn Thr Gln
                325                 330                 335
Ser Pro Leu Tyr Lys Lys Gly Ile Ala Tyr Thr Gly Ala Tyr Gly Asn
            340                 345                 350
Val Leu Ile Asp Gly Leu Ser Gln Leu Ile Pro Ile Ile Gln Lys Thr
        355                 360                 365
Lys Ser Pro Ile Glu Ala Asp Lys Met Ala Thr Ile Tyr His Trp Ile
    370                 375                 380
Asn His Ser Phe Phe Pro Ile Ile Val Arg Gly Glu Met Met Asp Met
385                 390                 395                 400
Thr Arg Gly Arg Ser Ile Ser Arg Phe Asn Ala Gln Ser His Val Ala
                405                 410                 415
Gly Ile Glu Ala Leu Arg Ala Ile Leu Arg Ile Ala Asp Met Ser Glu
            420                 425                 430
Glu Pro His Arg Leu Glu Leu Lys Thr Arg Ile Lys Thr Leu Val Thr
        435                 440                 445
Gln Gly Asn Ala Phe Tyr Asn Val Tyr Asp Asn Leu Lys Thr Tyr His
    450                 455                 460
Asp Ile Lys Leu Met Lys Glu Leu Leu Ser Asp Thr Ser Val Pro Val
465                 470                 475                 480
Gln Lys Leu Asp Ser Tyr Val Ala Ser Phe Asn Ser Met Asp Lys Leu
                485                 490                 495
Ala Leu Tyr Asn Asn Lys His Asp Phe Ala Phe Gly Leu Ser Met Phe
            500                 505                 510
Ser Asn Arg Thr Gln Asn Tyr Glu Ala Met Asn Asn Glu Asn Leu His
        515                 520                 525
Gly Trp Phe Thr Ser Asp Gly Met Phe Tyr Leu Tyr Asn Asn Asp Leu
    530                 535                 540
Gly His Tyr Ser Glu Asn Tyr Trp Ala Thr Val Asn Pro Tyr Arg Leu
545                 550                 555                 560
Pro Gly Thr Thr Glu Thr Glu Gln Lys Pro Leu Glu Gly Thr Pro Glu
                565                 570                 575
Asn Ile Lys Thr Asn Tyr Gln Gln Val Gly Met Thr Ser Leu Ser Asp
            580                 585                 590
Asp Ala Phe Val Ala Ser Lys Lys Leu Asn Asn Thr Ser Ala Leu Ala
        595                 600                 605
Ala Met Thr Phe Thr Asn Trp Asn Lys Ser Leu Thr Leu Asn Lys Gly
    610                 615                 620
Trp Phe Ile Leu Gly Asn Lys Ile Ile Phe Val Gly Ser Asn Ile Lys
625                 630                 635                 640
Asn Gln Ser Ser His Lys Ala Tyr Thr Thr Ile Glu Gln Arg Lys Glu
                645                 650                 655
Asn Gln Lys His Pro Tyr Cys Ser Tyr Val Asn Asn Gln Pro Val Asp
            660                 665                 670
Leu Asn Asn Gln Leu Val Asp Phe Thr Asn Thr Lys Ser Ile Phe Leu
        675                 680                 685
Glu Ser Asp Asp Pro Ala Gln Asn Ile Gly Tyr Tyr Phe Phe Lys Pro
    690                 695                 700
Arg Thr Leu Ser Ile Ser Lys Ala Leu Gln Thr Gly Lys Trp Gln Asn
705                 710                 715                 720
Ile Lys Ala Asp Asp Lys Ser Pro Glu Ala Ile Lys Glu Val Ser Asn
                725                 730                 735
Thr Phe Ile Thr Ile Met Gln Asn His Thr Gln Glu Gly Asp Arg Tyr
            740                 745                 750
Ala Tyr Met Met Leu Pro Asn Met Thr Arg Gln Glu Phe Glu Thr Tyr
        755                 760                 765
Ile Ser Lys Leu Asp Ile Asp Leu Leu Glu Asn Asn
    770                 775                 780
```

<210> 87
<211> 868
<212> PRT
<213> Streptococcus pyogenes serotype M4 (strain MGAS10750)

<220>
<223> hyaluronan lyase

<400> 87

Met Asn Thr Tyr Phe Cys Thr His His Lys Gln Leu Leu Leu Tyr Ser
 1   5   10   15
Asn Leu Phe Leu Ser Phe Ala Met Met Gly Gln Gly Thr Ala Ile Tyr
            20   25   30
Ala Asp Thr Leu Thr Ser Asn Ser Lys Pro Asn Asn Thr Tyr Phe Gln
        35   40   45
Thr Gln Thr Leu Thr Thr Thr Asp Ser Glu Lys Lys Val Val Gln Pro
    50   55   60
Gln Gln Lys Asp Tyr Tyr Thr Glu Leu Leu Asp Gln Trp Asn Ser Ile
65   70   75   80
Ile Ala Gly Asn Asp Ala Tyr Asp Lys Thr Asn Pro Asp Met Val Thr
                85   90   95
Phe His Asn Lys Ala Glu Lys Asp Ala Gln Asn Ile Ile Lys Ser Tyr
            100   105   110
Gln Glu Pro Asp His Glu Asn Arg Thr Tyr Leu Trp Glu His Ala Lys
        115   120   125
Asp Tyr Ser Ala Ser Ala Asn Ile Thr Lys Thr Tyr Arg Asn Ile Glu
    130   135   140
Lys Ile Ala Lys Gln Ile Thr Asn Pro Glu Ser Cys Tyr Tyr Gln Asp
145   150   155   160
Ser Lys Ala Ile Ala Ile Val Lys Asp Gly Met Ala Phe Met Tyr Glu
                165   170   175
His Ala Tyr Asn Leu Asp Arg Glu Asn His Gln Thr Thr Gly Lys Glu
            180   185   190
Asn Lys Glu Asn Trp Trp Asp Tyr Glu Ile Gly Thr Pro Arg Ala Ile
        195   200   205
Asn Asn Thr Leu Ser Leu Met Tyr Pro Tyr Phe Thr Gln Glu Glu Ile
    210   215   220
Leu Lys Tyr Thr Ala Pro Ile Glu Lys Phe Val Pro Asp Pro Thr Arg
225   230   235   240
Phe Arg Val Arg Ala Ala Asn Phe Pro Pro Phe Glu Ala Asn Ser Gly
                245   250   255
Asn Leu Ile Asp Met Gly Arg Val Lys Leu Ile Ser Gly Ile Leu Arg
            260   265   270
Lys Asp Asp Leu Glu Ile Ser Asp Thr Ile Lys Ala Ile Glu Lys Val
        275   280   285
Phe Thr Leu Val Asp Glu Gly Asn Gly Phe Tyr Gln Asp Gly Ser Leu
    290   295   300
Ile Asp His Val Val Thr Asn Ala Gln Ser Pro Leu Tyr Lys Lys Gly
305   310   315   320
Ile Ala Tyr Thr Gly Ala Tyr Gly Asn Val Leu Ile Asp Gly Leu Ser
                325   330   335
Gln Leu Ile Pro Ile Ile Gln Lys Thr Lys Ser Pro Ile Glu Ala Asp
            340   345   350
Lys Met Ala Thr Ile Tyr His Trp Ile Asn His Ser Phe Phe Pro Ile
        355   360   365
Ile Val Arg Gly Glu Met Met Asp Met Thr Arg Gly Arg Ser Ile Ser
    370   375   380
Arg Phe Asn Ala Gln Ser His Val Ala Gly Ile Glu Ala Leu Arg Ala
385   390   395   400
Ile Leu Arg Ile Ala Asp Met Ser Glu Glu Pro His Arg Leu Ala Leu
                405   410   415
Lys Thr Arg Ile Lys Thr Leu Val Thr Gln Gly Asn Val Phe Tyr Asn
            420   425   430
Val Tyr Asp Asn Leu Lys Thr Tyr His Asp Ile Lys Leu Met Lys Glu
        435   440   445
Leu Leu Ser Asp Thr Ser Val Pro Val Gln Lys Leu Asp Ser Tyr Val
    450   455   460
Ala Ser Phe Asn Ser Met Asp Lys Leu Ala Leu Tyr Asn Asn Lys His
465   470   475   480
Asp Phe Ala Phe Gly Leu Ser Met Phe Ser Asn Arg Thr Gln Asn Tyr
                485   490   495
Glu Ala Met Asn Asn Glu Asn Leu His Gly Trp Phe Thr Ser Asp Gly
            500   505   510
Met Phe Tyr Leu Tyr Asn Asn Asp Leu Gly His Tyr Ser Glu Asn Tyr
        515   520   525
Trp Ala Thr Val Asn Pro Tyr Arg Leu Pro Gly Thr Thr Glu Thr Glu

530 535 540
Gln Lys Pro Leu Glu Gly Thr Pro Glu Asn Ile Lys Thr Asn Tyr Gln
545 550 555 560
Gln Val Gly Met Thr Ser Leu Ser Asp Asp Ala Phe Val Ala Ser Lys
565 570 575
Lys Leu Asn Asn Thr Ser Ala Leu Ala Ala Met Thr Phe Thr Asn Trp
580 585 590
Asn Lys Ser Leu Thr Leu Asn Lys Gly Trp Phe Ile Leu Gly Asn Lys
595 600 605
Ile Ile Phe Val Gly Ser Asn Ile Lys Asn Gln Ser Ser His Lys Ala
610 615 620
Tyr Thr Thr Ile Glu Gln Arg Lys Glu Asn Gln Lys His Pro Tyr Cys
625 630 635 640
Ser Tyr Val Asn Asn Gln Pro Val Asp Leu Asn Asn Gln Leu Val Asp
645 650 655
Phe Thr Asn Thr Lys Ser Ile Phe Leu Glu Ser Asp Asp Pro Ala Gln
660 665 670
Asn Ile Gly Tyr Tyr Phe Phe Lys Pro Thr Thr Leu Ser Ile Ser Lys
675 680 685
Ala Leu Gln Thr Gly Lys Trp Gln Asn Ile Lys Ala Asp Asp Lys Ser
690 695 700
Pro Glu Ala Ile Lys Glu Val Ser Asn Thr Phe Ile Thr Ile Met Gln
705 710 715 720
Asn His Thr Gln Asp Gly Asp Arg Tyr Ala Tyr Met Met Leu Pro Asn
725 730 735
Met Thr Arg Gln Glu Phe Glu Thr Tyr Ile Ser Lys Leu Asp Ile Asp
740 745 750
Leu Leu Glu Asn Asn Asp Lys Leu Ala Ala Val Tyr Asp His Asp Ser
755 760 765
Gln Gln Met His Val Ile His Tyr Glu Lys Lys Ala Thr Thr Phe Ser
770 775 780
Asn His Asn Leu Ser His Gln Gly Phe Tyr Ser Phe Pro His Pro Val
785 790 795 800

Lys Gln Asn Gln Gln Gln Lys Leu Ala His Gln Gly Ile Ala Ala Lys
805 810 815
Asn Asn Ala Leu Asn Ser His Lys Ile Pro His Lys Arg Gln Arg Arg
820 825 830
Leu Pro Arg Thr Gly Tyr Gln Ser Ser Ser Leu Glu Phe Leu Gly Gly
835 840 845
Ala Leu Val Ala Ser Phe Asn His Ile Thr Lys Pro Phe Arg Lys Lys
850 855 860
Asp Leu Arg Ile
865

<210> 88
<211> 828
<212> PRT
<213> Streptococcus pyogenes serotype M6

<220>
<223> hyaluronan lyase

<400> 88
Met Val Tyr Phe Tyr Leu Val Asp Gln Phe Thr Phe Ile Ile Ser Phe
1 5 10 15
Leu Tyr Trp Arg Asn Leu Ser Val Asn Thr Tyr Phe Cys Thr His His
20 25 30
Lys Gln Leu Leu Leu Tyr Ser Asn Leu Phe Leu Ser Phe Ala Met Met
35 40 45
Gly Gln Gly Thr Ala Ile Tyr Ala Asp Thr Leu Thr Ser Asn Ser Glu
50 55 60
Pro Asn Asn Thr Tyr Phe Gln Thr Gln Thr Leu Thr Thr Thr Asp Ser
65 70 75 80
Glu Lys Lys Val Val Gln Pro Gln Gln Lys Asp Tyr Tyr Thr Glu Leu
85 90 95
Leu Asp Gln Trp Asn Ser Ile Ile Ala Gly Asn Asp Ala Tyr Asp Lys

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            100                 105                 110
Thr Asn Pro Asp Met Val Thr Phe His Asn Lys Ala Glu Lys Asp Ala
        115                 120                 125
Gln Asn Ile Ile Lys Ser Tyr Gln Gly Pro Asp His Glu Asn Arg Thr
    130                 135                 140
Tyr Leu Trp Glu His Ala Lys Asp Tyr Ser Ala Ser Thr Asn Ile Thr
145                 150                 155                 160
Lys Thr Tyr Arg Asn Ile Glu Lys Ile Ala Lys Gln Ile Thr Asn Pro
                165                 170                 175
Glu Ser Cys Tyr Tyr Gln Asp Ser Lys Ala Ile Ala Ile Val Lys Asp
            180                 185                 190
Gly Met Ala Phe Met Tyr Glu His Ala Tyr Asn Leu Asp Arg Glu Asn
        195                 200                 205
His Gln Thr Thr Gly Lys Glu Asn Lys Glu Asn Trp Trp Val Tyr Glu
    210                 215                 220
Ile Gly Thr Pro Arg Ala Ile Asn Asn Thr Leu Ser Leu Met Tyr Pro
225                 230                 235                 240
Tyr Phe Thr Gln Glu Glu Ile Leu Lys Tyr Thr Ala Pro Ile Glu Lys
                245                 250                 255
Phe Val Pro Asp Pro Thr Arg Phe Arg Val Arg Ala Ala Asn Phe Ser
            260                 265                 270
Pro Phe Glu Ala Asn Ser Gly Asn Leu Ile Asp Met Gly Arg Val Lys
        275                 280                 285
Leu Ile Ser Gly Ile Leu Arg Lys Asp Asp Leu Glu Ile Ser Asp Thr
    290                 295                 300
Ile Lys Ala Ile Glu Lys Val Phe Thr Leu Val Asp Glu Gly Asn Gly
305                 310                 315                 320
Phe Tyr Gln Asp Gly Ser Leu Ile Asp His Val Val Thr Asn Ala Gln
                325                 330                 335
Ser Pro Leu Tyr Lys Lys Gly Ile Ala Tyr Thr Gly Ala Tyr Gly Asn
            340                 345                 350
Val Leu Ile Asp Gly Leu Ser Gln Leu Ile Pro Ile Ile Gln Lys Thr
        355                 360                 365
Lys Ser Pro Ile Glu Ala Asp Lys Met Ala Thr Ile Tyr His Trp Ile
    370                 375                 380
Asn His Ser Phe Phe Pro Ile Ile Val Arg Gly Glu Met Met Asp Met
385                 390                 395                 400
Thr Arg Gly Arg Ser Ile Ser Arg Phe Asn Ala Gln Ser His Val Ala
                405                 410                 415
Gly Ile Glu Ala Leu Arg Ala Ile Leu Arg Ile Ala Asp Met Ser Glu
            420                 425                 430
Glu Pro His Arg Leu Ala Leu Lys Thr Arg Ile Lys Thr Leu Val Thr
        435                 440                 445
Gln Gly Asn Ala Phe Tyr Asn Val Tyr Asp Asn Leu Lys Thr Tyr His
    450                 455                 460
Asp Ile Lys Leu Met Lys Glu Leu Leu Ser Asp Thr Phe Val Pro Val
465                 470                 475                 480
Gln Lys Leu Asp Ser Tyr Val Ala Ser Phe Asn Ser Met Asp Lys Leu
                485                 490                 495
Ala Leu Tyr Asn Asn Lys His Asp Phe Ala Phe Gly Leu Ser Met Phe
            500                 505                 510
Ser Asn Arg Thr Gln Asn Tyr Glu Ala Met Asn Asn Glu Asn Leu His
        515                 520                 525
Gly Trp Phe Thr Ser Asp Gly Met Phe Tyr Leu Tyr Asn Asn Asp Leu
    530                 535                 540
Gly His Tyr Ser Glu Asn Tyr Trp Ala Thr Val Asn Pro Tyr Arg Leu
545                 550                 555                 560
Pro Gly Thr Thr Glu Thr Glu Gln Lys Pro Leu Glu Gly Thr Pro Glu
                565                 570                 575
Asn Ile Lys Thr Asp Tyr Gln Gln Val Gly Met Thr Ser Leu Ser Asp
            580                 585                 590
Asp Ala Phe Val Ala Ser Lys Lys Leu Asn Asn Thr Ser Ala Leu Ala
        595                 600                 605
Ala Met Thr Phe Thr Asn Trp Asn Lys Ser Leu Thr Leu Asn Lys Gly
    610                 615                 620
Trp Phe Ile Leu Gly Asn Lys Ile Ile Phe Val Gly Ser Asn Ile Lys
625                 630                 635                 640
Asn Gln Ser Ser His Lys Ala Tyr Thr Thr Ile Glu Gln Arg Lys Glu
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                645                 650                 655
Asn Gln Lys His Pro Tyr Cys Ser Tyr Val Asn Asn Gln Pro Val Asp
            660                 665                 670
Leu Asn Asn Gln Leu Val Asp Phe Thr Asn Thr Lys Ser Ile Phe Leu
        675                 680                 685
Glu Ser Asp Asp Pro Ala Gln Asn Ile Gly Tyr Tyr Phe Phe Lys Pro
    690                 695                 700
Thr Thr Leu Ser Ile Ser Lys Ala Leu Gln Thr Gly Lys Trp Gln Asn
705                 710                 715                 720
Ile Lys Ala Asp Asp Lys Ser Pro Glu Ala Ile Lys Glu Val Ser Asn
                725                 730                 735
Thr Phe Ile Thr Ile Met Gln Asn His Thr Gln Asp Gly Asp Arg Tyr
            740                 745                 750
Ala Tyr Met Met Leu Pro Asn Met Thr Arg Gln Glu Phe Glu Thr Tyr
        755                 760                 765
Ile Ser Lys Leu Asp Ile Asp Leu Leu Glu Asn Asn Asp Lys Leu Ala
    770                 775                 780
Ala Val Tyr Asp His Asp Ser Gln Gln Met His Val Ile His Tyr Glu
785                 790                 795                 800
Lys Lys Ala Thr Met Phe Ser Asn His Asn Leu Ser His Gln Gly Phe
                805                 810                 815
Tyr Ser Phe Pro His Pro Val Lys Gln Asn Gln Gln
            820                 825


<210> 89
<211> 556
<212> PRT
<213> Streptococcus pyogenes serotype M12 (strain MGAS2096)

<220>
<223> hyaluronan lyase

<400> 89
Met Val Tyr Phe Tyr Leu Val Asn Gln Ser Thr Phe Ile Ile Ser Phe
 1                5                  10                  15
Leu Tyr Trp Arg Asn Leu Ser Val Asn Thr Tyr Phe Cys Thr His His
            20                  25                  30
Lys Gln Leu Leu Leu Tyr Ser Asn Leu Phe Leu Ser Phe Ala Met Met
        35                  40                  45
Gly Gln Gly Thr Ala Ile Tyr Ala Asp Thr Leu Thr Ser Asn Ser Glu
    50                  55                  60
Pro Asn Asn Thr Tyr Phe Gln Thr Gln Thr Leu Thr Thr Thr Asp Ser
65                  70                  75                  80
Glu Lys Lys Val Val Gln Pro Gln Gln Lys Asp Tyr Tyr Thr Glu Leu
                85                  90                  95
Leu Asp Gln Trp Asn Ser Ile Ile Ala Gly Asn Asp Ala Tyr Asp Lys
            100                 105                 110
Thr Asn Pro Asp Met Val Thr Phe His Asn Lys Ala Glu Lys Asp Ala
        115                 120                 125
Gln Asn Ile Ile Lys Ser Tyr Gln Gly Pro Asp His Glu Asn Arg Thr
    130                 135                 140
Tyr Leu Gly Asn Met Gln Arg Ile Ile Pro Leu Leu Leu Ile Ser Arg
145                 150                 155                 160
Lys Leu Thr Ala Ile Leu Lys Lys Ile Ser Lys Met Lys Ser Leu Met
                165                 170                 175
Glu Asp Ser Thr Ile Ser Thr Asn Gly Leu Thr Gln Gln Leu Lys Ile
            180                 185                 190
Tyr Asn Asp Met Asp Arg Val Thr Tyr His Asn Lys Gly Leu Asp Phe
        195                 200                 205
Ala Phe Gly Leu Ser Met Thr Ser Lys Asn Val Ala Arg Tyr Glu Ser
    210                 215                 220
Ile Asn Gly Glu Asn Leu Lys Gly Trp His Thr Gly Ala Gly Met Ser
225                 230                 235                 240
Tyr Leu Tyr Asn Ser Asp Val Lys His Tyr Arg Asp Asn Phe Trp Ala
                245                 250                 255
Thr Ala Asp Met Lys Arg Leu Ala Gly Thr Thr Thr Leu Asp Asn Glu
            260                 265                 270
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Glu Pro Lys Ser Thr Asp Val Lys Lys Ser Ser Lys Thr Phe Val Gly
        275                 280                 285
Gly Thr Lys Phe Asp Asp Gln His Ala Ser Ile Gly Met Asp Phe Glu
    290                 295                 300
Asn Gln Asp Lys Thr Leu Thr Ala Lys Lys Ser Tyr Phe Ile Leu Asn
305                 310                 315                 320
Asp Lys Ile Val Phe Leu Gly Thr Gly Ile Lys Ser Thr Asp Ser Ser
                325                 330                 335
Lys Asn Pro Val Thr Thr Ile Glu Asn Arg Lys Ala Asn Asp Tyr Lys
            340                 345                 350
Leu Tyr Lys Asp Asp Thr Gln Thr Thr Asn Ser Asp Asn Gln Glu Thr
        355                 360                 365
Asn Ser Leu Phe Leu Glu Ser Thr Asn Ser Thr Gln Asn Asn Ile Gly
    370                 375                 380
Tyr His Phe Leu Asn Glu Ser Lys Ile Thr Val Lys Lys Glu Ser His
385                 390                 395                 400
Thr Gly Lys Trp Ser Asp Ile Asn Lys Ser Gln Lys Asp Ile Gln Lys
                405                 410                 415
Thr Asp Glu Tyr Tyr Glu Val Thr Gln Lys His Ser Asn Thr Asp Ser
            420                 425                 430
Lys Tyr Ala Tyr Val Leu Tyr Pro Gly Leu Ser Lys Asp Val Phe Lys
        435                 440                 445
Ser Lys Ala Ser Lys Val Thr Val Val Lys Gln Glu Asp Asp Phe His
    450                 455                 460
Val Val Lys Asp Asn Glu Ser Val Trp Ala Gly Ile Asn Tyr Ser Asp
465                 470                 475                 480
Ser Ala Lys Thr Phe Glu Ile Asn Asn Thr Lys Val Glu Val Lys Ala
                485                 490                 495
Lys Gly Met Phe Ile Leu Thr Lys Lys Asp Asp Asn Thr Tyr Glu Cys
            500                 505                 510
Ser Phe Tyr Asn Pro Glu Ser Thr Asn Ser Val Ser Asp Ile Glu Ser
        515                 520                 525
Lys Ile Ser Met Thr Gly Tyr Ser Ile Ile Asn Lys Asn Thr Ser Thr
    530                 535                 540
Ser Asn Glu Ser Gly Val Arg Phe Glu Leu Thr Lys
545                 550                 555
```

<210> 90
<211> 635
<212> PRT
<213> Streptococcus pyogenes serotype M12 (strain MGAS2096)

<220>
<223> hyaluronan lyase

<400> 90

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Met Ala Phe Met Tyr Glu His Ala Tyr Asn Leu Asn Arg Glu Asn His
 1               5                  10                  15
Gln Thr Thr Gly Lys Glu Asn Lys Glu Asn Trp Trp Val Tyr Glu Ile
            20                  25                  30
Gly Thr Pro Arg Ala Ile Asn Asn Thr Leu Ser Leu Met Tyr Pro Tyr
        35                  40                  45
Phe Thr Gln Glu Glu Ile Leu Lys Tyr Thr Ala Pro Ile Glu Lys Phe
    50                  55                  60
Val Pro Asp Pro Thr Arg Phe Arg Val Arg Ala Ala Asn Phe Ser Pro
65                  70                  75                  80
Phe Glu Ala Ser Ser Gly Asn Leu Ile Asp Met Gly Arg Val Lys Leu
                85                  90                  95
Ile Ser Gly Ile Leu Arg Lys Asp Asp Leu Glu Ile Ser Asp Thr Ile
            100                 105                 110
Lys Ala Ile Glu Lys Val Phe Thr Leu Val Asp Glu Gly Asn Gly Phe
        115                 120                 125
Tyr Gln Asp Gly Ser Leu Ile Asp His Val Val Thr Asn Ala Gln Ser
    130                 135                 140
Pro Leu Tyr Lys Lys Gly Ile Ala Tyr Thr Gly Ala Tyr Gly Asn Val
145                 150                 155                 160
Leu Ile Asp Gly Leu Ser Gln Leu Ile Pro Ile Ile Gln Lys Thr Lys
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                165                 170                 175
Ser Pro Ile Glu Ala Asp Lys Met Ala Thr Ile Tyr His Trp Ile Asn
            180                 185                 190
His Ser Phe Phe Pro Ile Ile Val Arg Gly Glu Met Met Asp Met Thr
        195                 200                 205
Arg Gly Arg Ser Ile Ser Arg Phe Asn Ala Gln Ser His Val Ala Gly
    210                 215                 220
Ile Glu Ala Leu Arg Ala Ile Leu Arg Ile Ala Asp Met Ser Glu Glu
225                 230                 235                 240
Pro His Arg Leu Ala Leu Lys Thr Arg Ile Lys Thr Leu Val Thr Gln
                245                 250                 255
Gly Asn Ala Phe Tyr Asn Val Tyr Asp Asn Leu Lys Thr Tyr His Asp
            260                 265                 270
Ile Lys Leu Met Lys Glu Leu Leu Ser Asp Thr Ser Val Pro Val Gln
        275                 280                 285
Lys Leu Asp Ser Tyr Val Ala Ser Phe Asn Ser Met Asp Lys Leu Ala
    290                 295                 300
Leu Tyr Asn Asn Lys His Asp Phe Ala Phe Gly Leu Ser Met Phe Ser
305                 310                 315                 320
Asn Arg Thr Gln Asn Tyr Glu Ala Met Asn Asn Glu Asn Leu His Gly
                325                 330                 335
Trp Phe Thr Ser Asp Gly Met Phe Tyr Leu Tyr Asn Asn Asp Leu Gly
            340                 345                 350
His Tyr Ser Glu Asn Tyr Trp Ala Thr Val Asn Pro Tyr Arg Leu Pro
        355                 360                 365
Gly Thr Thr Glu Thr Glu Gln Lys Pro Leu Glu Gly Thr Pro Glu Asn
    370                 375                 380
Ile Lys Thr Asn Tyr Gln Gln Val Gly Met Thr Ser Leu Ser Asp Asp
385                 390                 395                 400
Ala Phe Val Ala Ser Lys Lys Leu Asn Asn Thr Ser Ala Leu Ala Ala
                405                 410                 415
Met Thr Phe Thr Asn Trp Asn Lys Ser Leu Thr Leu Asn Lys Gly Trp
            420                 425                 430
Phe Ile Leu Gly Asn Lys Ile Ile Phe Val Gly Ser Asn Ile Lys Asn
        435                 440                 445
Gln Ser Ser His Lys Ala Tyr Thr Thr Ile Glu Gln Arg Lys Glu Asn
    450                 455                 460
Gln Lys His Pro Tyr Cys Ser Tyr Val Asn Asn Gln Pro Val Asp Leu
465                 470                 475                 480
Asn Asn Gln Leu Val Asp Phe Thr Asn Thr Lys Ser Ile Phe Leu Glu
                485                 490                 495
Ser Asp Asp Pro Ala Gln Asn Ile Gly Tyr Tyr Phe Phe Lys Pro Thr
            500                 505                 510
Thr Leu Ser Ile Ser Lys Ala Leu Gln Thr Gly Lys Trp Gln Asn Ile
        515                 520                 525
Lys Ala Asp Asp Lys Ser Pro Glu Ala Ile Lys Glu Val Ser Asn Thr
    530                 535                 540
Phe Ile Thr Ile Met Gln Asn His Thr Gln Asp Gly Asp Arg Tyr Ala
545                 550                 555                 560
Tyr Met Met Leu Pro Asn Met Thr Arg Gln Glu Phe Glu Thr Tyr Ile
                565                 570                 575
Ser Lys Leu Asp Ile Asp Leu Leu Glu Asn Asn Asp Lys Leu Ala Ala
            580                 585                 590
Val Tyr Asp His Asp Ser Gln Gln Met His Val Ile His Tyr Glu Lys
        595                 600                 605
Lys Ala Thr Met Phe Ser Asn His Asn Leu Ser His Gln Gly Phe Tyr
    610                 615                 620
Ser Phe Pro His Pro Val Lys Gln Asn Gln Gln
625                 630                 635
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<210> 91
<211> 828
<212> PRT
<213> Streptococcus pyogenes serotype M12 (strain MGAS9429)

<220>
<223> hyaluronan lyase

<400> 91
Met Val Tyr Phe Tyr Leu Val Asn Gln Ser Thr Phe Ile Ile Ser Phe
 1   5   10   15
Leu Tyr Trp Arg Asn Leu Ser Val Asn Thr Tyr Phe Cys Thr His His
            20   25   30
Lys Gln Leu Leu Leu Tyr Ser Asn Leu Phe Leu Ser Phe Ala Met Met
        35   40   45
Gly Gln Gly Thr Ala Ile Tyr Ala Asp Thr Leu Thr Ser Asn Ser Glu
    50   55   60
Pro Asn Asn Thr Tyr Phe Gln Thr Gln Thr Leu Thr Thr Thr Asp Ser
65   70   75   80
Glu Lys Lys Val Val Gln Pro Gln Gln Lys Asp Tyr Tyr Thr Glu Leu
                85   90   95
Leu Asp Gln Trp Asn Ser Ile Ile Ala Gly Asn Asp Ala Tyr Val Lys
            100   105   110
Thr Asn Pro Asp Met Val Thr Phe His Asn Lys Ala Glu Lys Asp Ala
        115   120   125
Gln Asn Ile Ile Lys Ser Tyr Gln Gly Pro Asp His Glu Asn Arg Thr
    130   135   140
Tyr Leu Trp Glu His Ala Lys Asp Tyr Ser Ala Ser Thr Asn Ile Thr
145   150   155   160
Lys Thr Tyr Arg Asn Ile Glu Lys Ile Ala Lys Gln Ile Thr Asn Pro
                165   170   175
Glu Ser Cys Tyr Tyr Gln Asp Ser Lys Ala Ile Ala Ile Val Lys Asp
            180   185   190
Gly Met Ala Phe Met Tyr Glu His Ala Tyr Asn Leu Asn Arg Glu Asn
        195   200   205
His Gln Thr Thr Gly Lys Glu Asn Lys Glu Asn Trp Trp Val Tyr Glu
    210   215   220
Ile Gly Thr Pro Arg Ala Ile Asn Asn Thr Leu Ser Leu Met Tyr Pro
225   230   235   240
Tyr Phe Thr Gln Glu Glu Ile Leu Lys Tyr Thr Ala Pro Ile Glu Lys
                245   250   255
Phe Val Pro Asp Pro Thr Arg Phe Arg Val Arg Ala Ala Asn Phe Ser
            260   265   270
Pro Phe Glu Ala Ser Ser Gly Asn Leu Ile Asp Met Gly Arg Val Lys
        275   280   285
Leu Ile Ser Gly Ile Leu Arg Lys Asp Asp Leu Glu Ile Ser Asp Thr
    290   295   300
Ile Lys Ala Ile Glu Lys Val Phe Thr Leu Val Asp Glu Gly Asn Gly
305   310   315   320
Phe Tyr Gln Asp Gly Ser Leu Ile Asp His Val Val Thr Asn Ala Gln
                325   330   335
Ser Pro Leu Tyr Lys Lys Gly Ile Ala Tyr Thr Gly Ala Tyr Gly Asn
            340   345   350
Val Leu Ile Asp Gly Leu Ser Gln Leu Ile Pro Ile Ile Gln Lys Thr
        355   360   365
Lys Ser Pro Ile Glu Ala Asp Lys Met Ala Thr Ile Tyr His Trp Ile
    370   375   380
Asn His Ser Phe Phe Pro Ile Ile Val Arg Gly Glu Met Met Asp Met
385   390   395   400
Thr Arg Gly Arg Ser Ile Ser Arg Phe Asn Ala Gln Ser His Val Ala
                405   410   415
Gly Ile Glu Ala Leu Arg Ala Ile Leu Arg Ile Ala Asp Met Ser Glu
            420   425   430
Glu Pro His Arg Leu Ala Leu Lys Thr Arg Ile Lys Thr Leu Val Thr
        435   440   445
Gln Gly Asn Ala Phe Tyr Asn Val Tyr Asp Asn Leu Lys Thr Tyr His
    450   455   460
Asp Ile Lys Leu Met Lys Glu Leu Leu Ser Asp Thr Ser Val Pro Val
465   470   475   480
Gln Lys Leu Asp Ser Tyr Val Ala Ser Phe Asn Ser Met Asp Lys Leu
                485   490   495
Ala Leu Tyr Asn Asn Lys His Asp Phe Ala Phe Gly Leu Ser Met Phe
            500   505   510
Ser Asn Arg Thr Gln Asn Tyr Glu Ala Met Asn Asn Glu Asn Leu His
        515   520   525

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Gly Trp Phe Thr Ser Asp Gly Met Phe Tyr Leu Tyr Asn Asn Asp Leu
    530                 535                 540
Gly His Tyr Ser Glu Asn Tyr Trp Ala Thr Val Asn Pro Tyr Arg Leu
545                 550                 555                 560
Pro Gly Thr Thr Glu Thr Glu Gln Lys Pro Leu Glu Gly Thr Pro Glu
                565                 570                 575
Asn Ile Lys Thr Asn Tyr Gln Gln Val Gly Met Thr Ser Leu Ser Asp
            580                 585                 590
Asp Ala Phe Val Ala Ser Lys Lys Leu Asn Asn Thr Ser Ala Leu Ala
        595                 600                 605
Ala Met Thr Phe Thr Asn Trp Asn Lys Ser Leu Thr Leu Asn Lys Gly
    610                 615                 620
Trp Phe Ile Leu Gly Asn Lys Ile Ile Phe Val Gly Ser Asn Ile Lys
625                 630                 635                 640
Asn Gln Ser Ser His Lys Ala Tyr Thr Thr Ile Glu Gln Arg Lys Glu
                645                 650                 655
Asn Gln Lys His Pro Tyr Cys Ser Tyr Val Asn Asn Gln Pro Val Asp
            660                 665                 670
Leu Asn Asn Gln Leu Val Asp Phe Thr Asn Thr Lys Ser Ile Phe Leu
        675                 680                 685
Glu Ser Asp Asp Pro Ala Gln Asn Ile Gly Tyr Tyr Phe Phe Lys Pro
    690                 695                 700
Thr Thr Leu Ser Ile Ser Lys Ala Leu Gln Thr Gly Lys Trp Gln Asn
705                 710                 715                 720
Ile Lys Ala Asp Asp Lys Ser Pro Glu Ala Ile Lys Glu Val Ser Asn
                725                 730                 735
Thr Phe Ile Thr Ile Met Gln Asn His Thr Gln Asp Gly Asp Arg Tyr
            740                 745                 750
Ala Tyr Met Met Leu Pro Asn Met Thr Arg Gln Glu Phe Glu Thr Tyr
        755                 760                 765
Ile Ser Lys Leu Asp Ile Asp Leu Leu Glu Asn Asn Asp Lys Leu Ala
    770                 775                 780
Ala Val Tyr Asp His Asp Ser Gln Gln Met His Val Ile His Tyr Glu
785                 790                 795                 800
Lys Lys Ala Thr Met Phe Ser Asn His Asn Leu Ser His Gln Gly Phe
                805                 810                 815
Tyr Ser Phe Pro His Pro Val Lys Gln Asn Gln Gln
            820                 825
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<210> 92
<211> 828
<212> PRT
<213> Streptococcus pyogenes serotype M28

<220>
<223> hyaluronan lyase

<400> 92

```
Met Val Tyr Phe Tyr Leu Val Asn Gln Phe Thr Phe Ile Ile Ser Phe
 1               5                  10                  15
Leu Tyr Arg Arg Asn Leu Ser Val Asn Thr Tyr Phe Cys Thr His His
            20                  25                  30
Lys Gln Leu Leu Leu Tyr Ser Asn Leu Phe Leu Ser Phe Ala Met Met
        35                  40                  45
Gly Gln Gly Thr Ala Ile Tyr Ala Asp Thr Leu Thr Ser Asn Ser Glu
    50                  55                  60
Pro Asn Asn Thr Tyr Phe Gln Thr Gln Met Leu Thr Thr Thr Asp Ser
65                  70                  75                  80
Glu Lys Lys Val Val Gln Pro Gln Gln Lys Asp Tyr Tyr Thr Glu Leu
                85                  90                  95
Leu Asp Gln Trp Asn Ser Ile Ile Ala Gly Asn Asp Ala Tyr Asp Lys
            100                 105                 110
Thr Asn Pro Asp Met Val Thr Phe His Asn Lys Ala Glu Lys Asp Ala
        115                 120                 125
Gln Asn Ile Ile Lys Ser Tyr Gln Gly Pro Asp His Glu Asn Arg Thr
    130                 135                 140
Tyr Leu Trp Glu His Ala Lys Asp Tyr Ser Ala Ser Ala Asn Ile Thr
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145                 150                 155                 160
Lys Thr Tyr Arg Asn Ile Glu Lys Ile Ala Lys Gln Ile Thr Asn Pro
                165                 170                 175
Glu Ser Cys Tyr Tyr Gln Asp Ser Lys Ala Ile Ala Ile Val Lys Asp
            180                 185                 190
Gly Met Ala Phe Met Tyr Glu His Ala Tyr Asn Leu Asp Arg Glu Asn
        195                 200                 205
His Gln Thr Thr Gly Lys Glu Asn Lys Glu Asn Trp Trp Val Tyr Glu
    210                 215                 220
Ile Gly Thr Pro Arg Ala Ile Asn Asn Thr Leu Ser Leu Met Tyr Pro
225                 230                 235                 240
Tyr Phe Thr Gln Glu Glu Ile Leu Lys Tyr Thr Ala Pro Ile Glu Lys
                245                 250                 255
Phe Val Pro Asp Pro Thr Arg Phe Arg Val Arg Ala Ala Asn Phe Ser
            260                 265                 270
Pro Phe Glu Ala Asn Ser Gly Asn Leu Ile Asp Met Gly Arg Val Lys
        275                 280                 285
Leu Ile Ser Gly Ile Leu Arg Lys Asp Asp Leu Glu Ile Ser Asp Thr
    290                 295                 300
Ile Lys Ala Ile Glu Lys Val Phe Thr Leu Val Asp Glu Gly Asn Gly
305                 310                 315                 320
Phe Tyr Gln Asp Gly Ser Leu Ile Asp His Val Val Thr Asn Ala Gln
                325                 330                 335
Ser Pro Leu Tyr Lys Lys Gly Ile Ala Tyr Thr Gly Ala Tyr Gly Asn
            340                 345                 350
Val Leu Ile Asp Gly Leu Ser Gln Leu Ile Pro Ile Ile Gln Lys Thr
        355                 360                 365
Lys Ser Ser Ile Glu Ala Asp Lys Met Ala Thr Ile Tyr His Trp Ile
    370                 375                 380
Asn His Ser Phe Phe Pro Ile Ile Val Arg Gly Glu Met Met Asp Met
385                 390                 395                 400
Thr Arg Gly Arg Ser Ile Ser Arg Phe Asn Ala Gln Ser His Val Ala
                405                 410                 415
Gly Ile Glu Ala Leu Arg Ala Ile Leu Arg Ile Ala Asp Met Ser Glu
            420                 425                 430
Glu Pro His Arg Leu Ala Leu Lys Thr Arg Ile Lys Thr Leu Val Thr
        435                 440                 445
Gln Gly Asn Ala Phe Tyr Asn Val Tyr Asp Asn Leu Lys Thr Tyr His
    450                 455                 460
Asp Ile Lys Leu Met Lys Glu Leu Leu Ser Asp Thr Ser Val Pro Val
465                 470                 475                 480
Gln Lys Leu Asp Ser Tyr Val Ala Ser Phe Asn Ser Met Asp Lys Leu
                485                 490                 495
Ala Leu Tyr Asn Asn Lys His Asp Phe Ala Phe Gly Leu Ser Met Phe
            500                 505                 510
Ser Asn Arg Thr Gln Asn Tyr Glu Ala Met Asn Asn Glu Asn Leu His
        515                 520                 525
Gly Trp Phe Thr Ser Asp Gly Met Phe Tyr Leu Tyr Asn Asn Asp Leu
    530                 535                 540
Gly His Tyr Ser Glu Asn Tyr Trp Ala Thr Val Asn Pro Tyr Arg Leu
545                 550                 555                 560
Pro Gly Thr Thr Glu Thr Glu Gln Lys Pro Leu Glu Gly Thr Pro Glu
                565                 570                 575
Asn Ile Lys Thr Asn Tyr Gln Gln Val Gly Met Thr Ser Leu Ser Asp
            580                 585                 590
Asp Ala Phe Val Ala Ser Lys Lys Leu Asn Asn Thr Ser Ala Leu Ala
        595                 600                 605
Ala Met Thr Phe Thr Asn Trp Asn Lys Ser Leu Thr Leu Asn Lys Gly
    610                 615                 620
Trp Phe Ile Leu Gly Asn Lys Ile Ile Phe Val Gly Ser Asn Ile Lys
625                 630                 635                 640
Asn Gln Ser Ser His Lys Ala Tyr Thr Thr Ile Glu Gln Arg Lys Glu
                645                 650                 655
Asn Gln Lys His Pro Tyr His Ala Tyr Val Asn Asn Gln Pro Val Asp
            660                 665                 670
Leu Asn Asn Gln Leu Val Asp Phe Thr Asn Thr Lys Ser Ile Phe Leu
        675                 680                 685
Glu Ser Asp Asp Ser Ala Gln Asn Ile Gly Tyr Tyr Phe Phe Lys Pro
```

690 695 700
Thr Thr Leu Ser Ile Ser Lys Ala Leu Gln Thr Gly Lys Trp Gln Asn
705 710 715 720
Ile Lys Ala Asp Asp Lys Ser Pro Glu Ala Ile Lys Glu Val Ser Asn
725 730 735
Thr Phe Ile Thr Ile Met Gln Asn His Thr Gln Asp Gly Asp Arg Tyr
740 745 750
Ala Tyr Met Met Leu Pro Asn Met Thr Arg Gln Glu Phe Glu Thr Tyr
755 760 765
Ile Ser Lys Leu Asp Ile Asp Leu Leu Glu Asn Asn Asp Lys Leu Ala
770 775 780
Ala Val Tyr Asp His Asp Ser Gln Gln Met His Val Ile His Tyr Glu
785 790 795 800
Lys Lys Ala Thr Met Phe Ser Asn His Asn Leu Ser His Gln Gly Phe
805 810 815
Tyr Ser Phe Pro His Pro Val Lys Gln Asn Gln Gln
820 825

<210> 93
<211> 522
<212> PRT
<213> Streptococcus suis

<220>
<223> hyaluronan lyase

<400> 93
Met Gly Phe Phe Ile Ser Gln Ser Lys Gln His Tyr Gly Ile Arg Lys
1 5 10 15
Tyr Lys Val Gly Val Cys Ser Ala Leu Ile Ala Leu Ser Ile Leu Gly
20 25 30
Thr Arg Val Ala Ala Asn Gln Leu Pro Ser Thr Glu Thr Ala Ser Pro
35 40 45
Gln Ser Ser Gln Leu Val Glu Thr Thr Pro Glu Thr Thr Glu Ala Val
50 55 60
Asn Leu Thr Thr Glu Ala Val Met Thr Ser Glu Val Ser Ser Glu Val
65 70 75 80
Ser Pro Val Thr Ser Thr Glu Thr Gln Pro Ser Ser Thr Ala Ala Glu
85 90 95
Thr Leu Ala Ser Pro Gln Ala Val Gln Ala Thr Lys Glu Glu Glu Lys
100 105 110
Asn Leu Val Ala Asn Gly Glu Phe Ala Ser Thr Thr Ala Ala Ser Gly
115 120 125
Asn Trp Ala Asp Pro Ala Ala Thr Asn Trp Glu Thr Trp Ile Pro Ala
130 135 140
Asn Val Lys Lys Glu Asn Gly Gln Val Arg Ile Asp Glu Gly Arg Leu
145 150 155 160
His Ile Ser Ser Thr Ala Ser Tyr Arg Val Ala Val His Gln Thr Val
165 170 175
Asp Val Asp Pro Asn Lys Arg Tyr Leu Phe Ser Tyr Asn Val Glu Thr
180 185 190
Lys Asp Leu Lys Gly Ser Gly Val Arg Val Arg Leu Arg Ser Leu Thr
195 200 205
Ala Glu Gly Lys Asp Leu Ser Pro Gln Glu Phe Ala Tyr Thr Pro Tyr
210 215 220
Lys Asn Gly Ser Gln Ala Glu His Ile Glu Gln Ile Leu Thr Val Ser
225 230 235 240
Pro Glu Thr Arg Lys Leu Lys Val Glu Leu Phe Phe Glu Asn Ser Val
245 250 255
Gly Gln Ala Trp Leu Asp Asn Ile Ser Leu Val Glu Tyr Val Glu Lys
260 265 270
Thr Pro Glu Thr Pro Glu Pro Ser Leu Glu Leu Val Gln Pro Glu Thr
275 280 285
Gly Gln Ile Ser Leu Ala Ser Asn Lys Val Tyr Leu Pro Val Arg Pro
290 295 300
Asp Leu Thr Tyr Arg Ile Ala Asp Ala Ala Val Ala Ile Val Glu Lys
305 310 315 320

```
Asn Met Ile Arg Pro Leu Ala Ala Gly Lys Thr Gln Val Asp Val Tyr
                325                 330                 335
Asp Lys Asp Thr Lys Leu Ser Ser Phe Glu Leu Thr Val Thr Glu His
            340                 345                 350
Gln Ala Thr Val Phe Asp Thr Leu Arg Asn Asn Trp Glu Asp Ile Ser
        355                 360                 365
Leu Ala Asn Lys Arg Tyr Gln Ser Asn Asp Thr Gln Met Lys Ala Phe
    370                 375                 380
Leu Gly Arg Leu Asp Ala Gly Val Ala Ser Ser Leu Lys Lys Trp Val
385                 390                 395                 400
Glu Pro Thr Asn Gln Gly Lys Thr Ile Phe Asn Asp Ile Asp Phe Ser
                405                 410                 415
Lys Ser Ser His Leu Thr Thr Val Tyr Arg Arg Leu Glu Gln Met Ala
            420                 425                 430
Gln Val Val Glu Asn Pro Asp Ser Ala Tyr Tyr His Asp Arg Ser Leu
        435                 440                 445
Ile Asp Leu Val Arg Lys Gly Met Asn Trp Leu Tyr Thr Asn Val Tyr
    450                 455                 460
Asn Glu Asn Lys Ser Ile Asp Gly Asn Trp Trp Asp Tyr Glu Ile Gly
465                 470                 475                 480
Thr Pro Arg Ala Val Val Asn Thr Leu Ile Tyr Met His Pro Tyr Phe
                485                 490                 495
Ser Gln Glu Glu Ile Leu Thr Tyr Thr Lys Pro Ile Ser Lys Phe Val
            500                 505                 510
Pro Asp Pro Thr Thr Ile Ser Val Lys His
        515                 520


<210> 94
<211> 1164
<212> PRT
<213> Streptococcus suis

<220>
<223> hyaluronan lyase

<400> 94
Met Gly Phe Phe Ile Ser Gln Ser Lys Gln His Tyr Gly Ile Arg Lys
 1               5                  10                  15
Tyr Lys Val Gly Val Cys Ser Ala Leu Ile Ala Leu Ser Ile Leu Gly
            20                  25                  30
Thr Arg Val Ala Ala Asn Gln Leu Pro Ser Thr Glu Thr Ala Ser Pro
        35                  40                  45
Gln Ser Ser Gln Leu Val Glu Thr Thr Pro Glu Thr Thr Glu Ala Val
    50                  55                  60
Met Thr Ser Glu Val Ser Ser Glu Val Ser Pro Val Thr Ser Thr Glu
65                  70                  75                  80
Thr Gln Pro Ser Ser Thr Thr Ala Glu Thr Leu Ala Ser Pro Gln Ala
                85                  90                  95
Val Gln Ala Thr Lys Glu Glu Lys Asn Leu Val Ala Asn Gly Glu Phe
            100                 105                 110
Ile Ser Thr Thr Ala Pro Ser Gly Asn Trp Lys Glu Leu Ala Ala Thr
        115                 120                 125
Asn Trp Glu Thr Trp Ile Pro Ala Asn Val Lys Lys Glu Asn Gly Gln
    130                 135                 140
Val Arg Ile Asp Glu Gly Arg Leu His Ile Ser Ser Thr Ala Ser Tyr
145                 150                 155                 160
Arg Val Ala Val His Gln Thr Val Asp Val Asp Pro Asn Lys Arg Tyr
                165                 170                 175
Leu Phe Ser Tyr Asp Val Glu Thr Lys Asp Leu Lys Gly Ser Gly Val
            180                 185                 190
Arg Val Arg Leu Arg Ser Leu Thr Ala Glu Gly Lys Asp Leu Ser Pro
        195                 200                 205
Gln Glu Phe Ala Tyr Thr Pro Tyr Lys Asn Gly Ser Gln Ala Glu His
    210                 215                 220
Ile Glu Gln Ile Leu Thr Val Ser Pro Glu Thr Arg Lys Leu Lys Val
225                 230                 235                 240
Glu Leu Phe Phe Glu Asn Ser Val Gly Gln Ala Trp Leu Asp Asn Ile
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                245                 250                 255
Ser Leu Val Glu Tyr Val Glu Lys Thr Pro Glu Thr Pro Glu Gln Ser
            260                 265                 270
Pro Glu Leu Val Gln Pro Glu Thr Gly Gln Ile Ser Leu Ala Ser Asn
        275                 280                 285
Lys Val Tyr Leu Pro Val Arg Pro Asp Leu Thr Tyr Arg Ile Ala Asp
    290                 295                 300
Ala Ala Val Ala Thr Val Glu Lys Asn Met Ile Arg Pro Leu Ala Ala
305                 310                 315                 320
Gly Lys Thr Gln Val Asp Val Tyr Asp Lys Asp Thr Lys Leu Ser Ser
                325                 330                 335
Phe Glu Leu Ile Val Thr Glu His Gln Ala Thr Val Phe Asp Thr Leu
            340                 345                 350
Arg Asn Asn Trp Glu Asp Ile Ser Leu Ala Asn Lys Arg Tyr Gln Ser
        355                 360                 365
Asn Asp Ala Gln Met Lys Ala Phe Leu Gly Arg Leu Asp Ala Gly Val
    370                 375                 380
Ala Ser Ser Leu Glu Lys Trp Val Glu Pro Thr Glu Gln Ser Lys Thr
385                 390                 395                 400
Ile Phe Asn Asp Ile Asp Phe Ser Lys Ser Ser His Leu Thr Thr Val
                405                 410                 415
Tyr Arg Arg Leu Glu Gln Met Ala Gln Val Val Glu Asn Pro Asp Ser
            420                 425                 430
Ala Tyr Tyr His Asp Arg Ser Leu Ile Asp Leu Val Arg Lys Gly Met
        435                 440                 445
Asn Trp Leu Tyr Ala Asn Val Tyr Asn Glu Asn Lys Ser Ile Asp Gly
    450                 455                 460
Asn Trp Trp Asp Tyr Glu Ile Gly Thr Ser Arg Ala Val Val Asn Thr
465                 470                 475                 480
Leu Ile Tyr Met His Pro Tyr Phe Ser Gln Glu Glu Ile Leu Thr Tyr
                485                 490                 495
Thr Lys Pro Ile Ser Lys Phe Val Pro Asp Pro Thr Thr Ile Arg Lys
            500                 505                 510
Thr Leu Thr Asn Pro Val Pro Ala Val Gly Gly Asn Gln Thr Asp Leu
        515                 520                 525
Ser Lys Val Ala Ile Leu Glu Gly Ala Leu Arg Glu Asp Ala Asp Arg
    530                 535                 540
Val Arg Ala Gly Ala Gln Gly Leu Thr Thr Ile Met Lys Phe Val Asp
545                 550                 555                 560
Lys Gly Glu Gly Phe Tyr Arg Asp Gly Ser Phe Ile Asp His Thr Asn
                565                 570                 575
Val Ala Tyr Thr Gly Ala Tyr Gly Asn Val Leu Ile Glu Gly Phe Ser
            580                 585                 590
Gln Leu Leu Pro Val Ile Gln Pro Thr Glu Phe Ala Leu Lys Glu Glu
        595                 600                 605
Gln Thr Asn Ile Leu Tyr Glu Trp Ile Glu Lys Ala Phe Met Pro Ile
    610                 615                 620
Leu Val Arg Gly Glu Leu Met Asp Met Thr Arg Gly Arg Ser Ile Ser
625                 630                 635                 640
Arg Ala Thr Gly Glu Ser His Val Gln Ala Met Glu Ile Leu Arg Ser
                645                 650                 655
Leu Val Arg Ile Ala Glu Ser Ala Gln Pro Glu Gln Lys Thr Lys Leu
            660                 665                 670
Leu Ser Phe Val Lys Ala Gln Leu Thr Ser Asp Thr Phe Tyr Asp Ser
        675                 680                 685
Tyr Arg Ser Leu Lys Ser Tyr Lys Asp Ile Asp Leu Val Asn Lys Leu
    690                 695                 700
Leu Ala Asp Asn Gln Ile Pro Ala Glu Val Asp Lys Asp Tyr Ile Ala
705                 710                 715                 720
Ala Phe Asn Asn Met Asp Lys Phe Val Tyr Arg Ser Ala Gln Glu Gly
                725                 730                 735
Phe Thr Phe Ala Leu Ser Met Tyr Ser Ser Arg Thr Gln Asn Tyr Glu
            740                 745                 750
Asp Met Asn Asn Glu Asn Arg Lys Gly Trp Tyr Thr Ala Asp Gly Met
        755                 760                 765
Val Tyr Leu Tyr Asn Asp Asp Leu Ser His Tyr Ser Asn His Tyr Trp
    770                 775                 780
Ala Thr Val Asp Pro Tyr Arg Leu Pro Gly Thr Thr Thr Thr Lys Asp
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785 790 795 800
Lys Arg Glu Asp Gly Ser Gly Glu Val Thr Leu Ala Ser Asp Phe Val
805 810 815
Gly Ala Ser Gln Leu Gly Asn Arg Leu Ala Thr Ile Ala Met Asp Phe
820 825 830
Asn Asn Trp Asn Asn Ser Leu Thr Ala Arg Lys Ala Trp Ile Val Leu
835 840 845
Gly Asn Lys Ile Val Phe Leu Gly Thr Asp Ile Gln His Gln Ser Ala
850 855 860
Gln Gly Ala Glu Thr Thr Ile Glu Asn Arg Lys Leu Leu Thr Gly Glu
865 870 875 880
Lys Tyr Ser Tyr Tyr Ile Asn Gly Gln Pro Val Asp Leu Ser Lys Glu
885 890 895
Val Val Thr Asp Lys Thr Gln Ser Phe Tyr Met Thr Asn Gly Lys Asp
900 905 910
Asn Gln Ser Ile Gly Tyr Val Phe Leu Asn Gln Leu Pro Thr His Ala
915 920 925
Lys Leu Asp Gln Arg Thr Gly Lys Trp Ser Asp Ile Asn Tyr Asn Gln
930 935 940
Ser Lys Glu Glu Val Ser Asn Ser Phe Val Ser Leu Trp His Glu His
945 950 955 960
Ala Gln Thr Ser Ser Asn Tyr Ala Tyr Val Leu Val Pro Asn Gln Ser
965 970 975
Met Glu Lys Val Asn Gln Ala Ala Ala Ser Val Lys Leu Leu His Gln
980 985 990
Asp Arg Asp Leu Gln Val Val Tyr Asp Gln Glu Gln Asn Val Trp Gly
995 1000 1005
Val Val Lys Tyr Thr Asp Thr Ala Tyr Lys Leu Thr Asp Asp Ile Thr
1010 1015 1020
Leu Thr Asp Ala Gly Leu Tyr Thr Ile Gln Lys Val Glu Gly Gly Tyr
1025 1030 1035 1040
Arg Ile Ala Phe Tyr Asn Pro Ser Thr Arg Thr Val Lys Asn Gly Ile
1045 1050 1055
Glu Leu Thr Lys Ala Gly Ser Ser Leu Thr Val Glu Met Glu Pro Thr
1060 1065 1070
Ala Ala Tyr Pro Ser Thr Val Trp Lys Val Thr Met Pro Glu Gly Ser
1075 1080 1085
Asp Lys Gln Thr Gly Ser Val Glu Lys Thr Glu Lys Glu Glu Lys Gln
1090 1095 1100
Leu Lys Glu Asn Gln Pro Ser Ser Glu Val Lys Gln Val Val His His
1105 1110 1115 1120
Ala Ala Glu Lys Thr Lys Pro Ser Lys Pro Arg Leu Pro Gln Thr Gly
1125 1130 1135
Glu Glu Ala Ser Leu Gly Leu Gly Phe Leu Gly Leu Leu Thr Leu Gly
1140 1145 1150
Ala Val Val Asp Phe Lys Cys Arg Arg Ser His Ser
1155 1160

<210> 95
<211> 1164
<212> PRT
<213> Streptococcus suis

<220>
<223> hyaluronan lyase

<400> 95
Met Gly Phe Phe Ile Ser Gln Ser Lys Gln His Tyr Gly Ile Arg Lys
1 5 10 15
Tyr Lys Val Gly Val Cys Ser Ala Leu Ile Ala Leu Ser Ile Leu Gly
20 25 30
Thr Arg Val Ala Ala Asn Gln Leu Pro Ser Thr Glu Thr Ala Ser Pro
35 40 45
Gln Ser Ser Gln Leu Val Glu Thr Thr Pro Glu Thr Thr Glu Ala Val
50 55 60
Met Thr Ser Glu Val Ser Ser Glu Val Ser Pro Val Thr Ser Thr Glu
65 70 75 80

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Thr Gln Pro Ser Ser Thr Thr Ala Glu Thr Leu Ala Ser Pro Gln Ala
                85                  90                  95
Val Gln Ala Thr Lys Glu Glu Lys Asn Leu Val Ala Asn Gly Glu Phe
            100                 105                 110
Ile Ser Thr Thr Ala Pro Ser Gly Asn Trp Lys Glu Leu Ala Ala Thr
        115                 120                 125
Asn Trp Glu Thr Trp Ile Pro Ala Asn Val Lys Lys Glu Asn Gly Gln
    130                 135                 140
Val Arg Ile Asp Glu Gly Arg Leu His Ile Ser Ser Thr Ala Ser Tyr
145                 150                 155                 160
Arg Val Ala Val His Gln Thr Val Asp Val Asp Pro Asn Lys Arg Tyr
                165                 170                 175
Leu Phe Ser Tyr Asp Val Glu Thr Lys Asp Leu Lys Gly Ser Gly Val
            180                 185                 190
Arg Val Arg Leu Arg Ser Leu Thr Ala Glu Gly Lys Asp Leu Ser Pro
        195                 200                 205
Gln Glu Phe Ala Tyr Thr Pro Tyr Lys Asn Gly Ser Gln Ala Glu His
    210                 215                 220
Ile Glu Gln Ile Leu Thr Val Ser Pro Glu Thr Arg Lys Leu Lys Val
225                 230                 235                 240
Glu Leu Phe Phe Glu Asn Ser Val Gly Gln Ala Trp Leu Asp Asn Ile
                245                 250                 255
Ser Leu Val Glu Tyr Val Glu Lys Thr Pro Glu Thr Pro Glu Gln Ser
            260                 265                 270
Pro Glu Leu Val Gln Pro Glu Thr Gly Gln Ile Ser Leu Ala Ser Asn
        275                 280                 285
Lys Val Tyr Leu Pro Val Arg Pro Asp Leu Thr Tyr Arg Ile Ala Asp
    290                 295                 300
Ala Ala Val Ala Thr Val Glu Lys Asn Met Ile Arg Pro Leu Ala Ala
305                 310                 315                 320
Gly Lys Thr Gln Val Asp Val Tyr Asp Lys Asp Thr Lys Leu Ser Ser
                325                 330                 335
Phe Glu Leu Ile Val Thr Glu His Gln Ala Thr Val Phe Asp Thr Leu
            340                 345                 350
Arg Asn Asn Trp Glu Asp Ile Ser Leu Ala Asn Lys Arg Tyr Gln Ser
        355                 360                 365
Asn Asp Ala Gln Met Lys Ala Phe Leu Gly Arg Leu Asp Ala Gly Val
    370                 375                 380
Ala Ser Ser Leu Glu Lys Trp Val Glu Pro Thr Glu Gln Ser Lys Thr
385                 390                 395                 400
Ile Phe Asn Asp Ile Asp Phe Ser Lys Ser Ser His Leu Thr Thr Val
                405                 410                 415
Tyr Arg Arg Leu Glu Gln Met Ala Gln Val Val Glu Asn Pro Asp Ser
            420                 425                 430
Ala Tyr Tyr His Asp Arg Ser Leu Ile Asp Leu Val Arg Lys Gly Met
        435                 440                 445
Asn Trp Leu Tyr Thr Asn Val Tyr Asn Glu Asn Lys Ser Ile Asp Gly
    450                 455                 460
Asn Trp Trp Asp Tyr Glu Ile Gly Thr Pro Arg Ala Val Val Asn Thr
465                 470                 475                 480
Leu Ile Tyr Met His Pro Tyr Phe Ser Gln Glu Glu Ile Leu Thr Tyr
                485                 490                 495
Thr Lys Pro Ile Ser Lys Phe Val Pro Asp Pro Thr Thr Ile Arg Lys
            500                 505                 510
Thr Leu Thr Asn Pro Val Pro Ala Val Gly Gly Asn Gln Thr Asp Leu
        515                 520                 525
Ser Lys Val Ala Ile Leu Glu Gly Ala Leu Arg Glu Asp Ala Asp Arg
    530                 535                 540
Val Arg Ala Gly Ala Gln Gly Leu Thr Thr Ile Met Lys Phe Val Asp
545                 550                 555                 560
Lys Gly Glu Gly Phe Tyr Arg Asp Gly Ser Phe Ile Asp His Thr Asn
                565                 570                 575
Val Ala Tyr Thr Gly Ala Tyr Gly Asn Val Leu Ile Glu Gly Phe Ser
            580                 585                 590
Gln Leu Leu Pro Val Ile Gln Pro Thr Glu Phe Ala Leu Lys Glu Glu
        595                 600                 605
Gln Thr Asn Ile Leu Tyr Glu Trp Ile Glu Lys Ala Phe Met Pro Ile
    610                 615                 620
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Leu Val Arg Gly Glu Leu Met Asp Met Thr Arg Gly Arg Ser Ile Ser
625                 630                 635                 640
Arg Ala Thr Gly Glu Ser His Val Gln Ala Met Glu Ile Leu Arg Ser
                645                 650                 655
Leu Val Arg Ile Ala Glu Ser Ala Gln Pro Glu Gln Lys Thr Lys Leu
            660                 665                 670
Leu Ser Phe Val Lys Ala Gln Leu Thr Ser Asp Thr Phe Tyr Asp Ser
        675                 680                 685
Tyr Arg Ser Leu Lys Ser Tyr Lys Asp Ile Asp Leu Val Asn Lys Leu
    690                 695                 700
Leu Ala Asp Asn Gln Ile Pro Ala Glu Val Asp Lys Asp Tyr Ile Ala
705                 710                 715                 720
Ala Phe Asn Asn Met Asp Lys Phe Val Tyr Arg Ser Ala Gln Glu Gly
                725                 730                 735
Phe Thr Phe Ala Leu Ser Met Tyr Ser Ser Arg Thr Gln Asn Tyr Glu
            740                 745                 750
Asp Met Asn Asn Glu Asn Arg Lys Gly Trp Tyr Thr Ala Asp Gly Met
        755                 760                 765
Val Tyr Leu Tyr Asn Asp Asp Leu Ser His Tyr Ser Asn His Tyr Trp
    770                 775                 780
Ala Thr Val Asp Pro Tyr Arg Leu Pro Gly Thr Thr Thr Thr Lys Asp
785                 790                 795                 800
Lys Arg Glu Asp Gly Ser Gly Glu Val Thr Leu Ala Ser Asp Phe Val
                805                 810                 815
Gly Ala Ser Gln Leu Gly Asn Arg Leu Ala Thr Ile Ala Met Asp Phe
            820                 825                 830
Asn Asn Trp Asn Asn Ser Leu Thr Ala Arg Lys Ala Trp Ile Val Leu
        835                 840                 845
Gly Asn Lys Ile Val Phe Leu Gly Thr Asp Ile Gln His Gln Ser Ala
    850                 855                 860
Gln Gly Ala Glu Thr Thr Ile Glu Asn Arg Lys Leu Leu Thr Gly Glu
865                 870                 875                 880
Lys Tyr Ser Tyr Tyr Ile Asn Gly Gln Pro Val Asp Leu Ser Lys Glu
                885                 890                 895
Val Val Thr Asp Lys Thr Gln Ser Phe Tyr Met Thr Asn Gly Lys Asp
            900                 905                 910
Asn Gln Ser Ile Gly Tyr Val Phe Leu Asn Gln Leu Pro Thr His Ala
        915                 920                 925
Lys Leu Asp Gln Arg Thr Gly Lys Trp Ser Asp Ile Asn Tyr Asn Gln
    930                 935                 940
Ser Lys Glu Glu Val Ser Asn Ser Phe Val Ser Leu Trp His Glu His
945                 950                 955                 960
Ala Gln Thr Ser Ser Asn Tyr Ala Tyr Val Leu Val Pro Asn Gln Ser
                965                 970                 975
Met Glu Lys Val Asn Gln Ala Ala Ala Ser Val Lys Leu Leu His Gln
            980                 985                 990
Asp Arg Asp Leu Gln Val Val Tyr Asp Gln Glu Gln Asn Val Trp Gly
        995                 1000                1005
Val Val Lys Tyr Thr Asp Thr Ala Tyr Lys Leu Thr Asp Asp Ile Thr
    1010                1015                1020
Leu Thr Asp Ala Gly Leu Tyr Thr Ile Gln Lys Val Glu Gly Gly Tyr
1025                1030                1035                1040
Arg Ile Ala Phe Tyr Asn Pro Ser Thr Arg Thr Val Lys Asn Gly Ile
                1045                1050                1055
Glu Leu Thr Lys Ala Gly Ser Ser Leu Thr Val Glu Met Glu Pro Thr
            1060                1065                1070
Ala Ala Tyr Pro Ser Thr Val Trp Lys Val Thr Met Pro Glu Gly Ser
        1075                1080                1085
Asp Lys Gln Thr Gly Ser Val Glu Lys Thr Glu Lys Glu Glu Lys Gln
    1090                1095                1100
Leu Lys Glu Asn Gln Pro Ser Ser Glu Val Lys Gln Val Val His His
1105                1110                1115                1120
Ala Ala Glu Lys Thr Lys Pro Ser Lys Pro Arg Leu Pro Gln Thr Gly
                1125                1130                1135
Glu Glu Ala Ser Leu Gly Leu Gly Phe Leu Gly Leu Leu Thr Leu Gly
            1140                1145                1150
Ala Val Val Asp Phe Lys Cys Arg Arg Ser His Ser
        1155                1160
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<210> 96
<211> 802
<212> PRT
<213> Vibrio fischeri (strain ATCC 700601 / ES114)

<220>
<223> hyaluronan lyase

<400> 96
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Met Tyr Met Ile Lys Lys His Arg Leu Asn Thr Ile Ala Leu Ser Met
 1               5                  10                  15
Leu Phe Leu Phe Thr Gly Asn Ala Tyr Ala Ala Lys Asn Thr Gln Thr
            20                  25                  30
Pro Gln Tyr Leu Pro Ser Asp Phe Glu Gln Val Arg Glu Asn Trp Ala
        35                  40                  45
Glu Asn Tyr Leu Gly Asp Pro Ala Ile Thr Phe Asp Gln Thr Leu Lys
    50                  55                  60
Asn Met Val Thr Ser Thr Asn Ser Ser Ala Gln Lys His Trp Asp Ser
65                  70                  75                  80
Met Thr Pro Gln Pro Asn Ala Ser Gly Ile Trp Asp Asp Leu Pro Leu
                85                  90                  95
Ile Asp Lys Asp Thr Thr Leu Gly Pro Asn Ile Arg Asn Ser Tyr Gln
            100                 105                 110
Arg Leu Phe Thr Met Ala Lys Ala Tyr Arg Leu Arg Asp Gly Asn Leu
        115                 120                 125
Glu Asn Asn Gln Leu Met Leu Asn Asp Ile Met Thr Ala Met Asn Tyr
    130                 135                 140
Ile Asn Gln Asn Phe Tyr Phe Val Asn Gln Leu Glu Tyr Gly Asn Trp
145                 150                 155                 160
Trp Gln Trp Glu Leu Ala Ile Pro Lys Asp Ile His Asn Ile Leu Val
                165                 170                 175
Leu Leu Phe Asp Asp Ile Lys Asp Asn Tyr Gln Thr Ile Ile Thr Asn
            180                 185                 190
His Leu Asn Ala Thr Arg Tyr Phe Thr Pro Asp Pro Thr His Leu Gly
        195                 200                 205
Val Ser Pro Gly Ala Ala Glu Ser Thr Asn Pro Asn Tyr Arg Glu Ser
    210                 215                 220
Thr Gly Gly Asn Arg Thr Asp Asn Ala Gln Val Val Leu Ile Arg Gly
225                 230                 235                 240
Met Leu Glu Asn Asn Ser Glu Glu Ile Ser Gln Ala Ile Ala Ala Leu
                245                 250                 255
Pro Ala Val Ile Glu Tyr Val Ser Glu Gly Asp Gly Tyr Tyr Thr Asp
            260                 265                 270
Gly Ser Phe Leu Gln His Ser Asp Ile Ala Tyr Asn Gly Thr Tyr Gly
        275                 280                 285
Asn Val Leu Leu Gly Gly Leu Gly Ile Gln Met Asn Ala Val Ala Gly
    290                 295                 300
Ser Pro Trp Ser Met Asp Asn Gln Thr Ile Ser Asn Val Tyr Asn Ile
305                 310                 315                 320
Ile Asn Gln Ser Tyr Glu Pro Leu Leu Tyr Lys Gly Ala Met Met Asp
                325                 330                 335
Met Val Asn Gly Arg Ser Ile Ser Arg Ser Ala Glu Gln Asn His Asp
            340                 345                 350
Val Gly Leu Asn Ile Val Asn Ser Met Leu Phe Tyr Thr Asn Gly Pro
        355                 360                 365
Asp Ser Asp Lys Asn Lys Gln Leu Ser Ser Leu Ile Lys Thr Gln Ile
    370                 375                 380
Thr Asp Asp Thr Tyr Gln Asn Phe Phe Asp Lys Ile Tyr Tyr Val Ser
385                 390                 395                 400
Thr Tyr Gln Ala Ala Gln His Ile Val Asn Asp Pro Thr Val Ser Leu
                405                 410                 415
Lys Asp Pro Leu Ile Gly Asn Phe Ser Tyr Pro Ser Met Asp Arg Ile
            420                 425                 430
Val His Arg Arg Thr Asp Trp Ala Phe Ala Leu Ala Met His Ser Tyr
        435                 440                 445
Arg Ile Gly Asn Tyr Glu Cys Met Asn Gly Glu Asn Leu Lys Gly Trp
```

```
    450                 455                 460
Phe Thr Gly Asp Gly Met Ile Tyr Leu Tyr Asn Asp Gln Leu Asp His
465                 470                 475                 480
Tyr Thr Gly Tyr Trp Pro Thr Val Asn Ala Ser Arg Met Pro Gly Thr
                485                 490                 495
Thr Val Asp Ser Gln Ile Met Ala Asp Cys Ser Gly Glu Arg Val Gly
            500                 505                 510
Gly Asn Val Asn Thr Asn Met Gln Trp Val Gly Ser Thr Ser Leu Asn
        515                 520                 525
Asn Tyr Gly Ile Ala Gly Met Gln Phe Tyr Asn Trp Ser Asp Thr Leu
    530                 535                 540
Ser Ala Tyr Lys Ser Trp Phe Met Phe Asp Asn Glu Val Val Met Leu
545                 550                 555                 560
Gly Ser Asn Ile Lys Asp Gln Ser Asn Ala Asn Asn Ile Thr Thr Ile
                565                 570                 575
Glu Asn Arg Lys Arg Leu Ala Glu Thr Lys Leu Phe Ile Asp Gly Thr
            580                 585                 590
Glu Gln Ala Ala Leu Pro Tyr Gln Gly Ala Pro Ala Thr Phe Ser Ile
        595                 600                 605
Arg Asn Lys Thr Leu Ala Asn Ser Asp Leu Ser Tyr Val Met Leu Thr
    610                 615                 620
Pro Lys Thr Ile Ser Ile Ser Gln Asn Asp Val Asp Gly Asn Trp Ser
625                 630                 635                 640
Asp Ile Gly Asn Ser Lys Gly Asp Val Ser Asp Ser Tyr Leu Gln Ala
                645                 650                 655
Thr Leu Thr Gln Val Asp Gln Ala Asp Tyr Gln Tyr Ala Leu Leu Pro
            660                 665                 670
Asn Gln Asn Asn Asp Thr Val Gln Asn Tyr Ala Gln His Pro Asp Val
        675                 680                 685
Thr Val Leu Arg Gln Asp Glu Gln Ala His Ala Val Gln Glu Asn Thr
    690                 695                 700
Leu Asn Ile Ile Ala Ala Asn Asn Trp Lys Asn Asn Pro Val Asn Ile
705                 710                 715                 720
Thr Asp Thr Ile Thr Leu Asn Ser Met Met Gly Phe Met Ile Lys Glu
                725                 730                 735
Glu Ser Ser Asn Thr Phe Thr Val Ala Val Ser Glu Pro Ile Gln Thr
            740                 745                 750
Ile Asp Ser Val Asn Phe Thr Phe Asp Lys Gln Gly Ile Val Ile Lys
        755                 760                 765
Glu Asp Ile Glu Asn Arg Val Val Leu Asn Gly Thr Thr Leu Thr Ile
    770                 775                 780
Asn Thr Ser Gly Leu Gln Gly Gln Ser Tyr Ser Phe Gln Val Thr Ile
785                 790                 795                 800
Gln Asp
```

<210> 97
<211> 517
<212> PRT
<213> Synechococcus sp. (strain RCC307)

<220>
<223> hyaluronan lyase

<400> 97

```
Met Gly Ala Pro Ala Ile Pro Lys Val Pro Arg Ser Pro Ser Lys Ser
 1               5                  10                  15
Glu His Thr Thr Arg Pro Asn Ser Gly Glu Ser Glu Val Val Arg Gln
            20                  25                  30
Ala Glu Glu Leu Phe Glu Gln Thr Leu Val Asn Val Arg Gly Gln Leu
        35                  40                  45
Ala Gly Ala Val Ala Ala Leu Glu Ser Ser Val His Asp Ser Glu Leu
    50                  55                  60
Asn Tyr Gly Glu Ile Phe Leu Arg Asp Asn Val Pro Val Met Val Tyr
65                  70                  75                  80
Leu Leu Leu Arg Gly Arg Phe Glu Ile Val Arg His Phe Leu Asp Leu
                85                  90                  95
```

```
Cys Leu Glu Leu Gln Ser Arg Ser Tyr Arg Thr Arg Gly Val Phe Pro
            100                 105                 110
Thr Ser Phe Val Glu Glu Asp Asp Lys Ile Leu Ala Asp Tyr Gly Gln
        115                 120                 125
Arg Ser Ile Gly Arg Ile Thr Ser Val Asp Ala Ser Leu Trp Trp Pro
    130                 135                 140
Val Leu Cys Trp Met Tyr Val Arg Ala Ser Gly Asp Thr Ser Tyr Gly
145                 150                 155                 160
Thr Ser Pro Lys Val Gln Arg Ala Val Gln Leu Leu Leu Asp Leu Val
                165                 170                 175
Leu Gln Pro Ser Phe Tyr Glu Pro Pro Val Leu Phe Val Pro Asp Cys
            180                 185                 190
Ala Phe Met Ile Asp Arg Pro Met Asp Val Trp Gly Ala Pro Leu Glu
        195                 200                 205
Val Glu Val Leu Leu Phe Gly Cys Leu Lys Ser Cys Cys Gln Leu Met
    210                 215                 220
Ser Leu Val Glu Gly Gly Gly His Gly Gly Pro Leu Ile Gln Gln Arg
225                 230                 235                 240
Leu Glu Leu Thr Arg Thr Trp Met Arg Asp Leu Arg Val Tyr Leu Leu
                245                 250                 255
Asn His Tyr Trp Val Thr Ser Lys Thr Met Gln Val Leu Arg Arg Arg
            260                 265                 270
Pro Thr Glu Gln Tyr Gly Asp Tyr Gln Ser Arg Asn Glu Phe Asn Val
        275                 280                 285
Gln Pro Glu Val Ile Pro His Trp Leu Gln Glu Trp Leu Asp Asp Arg
    290                 295                 300
Gly Gly Tyr Leu Ile Gly Asn Met Arg Thr Gly Arg Pro Asp Phe Arg
305                 310                 315                 320
Phe Tyr Ser Leu Gly Asn Ala Leu Gly Ser Leu Phe Gly Leu Leu Thr
                325                 330                 335
Gly Pro Gln Gln Leu Ala Leu Phe Arg Leu Val Ile His Asn Arg Gln
            340                 345                 350
His Leu Met Ala Glu Met Pro Met Arg Ile Cys His Pro Pro Met Asp
        355                 360                 365
Gln Asp Glu Trp Ile Thr Asn Thr Gly Met Asp Pro Lys Asn Trp Pro
    370                 375                 380
Trp Ser Tyr His Asn Gly Gly His Trp Pro Ser Leu Leu Trp Pro Met
385                 390                 395                 400
Ala Ala Ala Val Leu Met His Gln Arg Leu Tyr Pro Asn Asp Asp Leu
                405                 410                 415
Leu Leu Leu Gly Gln Thr Arg Thr Met Leu Glu Glu Cys Tyr Trp Gln
            420                 425                 430
Gln Leu Asn Gln Leu Pro Arg Gln Gln Trp Ala Glu Tyr Phe Asp Gly
        435                 440                 445
Pro Thr Gly Thr Trp Val Gly Gln Gln Ala Arg Ile Asn Gln Thr Trp
    450                 455                 460
Thr Ile Val Gly Phe Leu Leu Leu His His Leu Met Arg Lys Ala Pro
465                 470                 475                 480
Gln Asp Val Lys Leu Leu Asp Leu Asp Asp Val Gly Pro Leu Arg Leu
                485                 490                 495
Ser Phe Gln Asp Gln His Glu Lys Ser Ser Thr Asp Glu Arg Lys Ser
            500                 505                 510
Phe Leu Lys Asn Tyr
        515
```

<210> 98
<211> 1021
<212> PRT
<213> Proteus vulgaris

<220>
<223> chondroitin-sulfate-ABC endolyase

<400> 98

```
Met Pro Ile Phe Arg Phe Thr Ala Leu Ala Met Thr Leu Gly Leu Leu
 1               5                  10                  15
Ser Ala Pro Tyr Asn Ala Met Ala Ala Thr Ser Asn Pro Ala Phe Asp
```

```
            20                  25                  30
Pro Lys Asn Leu Met Gln Ser Glu Ile Tyr His Phe Ala Gln Asn Asn
        35                  40                  45
Pro Leu Ala Asp Phe Ser Ser Asp Lys Asn Ser Ile Leu Thr Leu Ser
    50                  55                  60
Asp Lys Arg Ser Ile Met Gly Asn Gln Ser Leu Leu Trp Lys Trp Lys
65                  70                  75                  80
Gly Gly Ser Ser Phe Thr Leu His Lys Lys Leu Ile Val Pro Thr Asp
                85                  90                  95
Lys Glu Ala Ser Lys Ala Trp Gly Arg Ser Ser Thr Pro Val Phe Ser
            100                 105                 110
Phe Trp Leu Tyr Asn Glu Lys Pro Ile Asp Gly Tyr Leu Thr Ile Asp
        115                 120                 125
Phe Gly Glu Lys Leu Ile Ser Thr Ser Glu Ala Gln Ala Gly Phe Lys
    130                 135                 140
Val Lys Leu Asp Phe Thr Gly Trp Arg Ala Val Gly Val Ser Leu Asn
145                 150                 155                 160
Asn Asp Leu Glu Asn Arg Glu Met Thr Leu Asn Ala Thr Asn Thr Ser
                165                 170                 175
Ser Asp Gly Thr Gln Asp Ser Ile Gly Arg Ser Leu Gly Ala Lys Val
            180                 185                 190
Asp Ser Ile Arg Phe Lys Ala Pro Ser Asn Val Ser Gln Gly Glu Ile
        195                 200                 205
Tyr Ile Asp Arg Ile Met Phe Ser Val Asp Asp Ala Arg Tyr Gln Trp
    210                 215                 220
Ser Asp Tyr Gln Val Lys Thr Arg Leu Ser Glu Pro Glu Ile Gln Phe
225                 230                 235                 240
His Asn Val Lys Pro Gln Leu Pro Val Thr Pro Glu Asn Leu Ala Ala
                245                 250                 255
Ile Asp Leu Ile Arg Gln Arg Leu Ile Asn Glu Phe Val Gly Gly Glu
            260                 265                 270
Lys Glu Thr Asn Leu Ala Leu Glu Glu Asn Ile Ser Lys Leu Lys Ser
        275                 280                 285
Asp Phe Asp Ala Leu Asn Ile His Thr Leu Ala Asn Gly Gly Thr Gln
    290                 295                 300
Gly Arg His Leu Ile Thr Asp Lys Gln Ile Ile Ile Tyr Gln Pro Glu
305                 310                 315                 320
Asn Leu Asn Ser Gln Asp Lys Gln Leu Phe Asp Asn Tyr Val Ile Leu
                325                 330                 335
Gly Asn Tyr Thr Thr Leu Met Phe Asn Ile Ser Arg Ala Tyr Val Leu
            340                 345                 350
Glu Lys Asp Pro Thr Gln Lys Ala Gln Leu Lys Gln Met Tyr Leu Leu
        355                 360                 365
Met Thr Lys His Leu Leu Asp Gln Gly Phe Val Lys Gly Ser Ala Leu
    370                 375                 380
Val Thr Thr His His Trp Gly Tyr Ser Ser Arg Trp Trp Tyr Ile Ser
385                 390                 395                 400
Thr Leu Leu Met Ser Asp Ala Leu Lys Glu Ala Asn Leu Gln Thr Gln
                405                 410                 415
Val Tyr Asp Ser Leu Leu Trp Tyr Ser Arg Glu Phe Lys Ser Ser Phe
            420                 425                 430
Asp Met Lys Val Ser Ala Asp Ser Ser Asp Leu Asp Tyr Phe Asn Thr
        435                 440                 445
Leu Ser Arg Gln His Leu Ala Leu Leu Leu Leu Glu Pro Asp Asp Gln
    450                 455                 460
Lys Arg Ile Asn Leu Val Asn Thr Phe Ser His Tyr Ile Thr Gly Ala
465                 470                 475                 480
Leu Thr Gln Val Pro Pro Gly Gly Lys Asp Gly Leu Arg Pro Asp Gly
                485                 490                 495
Thr Ala Trp Arg His Glu Gly Asn Tyr Pro Gly Tyr Ser Phe Pro Ala
            500                 505                 510
Phe Lys Asn Ala Ser Gln Leu Ile Tyr Leu Leu Arg Asp Thr Pro Phe
        515                 520                 525
Ser Val Gly Glu Ser Gly Trp Asn Asn Leu Lys Lys Ala Met Val Ser
    530                 535                 540
Ala Trp Ile Tyr Ser Asn Pro Glu Val Gly Leu Pro Leu Ala Gly Arg
545                 550                 555                 560
His Pro Phe Asn Ser Pro Ser Leu Lys Ser Val Ala Gln Gly Tyr Tyr
```

565 570 575
Trp Leu Ala Met Ser Ala Lys Ser Ser Pro Asp Lys Thr Leu Ala Ser
580 585 590
Ile Tyr Leu Ala Ile Ser Asp Lys Thr Gln Asn Glu Ser Thr Ala Ile
595 600 605
Phe Gly Glu Thr Ile Thr Pro Ala Ser Leu Pro Gln Gly Phe Tyr Ala
610 615 620
Phe Asn Gly Gly Ala Phe Gly Ile His Arg Trp Gln Asp Lys Met Val
625 630 635 640
Thr Leu Lys Ala Tyr Asn Thr Asn Val Trp Ser Ser Glu Ile Tyr Asn
645 650 655
Lys Asp Asn Arg Tyr Gly Arg Tyr Gln Ser His Gly Val Ala Gln Ile
660 665 670
Val Ser Asn Gly Ser Gln Leu Ser Gln Gly Tyr Gln Gln Glu Gly Trp
675 680 685
Asp Trp Asn Arg Met Glu Gly Ala Thr Thr Ile His Leu Pro Leu Lys
690 695 700
Asp Leu Asp Ser Pro Lys Pro His Thr Leu Met Gln Arg Gly Glu Arg
705 710 715 720
Gly Phe Ser Gly Thr Ser Ser Leu Glu Gly Gln Tyr Gly Met Met Ala
725 730 735
Phe Asn Leu Ile Tyr Pro Ala Asn Leu Glu Arg Phe Asp Pro Asn Phe
740 745 750
Thr Ala Lys Lys Ser Val Leu Ala Ala Asp Asn His Leu Ile Phe Ile
755 760 765
Gly Ser Asn Ile Asn Ser Ser Asp Lys Asn Lys Asn Val Glu Thr Thr
770 775 780
Leu Phe Gln His Ala Ile Thr Pro Thr Leu Asn Thr Leu Trp Ile Asn
785 790 795 800
Gly Gln Lys Ile Glu Asn Met Pro Tyr Gln Thr Thr Leu Gln Gln Gly
805 810 815
Asp Trp Leu Ile Asp Ser Asn Gly Asn Gly Tyr Leu Ile Thr Gln Ala
820 825 830
Glu Lys Val Asn Val Ser Arg Gln His Gln Val Ser Ala Glu Asn Lys
835 840 845
Asn Arg Gln Pro Thr Glu Gly Asn Phe Ser Ser Ala Trp Ile Asp His
850 855 860
Ser Thr Arg Pro Lys Asp Ala Ser Tyr Glu Tyr Met Val Phe Leu Asp
865 870 875 880
Ala Thr Pro Glu Lys Met Gly Glu Met Ala Gln Lys Phe Arg Glu Asn
885 890 895
Asn Gly Leu Tyr Gln Val Leu Arg Lys Asp Lys Asp Val His Ile Ile
900 905 910
Leu Asp Lys Leu Ser Asn Val Thr Gly Tyr Ala Phe Tyr Gln Pro Ala
915 920 925
Ser Ile Glu Asp Lys Trp Ile Lys Lys Val Asn Lys Pro Ala Ile Val
930 935 940
Met Thr His Arg Gln Lys Asp Thr Leu Ile Val Ser Ala Val Thr Pro
945 950 955 960
Asp Leu Asn Met Thr Arg Gln Lys Ala Ala Thr Pro Val Thr Ile Asn
965 970 975
Val Thr Ile Asn Gly Lys Trp Gln Ser Ala Asp Lys Asn Ser Glu Val
980 985 990
Lys Tyr Gln Val Ser Gly Asp Asn Thr Glu Leu Thr Phe Thr Ser Tyr
995 1000 1005
Phe Gly Ile Pro Gln Glu Ile Lys Leu Ser Pro Leu Pro
1010 1015 1020

<210> 99
<211> 700
<212> PRT
<213> Pedobacter heparinus

<220>
<223> chondroitin AC lyase

<400> 99

```
Met Lys Lys Leu Phe Val Thr Cys Ile Val Phe Phe Ser Ile Leu Ser
 1               5                  10                  15
Pro Ala Leu Leu Ile Ala Gln Gln Thr Gly Thr Ala Glu Leu Ile Met
            20                  25                  30
Lys Arg Val Met Leu Asp Leu Lys Lys Pro Leu Arg Asn Met Asp Lys
        35                  40                  45
Val Ala Glu Lys Asn Leu Asn Thr Leu Gln Pro Asp Gly Ser Trp Lys
    50                  55                  60
Asp Val Pro Tyr Lys Asp Asp Ala Met Thr Asn Trp Leu Pro Asn Asn
65                  70                  75                  80
His Leu Leu Gln Leu Glu Thr Ile Ile Gln Ala Tyr Ile Glu Lys Asp
                85                  90                  95
Ser His Tyr Tyr Gly Asp Asp Lys Val Phe Asp Gln Ile Ser Lys Ala
            100                 105                 110
Phe Lys Tyr Trp Tyr Asp Ser Asp Pro Lys Ser Arg Asn Trp Trp His
        115                 120                 125
Asn Glu Ile Ala Thr Pro Gln Ala Leu Gly Glu Met Leu Ile Leu Met
    130                 135                 140
Arg Tyr Gly Lys Lys Pro Leu Asp Glu Ala Leu Val His Lys Leu Thr
145                 150                 155                 160
Glu Arg Met Lys Arg Gly Glu Pro Glu Lys Lys Thr Gly Ala Asn Lys
                165                 170                 175
Thr Asp Ile Ala Leu His Tyr Phe Tyr Arg Ala Leu Leu Thr Ser Asp
            180                 185                 190
Glu Ala Leu Leu Ser Phe Ala Val Lys Glu Leu Phe Tyr Pro Val Gln
        195                 200                 205
Phe Val His Tyr Glu Glu Gly Leu Gln Tyr Asp Tyr Ser Tyr Leu Gln
    210                 215                 220
His Gly Pro Gln Leu Gln Ile Ser Ser Tyr Gly Ala Val Phe Ile Thr
225                 230                 235                 240
Gly Val Leu Lys Leu Ala Asn Tyr Val Arg Asp Thr Pro Tyr Ala Leu
                245                 250                 255
Ser Thr Glu Lys Leu Ala Ile Phe Ser Lys Tyr Tyr Arg Asp Ser Tyr
            260                 265                 270
Leu Lys Ala Ile Arg Gly Ser Tyr Met Asp Phe Asn Val Glu Gly Arg
        275                 280                 285
Gly Val Ser Arg Pro Asp Ile Leu Asn Lys Lys Ala Glu Lys Lys Arg
    290                 295                 300
Leu Leu Val Ala Lys Met Ile Asp Leu Lys His Thr Glu Glu Trp Ala
305                 310                 315                 320
Asp Ala Ile Ala Arg Thr Asp Ser Thr Val Ala Ala Gly Tyr Lys Ile
                325                 330                 335
Glu Pro Tyr His His Gln Phe Trp Asn Gly Asp Tyr Val Gln His Leu
            340                 345                 350
Arg Pro Ala Tyr Ser Phe Asn Val Arg Met Val Ser Lys Arg Thr Arg
        355                 360                 365
Arg Ser Glu Ser Gly Asn Lys Glu Asn Leu Leu Gly Arg Tyr Leu Ser
    370                 375                 380
Asp Gly Ala Thr Asn Ile Gln Leu Arg Gly Pro Glu Tyr Tyr Asn Ile
385                 390                 395                 400
Met Pro Val Trp Glu Trp Asp Lys Ile Pro Gly Ile Thr Ser Arg Asp
                405                 410                 415
Tyr Leu Thr Asp Arg Pro Leu Thr Lys Leu Trp Gly Glu Gln Gly Ser
            420                 425                 430
Asn Asp Phe Ala Gly Gly Val Ser Asp Gly Val Tyr Gly Ala Ser Ala
        435                 440                 445
Tyr Ala Leu Asp Tyr Asp Ser Leu Gln Ala Lys Lys Ala Trp Phe Phe
    450                 455                 460
Phe Asp Lys Glu Ile Val Cys Leu Gly Ala Gly Ile Asn Ser Asn Ala
465                 470                 475                 480
Pro Glu Asn Ile Thr Thr Thr Leu Asn Gln Ser Trp Leu Asn Gly Pro
                485                 490                 495
Val Ile Ser Thr Ala Gly Lys Thr Gly Arg Gly Lys Ile Thr Thr Phe
            500                 505                 510
Lys Ala Gln Gly Gln Phe Trp Leu Leu His Asp Ala Ile Gly Tyr Tyr
        515                 520                 525
Phe Pro Glu Gly Ala Asn Leu Ser Leu Ser Thr Gln Ser Gln Lys Gly
    530                 535                 540
```

```
Asn Trp Phe His Ile Asn Asn Ser His Ser Lys Asp Glu Val Ser Gly
545                 550                 555                 560
Asp Val Phe Lys Leu Trp Ile Asn His Gly Ala Arg Pro Glu Asn Ala
                565                 570                 575
Gln Tyr Ala Tyr Ile Val Leu Pro Gly Ile Asn Lys Pro Glu Glu Ile
            580                 585                 590
Lys Lys Tyr Asn Gly Thr Ala Pro Lys Val Leu Ala Asn Thr Asn Gln
        595                 600                 605
Leu Gln Ala Val Tyr His Gln Gln Leu Asp Met Val Gln Ala Ile Phe
    610                 615                 620
Tyr Thr Ala Gly Lys Leu Ser Val Ala Gly Ile Glu Ile Glu Thr Asp
625                 630                 635                 640
Lys Pro Cys Ala Val Leu Ile Lys His Ile Asn Gly Lys Gln Val Ile
                645                 650                 655
Trp Ala Ala Asp Pro Leu Gln Lys Glu Lys Thr Ala Val Leu Ser Ile
            660                 665                 670
Arg Asp Leu Lys Thr Gly Lys Thr Asn Arg Val Lys Ile Asp Phe Pro
        675                 680                 685
Gln Gln Glu Phe Ala Gly Ala Thr Val Glu Leu Lys
    690                 695                 700


<210> 100
<211> 844
<212> PRT
<213> Victivallis vadensis ATCC BAA-548

<220>
<223> chondroitin AC lyase

<400> 100
Met His Arg Lys Ile Val Phe Pro Leu Phe Val Leu Leu Phe Thr Ala
 1               5                  10                  15
Phe Ala Ala Phe Ala Ala Ser Gln Pro Gln Trp Lys Trp Arg Gln Ser
            20                  25                  30
Lys Tyr Cys Arg Ile Thr Glu His Ala Gly Lys Lys Leu Leu Thr Val
        35                  40                  45
Ala Ile Pro Ala Asp Ala Pro Glu Ile Ser Arg Arg Asn Thr Ala Asp
    50                  55                  60
Thr Pro Val Gly Leu Thr Cys Phe Glu Gly Gln Pro Ile Glu Phe Ser
65                  70                  75                  80
Ile Arg Leu Arg Asn Ser Gly Ile Ser Arg Pro Asp Asn Leu His Asn
                85                  90                  95
Gly Ile Lys Phe Gln Leu Tyr Phe Arg Asp Gly Asn Gly Thr Glu Arg
            100                 105                 110
Trp Thr Gln Ala Glu Ile Pro His Glu Pro Phe Pro Trp Arg Arg Phe
        115                 120                 125
Arg Phe Ser Glu Arg Ile Pro Ala Asp Ala Thr Ser Gly Thr Leu Arg
    130                 135                 140
Leu Gly Leu Gln Gly Ser Ser Gly Asn Val Glu Phe Asp Leu Asp Ser
145                 150                 155                 160
Leu Gln Ile Arg Pro Leu Asp Pro Glu Leu Ser Pro Val Pro Pro Thr
                165                 170                 175
Pro Glu Gln Ile Arg Glu Cys Glu Leu Ile Glu Ser Arg Leu Arg Ala
            180                 185                 190
Gly Leu Pro Ala Gln Gln Pro Pro Pro Gly Pro Glu Val Lys Lys Leu
        195                 200                 205
Leu Glu Arg Gln Asn Gly Asp Gly Thr Phe Arg Asp Leu Asp Tyr Ser
    210                 215                 220
Ala Arg Asn Arg Ser Ile Trp Pro Ala Ala Arg His Leu Lys Arg Thr
225                 230                 235                 240
Phe Glu Leu Ala Leu Ala Arg Ala Thr Pro Gly His Ala Leu Tyr Arg
                245                 250                 255
Asp Pro Ala Ala Gly Glu Ala Val Arg Lys Ala Val Ala Trp Trp Ala
            260                 265                 270
Glu Arg Gln Pro Gln Asn Gly Asn Trp Trp Trp Asn Asp Met Tyr Val
        275                 280                 285
Pro Lys Ile Leu Gly Asn Ile Leu Leu Leu Ser Pro Glu Leu Phe Pro
```

```
    290                 295                 300
Asp Gly Pro Arg Arg Arg Ala Ala Leu Asn Val Cys Arg Gln Ala Cys
305                 310                 315                 320
Phe Leu Ser Arg Tyr Thr Gly Asn Asn Arg Val Phe Ile Ala Ala Asn
                325                 330                 335
Ile Phe Cys Arg Ala Leu Leu Glu Arg Asn Leu Pro Val Met Asp Arg
            340                 345                 350
Ala Ala Ala Val Leu Ser Glu Glu Ile Arg Phe Ala Pro Ala Glu His
        355                 360                 365
Lys Thr Ala Trp Ser Phe Gly Gly Ile Arg Ala Asp Gly Cys Tyr His
    370                 375                 380
Gln His Gly Pro Gln Ile Gln Phe Gly Asn Tyr Gly Gly Glu Phe Leu
385                 390                 395                 400
Ala Asn Ile Ala Tyr Trp Ser Asn Ile Leu Lys Glu Thr His Trp Glu
                405                 410                 415
Leu Ser Pro Glu Gln Trp Arg Ile Met Arg His Leu Thr Phe Asn Gly
            420                 425                 430
Phe Gln Trp Val Leu Trp Arg Gly Arg Met Asp Leu Leu Ala Cys Gly
        435                 440                 445
Arg Gln Leu Gly Arg Asn Ala Ala Glu Thr Lys Gly Glu Arg Thr Leu
    450                 455                 460
Asn Ala Phe Ala Gln Leu Arg Asn Ala Asp Pro Gly Asp Arg Ala Pro
465                 470                 475                 480
Tyr Asp Ala Ala Leu Arg Arg Asn Arg Asp Gly Glu Asn Thr Leu Thr
                485                 490                 495
Gly Asn Arg His Phe Trp Asn Ser Asp Tyr Met Val His Arg Arg Pro
            500                 505                 510
Thr Trp Tyr Ala Ser Val Arg Met Asn Ser Val Arg Val Arg Pro Ile
        515                 520                 525
Glu Asp Asp Thr Asn Trp Asp Asn Ala Leu Gly Arg Tyr Phe Ser Asp
    530                 535                 540
Gly Ala Cys Leu Val Met Arg Ser Gly Arg Glu Tyr Glu Asn Ile Thr
545                 550                 555                 560
Ala Cys Trp Asp Trp Thr Arg Leu Pro Gly Thr Thr Leu Pro Lys Thr
                565                 570                 575
Pro Val Tyr Thr Gly Glu Asp Ala Arg Arg Phe Gly Leu Lys Ile Gly
            580                 585                 590
Gly Gly Asp Leu Pro Arg Trp Thr His Ser Arg Asn Trp Arg Gln Leu
        595                 600                 605
Gly Glu Thr Gly Phe Val Gly Gly Val Thr Asp Gly Glu Arg Gly Ala
    610                 615                 620
Ala Val Tyr Thr Gln Asp Leu Asp Gly Val Arg Ala Arg Lys Ala Trp
625                 630                 635                 640
Phe Phe Asp Arg Asp Ala Ile Tyr Cys Leu Gly Ser Gly Ile Thr Ser
                645                 650                 655
Thr Ser Pro Tyr Glu Val Ala Thr Thr Val Asn Ser Cys Leu Arg Asn
            660                 665                 670
Gly Glu Ile Gln Gln Gly Asp Gly Trp Phe Arg His Asp Gly Ile Gly
        675                 680                 685
Tyr Arg Gly Glu Asn Leu Lys Leu Thr Ala Gly Pro Arg Thr Gly Asp
    690                 695                 700
Trp Arg Tyr Val Glu Gly Gly Leu Thr Arg Pro Val Pro Glu Thr Lys
705                 710                 715                 720
Glu Leu Phe Thr Leu Thr Val Glu His Gly Val Lys Pro Arg Asn Ala
                725                 730                 735
Ser Tyr Ile Tyr Thr Ile Leu Pro Gly Ala Thr Pro Glu Glu Thr Ala
            740                 745                 750
Gly Thr Pro Pro Gly Arg Val Leu Arg Asn Thr Pro Glu Cys Gln Ala
        755                 760                 765
Val Glu Phe Ala Asp Gly Val Arg Ala Ala Ile Phe Tyr Glu Pro Gly
    770                 775                 780
Arg Leu Asp Asp Phe Glu Thr Asp Thr Pro Gly Val Phe Leu Ile Gly
785                 790                 795                 800
Lys Gly Thr Val His Ala Ala Asp Pro Thr Gly Arg His Ser Ser Phe
                805                 810                 815
Thr Leu Lys Leu Asn Gly Val Ser Arg Lys Val Pro Leu Pro Ala Gly
            820                 825                 830
Glu Phe Ala Gly Gln Ser Val Lys Ile Val Leu Lys
```

835 840

<210> 101
<211> 110
<212> PRT
<213> Homo sapiens

<220>
<223> Preproinsulin

<220>
<221> SIGNAL
<222> (1)...(24)
<223> Signal sequence

<220>
<221> CHAIN
<222> (25)...(54)
<223> Insulin B Chain

<220>
<221> PROPEP
<222> (57)...(87)
<223> Insulin C peptide

<220>
<221> CHAIN
<222> (90)...(110)
<223> Insulin A chain

<400> 101
Met Ala Leu Trp Met Arg Leu Leu Pro Leu Leu Ala Leu Leu Ala Leu
1 5 10 15
Trp Gly Pro Asp Pro Ala Ala Ala Phe Val Asn Gln His Leu Cys Gly
20 25 30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
35 40 45
Phe Tyr Thr Pro Lys Thr Arg Arg Glu Ala Glu Asp Leu Gln Val Gly
50 55 60
Gln Val Glu Leu Gly Gly Gly Pro Gly Ala Gly Ser Leu Gln Pro Leu
65 70 75 80
Ala Leu Glu Gly Ser Leu Gln Lys Arg Gly Ile Val Glu Gln Cys Cys
85 90 95
Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
100 105 110

<210> 102
<211> 86
<212> PRT
<213> Homo sapiens

<220>
<223> Proinsulin

<220>
<221> CHAIN
<222> (66)...(86)
<223> Insulin B Chain

<220>
<221> PROPEP
<222> (33)...(63)
<223> Insulin C peptide

<220>
<221> CHAIN
<222> (1)...(30)

<223> Insulin A chain

<400> 102
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
 1   5   10   15
Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr Arg Arg
            20   25   30
Glu Ala Glu Asp Leu Gln Val Gly Gln Val Glu Leu Gly Gly Gly Pro
        35   40   45
Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser Leu Gln Lys
    50   55   60
Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln
65   70   75   80
Leu Glu Asn Tyr Cys Asn
                85

<210> 103
<211> 21
<212> PRT
<213> Homo sapiens

<220>
<223> Insulin A chain

<400> 103
Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu
 1   5   10   15
Glu Asn Tyr Cys Asn
            20

<210> 104
<211> 30
<212> PRT
<213> Homo sapiens

<220>
<223> Insulin B chain

<400> 104
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
 1   5   10   15
Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr
            20   25   30

<210> 105
<211> 110
<212> PRT
<213> Gorilla gorilla

<220>
<223> Preproinsulin

<220>
<221> CHAIN
<222> (66)...(86)
<223> Insulin B Chain

<220>
<221> CHAIN
<222> (1)...(30)
<223> Insulin A chain

<400> 105
Met Ala Leu Trp Met Arg Leu Leu Pro Leu Leu Ala Leu Leu Ala Leu
 1   5   10   15

```
Trp Gly Pro Asp Pro Ala Ala Ala Phe Val Asn Gln His Leu Cys Gly
            20                  25                  30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
        35                  40                  45
Phe Tyr Thr Pro Lys Thr Arg Arg Glu Ala Glu Asp Leu Gln Val Gly
    50                  55                  60
Gln Val Glu Leu Gly Gly Gly Pro Gly Ala Gly Ser Leu Gln Pro Leu
65                  70                  75                  80
Ala Leu Glu Gly Ser Leu Gln Lys Arg Gly Ile Val Glu Gln Cys Cys
                85                  90                  95
Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
            100                 105                 110
```

<210> 106
<211> 110
<212> PRT
<213> Pongo pygmaeus

<220>
<223> Preproinsulin

<400> 106

```
Met Ala Leu Trp Met Arg Leu Leu Pro Leu Leu Ala Leu Leu Ala Leu
 1               5                  10                  15
Trp Gly Pro Asp Pro Ala Gln Ala Phe Val Asn Gln His Leu Cys Gly
            20                  25                  30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
        35                  40                  45
Phe Tyr Thr Pro Lys Thr Arg Arg Glu Ala Glu Asp Leu Gln Val Gly
    50                  55                  60
Gln Val Glu Leu Gly Gly Gly Pro Gly Ala Gly Ser Leu Gln Pro Leu
65                  70                  75                  80
Ala Leu Glu Gly Ser Leu Gln Lys Arg Gly Ile Val Glu Gln Cys Cys
                85                  90                  95
Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
            100                 105                 110
```

<210> 107
<211> 110
<212> PRT
<213> Pan troglodytes

<220>
<223> Preproinsulin

<400> 107

```
Met Ala Leu Trp Met Arg Leu Leu Pro Leu Leu Val Leu Leu Ala Leu
 1               5                  10                  15
Trp Gly Pro Asp Pro Ala Ser Ala Phe Val Asn Gln His Leu Cys Gly
            20                  25                  30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
        35                  40                  45
Phe Tyr Thr Pro Lys Thr Arg Arg Glu Ala Glu Asp Leu Gln Val Gly
    50                  55                  60
Gln Val Glu Leu Gly Gly Gly Pro Gly Ala Gly Ser Leu Gln Pro Leu
65                  70                  75                  80
Ala Leu Glu Gly Ser Leu Gln Lys Arg Gly Ile Val Glu Gln Cys Cys
                85                  90                  95
Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
            100                 105                 110
```

<210> 108
<211> 110
<212> PRT
<213> Macaca fascicularis

<220>
<223> Preproinsulin

<400> 108
Met Ala Leu Trp Met Arg Leu Leu Pro Leu Leu Ala Leu Leu Ala Leu
 1   5   10   15
Trp Gly Pro Asp Pro Ala Pro Ala Phe Val Asn Gln His Leu Cys Gly
            20   25   30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
        35   40   45
Phe Tyr Thr Pro Lys Thr Arg Arg Glu Ala Glu Asp Pro Gln Val Gly
    50   55   60
Gln Val Glu Leu Gly Gly Gly Pro Gly Ala Gly Ser Leu Gln Pro Leu
65   70   75   80
Ala Leu Glu Gly Ser Leu Gln Lys Arg Gly Ile Val Glu Gln Cys Cys
                85   90   95
Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
            100   105   110

<210> 109
<211> 110
<212> PRT
<213> Cercopithecus aethiops

<220>
<223> Preproinsulin

<400> 109
Met Ala Leu Trp Met Arg Leu Leu Pro Leu Leu Ala Leu Leu Ala Leu
 1   5   10   15
Trp Gly Pro Asp Pro Val Pro Ala Phe Val Asn Gln His Leu Cys Gly
            20   25   30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
        35   40   45
Phe Tyr Thr Pro Lys Thr Arg Arg Glu Ala Glu Asp Pro Gln Val Gly
    50   55   60
Gln Val Glu Leu Gly Gly Gly Pro Gly Ala Gly Ser Leu Gln Pro Leu
65   70   75   80
Ala Leu Glu Gly Ser Leu Gln Lys Arg Gly Ile Val Glu Gln Cys Cys
                85   90   95
Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
            100   105   110

<210> 110
<211> 110
<212> PRT
<213> Canis familiaris

<220>
<223> Preproinsulin

<400> 110
Met Ala Leu Trp Met Arg Leu Leu Pro Leu Leu Ala Leu Leu Ala Leu
 1   5   10   15
Trp Ala Pro Ala Pro Thr Arg Ala Phe Val Asn Gln His Leu Cys Gly
            20   25   30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
        35   40   45
Phe Tyr Thr Pro Lys Ala Arg Arg Glu Val Glu Asp Leu Gln Val Arg
    50   55   60
Asp Val Glu Leu Ala Gly Ala Pro Gly Glu Gly Gly Leu Gln Pro Leu
65   70   75   80
Ala Leu Glu Gly Ala Leu Gln Lys Arg Gly Ile Val Glu Gln Cys Cys
                85   90   95
Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn

100 105 110

<210> 111
<211> 110
<212> PRT
<213> Spermophilus tridecemlineatus

<220>
<223> Preproinsulin

<400> 111
Met Ala Leu Trp Thr Arg Leu Leu Pro Leu Leu Ala Leu Leu Ala Leu
1 5 10 15
Leu Gly Pro Asp Pro Ala Gln Ala Phe Val Asn Gln His Leu Cys Gly
20 25 30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
35 40 45
Phe Tyr Thr Pro Lys Ser Arg Arg Glu Val Glu Glu Gln Gln Gly Gly
50 55 60
Gln Val Glu Leu Gly Gly Gly Pro Gly Ala Gly Leu Pro Gln Pro Leu
65 70 75 80
Ala Leu Glu Met Ala Leu Gln Lys Arg Gly Ile Val Glu Gln Cys Cys
85 90 95
Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
100 105 110

<210> 112
<211> 110
<212> PRT
<213> Volemys kikuchii

<220>
<223> Preproinsulin

<400> 112
Met Ala Leu Trp Met Arg Leu Leu Pro Leu Leu Ala Leu Leu Val Leu
1 5 10 15
Trp Glu Pro Asn Pro Ala Gln Ala Phe Val Asn Gln His Leu Cys Gly
20 25 30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
35 40 45
Phe Tyr Thr Pro Lys Ser Arg Arg Gly Val Glu Asp Pro Gln Val Ala
50 55 60
Gln Leu Glu Leu Gly Gly Gly Pro Gly Ala Gly Asp Leu Gln Thr Leu
65 70 75 80
Ala Leu Glu Val Ala Gln Gln Lys Arg Gly Ile Val Asp Gln Cys Cys
85 90 95
Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
100 105 110

<210> 113
<211> 108
<212> PRT
<213> Aotus trivirgatus

<220>
<223> Preproinsulin

<400> 113
Met Ala Leu Trp Met His Leu Leu Pro Leu Leu Ala Leu Leu Ala Leu
1 5 10 15
Trp Gly Pro Glu Pro Ala Pro Ala Phe Val Asn Gln His Leu Cys Gly
20 25 30
Pro His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
35 40 45

Phe Tyr Ala Pro Lys Thr Arg Arg Glu Ala Glu Asp Leu Gln Val Gly
50 55 60
Gln Val Glu Leu Gly Gly Gly Ser Ile Thr Gly Ser Leu Pro Pro Leu
65 70 75 80
Glu Gly Pro Met Gln Lys Arg Gly Val Val Asp Gln Cys Cys Thr Ser
85 90 95
Ile Cys Ser Leu Tyr Gln Leu Gln Asn Tyr Cys Asn
100 105

<210> 114
<211> 110
<212> PRT
<213> Apodemus semotus

<220>
<223> Preproinsulin 1

<400> 114
Met Ala Leu Cys Val Arg Phe Leu Ser Leu Leu Ile Leu Leu Ile Leu
1 5 10 15
Trp Glu Pro Asn Pro Ala Gln Ala Phe Val Lys Gln His Leu Cys Gly
20 25 30
Pro His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
35 40 45
Phe Tyr Thr Pro Lys Ser Arg Arg Glu Val Glu Asp Pro Gln Val Glu
50 55 60
Gln Leu Glu Leu Gly Gly Ala Pro Gly Thr Gly Asp Leu Glu Thr Leu
65 70 75 80
Ala Leu Glu Val Ala Arg Gln Lys Arg Gly Ile Val Asp Gln Cys Cys
85 90 95
Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
100 105 110

<210> 115
<211> 110
<212> PRT
<213> Apodemus semotus

<220>
<223> Preproinsulin 2

<400> 115
Met Ala Leu Trp Met Arg Phe Leu Pro Leu Leu Val Leu Leu Phe Leu
1 5 10 15
Trp Glu Pro Asn Pro Ala Gln Ala Phe Val Lys Gln His Leu Cys Gly
20 25 30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
35 40 45
Phe Tyr Thr Pro Met Ser Arg Arg Glu Val Glu Asp Pro Gln Val Ala
50 55 60
Gln Leu Glu Leu Gly Gly Gly Pro Gly Ala Gly Asp Leu Gln Thr Leu
65 70 75 80
Ala Leu Glu Val Ala Arg Gln Lys Arg Gly Ile Val Asp Gln Cys Cys
85 90 95
Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
100 105 110

<210> 116
<211> 110
<212> PRT
<213> Cricetulus longicaudatus

<220>
<223> Preproinsulin

<400> 116
Met Thr Leu Trp Met Arg Leu Leu Pro Leu Leu Thr Leu Leu Val Leu
 1   5   10   15
Trp Glu Pro Asn Pro Ala Gln Ala Phe Val Asn Gln His Leu Cys Gly
            20   25   30

Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
        35   40   45
Phe Tyr Thr Pro Lys Ser Arg Arg Gly Val Glu Asp Pro Gln Val Ala
    50   55   60
Gln Leu Glu Leu Gly Gly Gly Pro Gly Ala Asp Asp Leu Gln Thr Leu
65   70   75   80
Ala Leu Glu Val Ala Gln Gln Lys Arg Gly Ile Val Asp Gln Cys Cys
                85   90   95
Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
            100   105   110

<210> 117
<211> 110
<212> PRT
<213> Rattus norvegicus

<220>
<223> Preproinsulin 1

<400> 117
Met Ala Leu Trp Met Arg Phe Leu Pro Leu Leu Ala Leu Leu Val Leu
 1   5   10   15
Trp Glu Pro Lys Pro Ala Gln Ala Phe Val Lys Gln His Leu Cys Gly
            20   25   30
Pro His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
        35   40   45
Phe Tyr Thr Pro Lys Ser Arg Arg Glu Val Glu Asp Pro Gln Val Pro
    50   55   60
Gln Leu Glu Leu Gly Gly Gly Pro Glu Ala Gly Asp Leu Gln Thr Leu
65   70   75   80
Ala Leu Glu Val Ala Arg Gln Lys Arg Gly Ile Val Asp Gln Cys Cys
                85   90   95
Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
            100   105   110

<210> 118
<211> 110
<212> PRT
<213> Rattus norvegicus

<220>
<223> Preproinsulin 2

<400> 118
Met Ala Leu Trp Ile Arg Phe Leu Pro Leu Leu Ala Leu Leu Ile Leu
 1   5   10   15
Trp Glu Pro Arg Pro Ala Gln Ala Phe Val Lys Gln His Leu Cys Gly
            20   25   30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
        35   40   45
Phe Tyr Thr Pro Met Ser Arg Arg Glu Val Glu Asp Pro Gln Val Ala
    50   55   60
Gln Leu Glu Leu Gly Gly Gly Pro Gly Ala Gly Asp Leu Gln Thr Leu
65   70   75   80
Ala Leu Glu Val Ala Arg Gln Lys Arg Gly Ile Val Asp Gln Cys Cys
                85   90   95
Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
            100   105   110

<210> 119
<211> 110
<212> PRT
<213> Rattus losea

<220>
<223> Preproinsulin 1

<400> 119
Met Ala Leu Trp Met Arg Phe Leu Pro Leu Leu Ala Leu Leu Val Val
 1   5   10   15
Trp Glu Pro Lys Pro Ala Gln Ala Phe Val Lys Gln His Leu Cys Gly
            20   25   30
Pro His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
        35   40   45
Phe Tyr Thr Pro Lys Ser Arg Arg Glu Val Glu Asp Pro Gln Val Pro
    50   55   60
Gln Leu Glu Leu Gly Gly Ser Pro Glu Ala Gly Asp Leu Gln Thr Leu
65   70   75   80
Ala Leu Glu Val Ala Arg Gln Lys Arg Gly Ile Val Asp Gln Cys Cys
                85   90   95
Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
            100   105   110

<210> 120
<211> 110
<212> PRT
<213> Rattus losea

<220>
<223> Preproinsulin 2

<400> 120
Met Ala Leu Trp Ile Arg Phe Leu Pro Leu Leu Ala Leu Leu Val Leu
 1   5   10   15
Trp Glu Pro Arg Pro Ala Gln Ala Phe Val Lys Gln His Leu Cys Gly
            20   25   30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
        35   40   45
Phe Tyr Thr Pro Val Ser Arg Arg Glu Val Glu Asp Pro Gln Val Ala
    50   55   60
Gln Gln Glu Leu Gly Gly Gly Pro Gly Ala Gly Asp Leu Gln Thr Leu
65   70   75   80
Ala Leu Glu Val Ala Arg Gln Lys Arg Gly Ile Val Asp Gln Cys Cys
                85   90   95
Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
            100   105   110

<210> 121
<211> 110
<212> PRT
<213> Niviventer coxingi

<220>
<223> Preproinsulin 1

<400> 121
Met Ala Leu Trp Met Arg Phe Leu Pro Leu Leu Ala Leu Leu Val Leu
 1   5   10   15
Trp Glu Pro Asn Pro Ala Gln Ala Phe Val Lys Gln His Leu Cys Gly
            20   25   30
Pro His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
        35   40   45
Phe Tyr Thr Pro Lys Ser Arg Arg Glu Val Glu Asp Pro Gln Val Ala
    50   55   60
Gln Leu Glu Leu Gly Glu Gly Pro Glu Ala Gly Asp Leu Gln Thr Leu

65 70 75 80
Ala Leu Glu Val Ala Arg Gln Lys Arg Gly Ile Val Asp Gln Cys Cys
85 90 95
Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
100 105 110

<210> 122
<211> 110
<212> PRT
<213> Niviventer coxingi

<220>
<223> Preproinsulin 2

<400> 122
Met Ala Leu Trp Ile Arg Phe Leu Pro Leu Leu Ala Leu Leu Val Leu
1 5 10 15
Trp Glu Pro His Pro Ala Gln Ala Phe Val Lys Gln His Leu Cys Gly
20 25 30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
35 40 45
Phe Tyr Thr Pro Met Ser Arg Arg Glu Val Glu Asp Pro Gln Val Pro
50 55 60
Gln Leu Glu Leu Gly Gly Gly Pro Gly Thr Gly Asp Leu Gln Thr Leu
65 70 75 80
Ala Leu Glu Val Ala Arg Gln Lys Arg Gly Ile Val Asp Gln Cys Cys
85 90 95
Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
100 105 110

<210> 123
<211> 108
<212> PRT
<213> Sus scrofa

<220>
<223> Preproinsulin

<400> 123
Met Ala Leu Trp Thr Arg Leu Leu Pro Leu Leu Ala Leu Leu Ala Leu
1 5 10 15
Trp Ala Pro Ala Pro Ala Gln Ala Phe Val Asn Gln His Leu Cys Gly
20 25 30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
35 40 45
Phe Tyr Thr Pro Lys Ala Arg Arg Glu Ala Glu Asn Pro Gln Ala Gly
50 55 60
Ala Val Glu Leu Gly Gly Gly Leu Gly Gly Leu Gln Ala Leu Ala Leu
65 70 75 80
Glu Gly Pro Pro Gln Lys Arg Gly Ile Val Glu Gln Cys Cys Thr Ser
85 90 95
Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
100 105

<210> 124
<211> 110
<212> PRT
<213> Meriones unguiculatus

<220>
<223> Preproinsulin

<400> 124
Met Ala Leu Trp Met Arg Leu Leu Pro Leu Leu Ala Phe Leu Ile Leu
1 5 10 15

Trp Glu Pro Thr Pro Ala His Ala Phe Val Asn Gln His Leu Cys Gly
20 25 30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
35 40 45
Phe Tyr Thr Pro Lys Phe Arg Arg Gly Val Glu Asp Pro Gln Met Pro
50 55 60
Gln Leu Glu Leu Gly Gly Ser Pro Gly Ala Gly Asp Leu Gln Ala Leu
65 70 75 80
Ala Leu Glu Val Ala Arg Gln Lys Arg Gly Ile Val Glu Gln Cys Cys
85 90 95
Thr Gly Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
100 105 110

<210> 125
<211> 110
<212> PRT
<213> Oryctolagus cuniculus

<220>
<223> Preproinsulin

<400> 125
Met Ala Ser Leu Ala Ala Leu Leu Pro Leu Leu Ala Leu Leu Val Leu
1 5 10 15
Cys Arg Leu Asp Pro Ala Gln Ala Phe Val Asn Gln His Leu Cys Gly
20 25 30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
35 40 45
Phe Tyr Thr Pro Lys Ser Arg Arg Glu Val Glu Glu Leu Gln Val Gly
50 55 60
Gln Ala Glu Leu Gly Gly Gly Pro Gly Ala Gly Gly Leu Gln Pro Ser
65 70 75 80
Ala Leu Glu Leu Ala Leu Gln Lys Arg Gly Ile Val Glu Gln Cys Cys
85 90 95
Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
100 105 110

<210> 126
<211> 108
<212> PRT

<213> Mus musculus

<220>
<223> Preproinsulin 1

<400> 126
Met Ala Leu Leu Val His Phe Leu Pro Leu Leu Ala Leu Leu Ala Leu
1 5 10 15
Trp Glu Pro Lys Pro Thr Gln Ala Phe Val Lys Gln His Leu Cys Gly
20 25 30
Pro His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
35 40 45
Phe Tyr Thr Pro Lys Ser Arg Arg Glu Val Glu Asp Pro Gln Val Glu
50 55 60
Gln Leu Glu Leu Gly Gly Ser Pro Gly Asp Leu Gln Thr Leu Ala Leu
65 70 75 80
Glu Val Ala Arg Gln Lys Arg Gly Ile Val Asp Gln Cys Cys Thr Ser
85 90 95
Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
100 105

<210> 127
<211> 110
<212> PRT

<213> Mus musculus

<220>
<223> Preproinsulin 2

<400> 127
Met Ala Leu Trp Met Arg Phe Leu Pro Leu Leu Ala Leu Leu Phe Leu
 1   5   10   15
Trp Glu Ser His Pro Thr Gln Ala Phe Val Lys Gln His Leu Cys Gly
            20   25   30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
        35   40   45
Phe Tyr Thr Pro Met Ser Arg Arg Glu Val Glu Asp Pro Gln Val Ala
    50   55   60
Gln Leu Glu Leu Gly Gly Gly Pro Gly Ala Gly Asp Leu Gln Thr Leu
65   70   75   80
Ala Leu Glu Val Ala Gln Gln Lys Arg Gly Ile Val Asp Gln Cys Cys
                85   90   95
Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
            100   105   110

<210> 128
<211> 110
<212> PRT
<213> Mus spretus

<220>
<223> Preproinsulin 2

<400> 128
Met Ala Leu Trp Met Arg Phe Leu Pro Leu Leu Ala Leu Leu Phe Leu
 1   5   10   15
Trp Glu Ser His Pro Thr Gln Ala Phe Val Lys Gln His Leu Cys Gly
            20   25   30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
        35   40   45
Phe Tyr Thr Pro Met Ser Arg Arg Glu Val Glu Asp Pro Gln Val Ala
    50   55   60
Gln Leu Glu Leu Gly Gly Gly Pro Gly Ala Gly Asp Leu Gln Thr Leu
65   70   75   80
Ala Leu Glu Val Ala Gln Gln Lys Arg Gly Ile Val Asp Gln Cys Cys
                85   90   95
Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
            100   105   110

<210> 129
<211> 108
<212> PRT
<213> Mus caroli

<220>
<223> Preproinsulin 1

<400> 129
Met Ala Leu Leu Val Arg Phe Leu Pro Leu Leu Ala Leu Leu Ala Leu
 1   5   10   15
Trp Glu Pro Lys Pro Thr Gln Ala Phe Val Lys Gln His Leu Cys Gly
            20   25   30
Pro His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
        35   40   45
Phe Tyr Ser Pro Lys Ser Arg Arg Glu Val Glu Asp Pro Gln Val Glu
    50   55   60
Gln Leu Glu Leu Gly Gly Ser Pro Gly Asp Leu Gln Thr Leu Ala Leu
65   70   75   80
Glu Val Ala Arg Glu Lys Arg Gly Ile Val Asp Gln Cys Cys Thr Ser
                85   90   95

Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
100 105

<210> 130
<211> 110
<212> PRT
<213> Mus caroli

<220>
<223> Preproinsulin 2

<400> 130
Met Ala Leu Trp Met Arg Phe Leu Pro Leu Val Ala Leu Leu Phe Leu
1 5 10 15
Trp Glu Ser His Pro Thr Gln Ala Phe Val Lys Gln His Leu Cys Gly
20 25 30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
35 40 45
Phe Tyr Thr Pro Met Ser Arg Arg Glu Val Glu Asp Pro Gln Val Ala
50 55 60
Gln Leu Glu Leu Gly Gly Gly Pro Gly Ala Gly Asp Leu Gln Thr Leu
65 70 75 80
Ala Leu Glu Val Ala Gln Gln Lys Arg Gly Ile Val Asp Gln Cys Cys
85 90 95
Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
100 105 110

<210> 131
<211> 110
<212> PRT
<213> Psammomys obesus

<220>
<223> Preproinsulin

<400> 131
Met Ala Leu Trp Met Arg Leu Leu Pro Leu Leu Ala Phe Leu Ile Leu
1 5 10 15
Trp Glu Pro Ser Pro Ala His Ala Phe Val Asn Gln His Leu Cys Gly
20 25 30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
35 40 45
Phe Tyr Thr Pro Lys Phe Arg Arg Gly Val Asp Asp Pro Gln Met Pro
50 55 60
Gln Leu Glu Leu Gly Gly Ser Pro Gly Ala Gly Asp Leu Arg Ala Leu
65 70 75 80
Ala Leu Glu Val Ala Arg Gln Lys Arg Gly Ile Val Glu Gln Cys Cys
85 90 95
Thr Gly Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
100 105 110

<210> 132
<211> 110
<212> PRT
<213> Felis silvestris catus

<220>
<223> Preproinsulin

<400> 132
Met Ala Pro Trp Thr Arg Leu Leu Pro Leu Leu Ala Leu Leu Ser Leu
1 5 10 15
Trp Ile Pro Ala Pro Thr Arg Ala Phe Val Asn Gln His Leu Cys Gly
20 25 30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe

```
        35                  40                  45
Phe Tyr Thr Pro Lys Ala Arg Arg Glu Ala Glu Asp Leu Gln Gly Lys
    50                  55                  60
Asp Ala Glu Leu Gly Glu Ala Pro Gly Ala Gly Gly Leu Gln Pro Ser
65                  70                  75                  80
Ala Leu Glu Ala Pro Leu Gln Lys Arg Gly Ile Val Glu Gln Cys Cys
                85                  90                  95
Ala Ser Val Cys Ser Leu Tyr Gln Leu Glu His Tyr Cys Asn
            100                 105                 110
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<210> 133
<211> 105
<212> PRT
<213> Bos taurus

<220>
<223> Preproinsulin

<400> 133

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Met Ala Leu Trp Thr Arg Leu Arg Pro Leu Leu Ala Leu Leu Ala Leu
 1               5                  10                  15
Trp Pro Pro Pro Pro Ala Arg Ala Phe Val Asn Gln His Leu Cys Gly
            20                  25                  30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
        35                  40                  45
Phe Tyr Thr Pro Lys Ala Arg Arg Glu Val Glu Gly Pro Gln Val Gly
    50                  55                  60
Ala Leu Glu Leu Ala Gly Gly Pro Gly Ala Gly Gly Leu Glu Gly Pro
65                  70                  75                  80
Pro Gln Lys Arg Gly Ile Val Glu Gln Cys Cys Ala Ser Val Cys Ser
                85                  90                  95
Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
            100                 105
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<210> 134
<211> 105
<212> PRT
<213> Ovis aries

<220>
<223> Preproinsulin

<400> 134

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Met Ala Leu Trp Thr Arg Leu Val Pro Leu Leu Ala Leu Leu Ala Leu
 1               5                  10                  15
Trp Ala Pro Ala Pro Ala His Ala Phe Val Asn Gln His Leu Cys Gly
            20                  25                  30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
        35                  40                  45
Phe Tyr Thr Pro Lys Ala Arg Arg Glu Val Glu Gly Pro Gln Val Gly
    50                  55                  60
Ala Leu Glu Leu Ala Gly Gly Pro Gly Ala Gly Gly Leu Glu Gly Pro
65                  70                  75                  80
Pro Gln Lys Arg Gly Ile Val Glu Gln Cys Cys Ala Gly Val Cys Ser
                85                  90                  95
Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
            100                 105
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<210> 135
<211> 110
<212> PRT
<213> Cavia porcellus

<220>
<223> Preproinsulin

<400> 135
Met Ala Leu Trp Met His Leu Leu Thr Val Leu Ala Leu Leu Ala Leu
 1   5   10   15
Trp Gly Pro Asn Thr Gly Gln Ala Phe Val Ser Arg His Leu Cys Gly
            20   25   30
Ser Asn Leu Val Glu Thr Leu Tyr Ser Val Cys Gln Asp Asp Gly Phe
        35   40   45
Phe Tyr Ile Pro Lys Asp Arg Arg Glu Leu Glu Asp Pro Gln Val Glu
    50   55   60
Gln Thr Glu Leu Gly Met Gly Leu Gly Ala Gly Gly Leu Gln Pro Leu
65   70   75   80
Ala Leu Glu Met Ala Leu Gln Lys Arg Gly Ile Val Asp Gln Cys Cys
                85   90   95
Thr Gly Thr Cys Thr Arg His Gln Leu Gln Ser Tyr Cys Asn
            100   105   110

<210> 136
<211> 86
<212> PRT
<213> Equus caballus

<220>
<223> Proinsulin

<220>
<221> UNSURE
<222> 31
<223> Xaa=Basic residue assumed by homology

<220>
<221> UNSURE
<222> 32
<223> Xaa=Basic residue assumed by homology

<220>
<221> UNSURE
<222> 64
<223> Xaa=Basic residue assumed by homology

<220>
<221> UNSURE
<222> 65
<223> Xaa=Basic residue assumed by homology

<400> 136
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
 1   5   10   15
Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Ala Xaa Xaa
            20   25   30
Glu Ala Glu Asp Pro Gln Val Gly Glu Val Glu Leu Gly Gly Gly Pro
        35   40   45
Gly Leu Gly Gly Leu Gln Pro Leu Ala Leu Ala Gly Pro Gln Gln Xaa
    50   55   60
Xaa Gly Ile Val Glu Gln Cys Cys Thr Gly Ile Cys Ser Leu Tyr Gln
65   70   75   80
Leu Glu Asn Tyr Cys Asn
                85

<210> 137
<211> 109
<212> PRT
<213> Octodon degus

<220>
<223> Preproinsulin

<400> 137
Met Ala Pro Trp Met His Leu Leu Thr Val Leu Ala Leu Leu Ala Leu
 1   5   10   15
Trp Gly Pro Asn Ser Val Gln Ala Tyr Ser Ser Gln His Leu Cys Gly
            20   25   30
Ser Asn Leu Val Glu Ala Leu Tyr Met Thr Cys Gly Arg Ser Gly Phe
        35   40   45
Tyr Arg Pro His Asp Arg Arg Glu Leu Glu Asp Leu Gln Val Glu Gln
    50   55   60
Ala Glu Leu Gly Leu Glu Ala Gly Gly Leu Gln Pro Ser Ala Leu Glu
65   70   75   80
Met Ile Leu Gln Lys Arg Gly Ile Val Asp Gln Cys Cys Asn Asn Ile
                85   90   95
Cys Thr Phe Asn Gln Leu Gln Asn Tyr Cys Asn Val Pro
            100   105

<210> 138
<211> 86
<212> PRT
<213> Chinchilla brevicaudata

<220>
<223> Proinsulin

<220>
<221> UNSURE
<222> 31
<223> Xaa=Basic residue assumed by homology

<220>
<221> UNSURE
<222> 32
<223> Xaa=Basic residue assumed by homology

<220>
<221> UNSURE
<222> 64
<223> Xaa=Basic residue assumed by homology

<220>
<221> UNSURE
<222> 65
<223> Xaa=Basic residue assumed by homology

<400> 138
Phe Val Asn Lys His Leu Cys Gly Ser His Leu Val Asp Ala Leu Tyr
 1   5   10   15
Leu Val Cys Gly Asp Arg Gly Phe Phe Tyr Thr Pro Met Ala Xaa Xaa
            20   25   30
Glu Leu Glu Asp Pro Gln Val Gly Gln Ala Asp Pro Gly Val Val Pro
        35   40   45
Glu Ala Gly Arg Leu Gln Pro Leu Ala Leu Glu Met Thr Leu Gln Xaa
    50   55   60
Xaa Gly Ile Val Asp Gln Cys Cys Thr Ser Ile Cys Thr Leu Tyr Gln
65   70   75   80
Leu Glu Asn Tyr Cys Asn
                85

<210> 139
<211> 107
<212> PRT
<213> Gallus gallus

<220>
<223> Preproinsulin

<400> 139
Met Ala Leu Trp Ile Arg Ser Leu Pro Leu Leu Ala Leu Leu Val Phe
 1   5   10   15
Ser Gly Pro Gly Thr Ser Tyr Ala Ala Ala Asn Gln His Leu Cys Gly
            20   25   30
Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe
        35   40   45
Phe Tyr Ser Pro Lys Ala Arg Arg Asp Val Glu Gln Pro Leu Val Ser
    50   55   60
Ser Pro Leu Arg Gly Glu Ala Gly Val Leu Pro Phe Gln Gln Glu Glu
65   70   75   80
Tyr Glu Lys Val Lys Arg Gly Ile Val Glu Gln Cys Cys His Asn Thr
                85   90   95
Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
            100   105

<210> 140
<211> 103
<212> PRT
<213> Selasphorus rufus

<220>
<223> Proinsulin

<400> 140
Ile Gln Ser Leu Pro Leu Leu Ala Leu Leu Ala Leu Ser Gly Pro Gly
 1   5   10   15
Thr Ser His Ala Ala Val Asn Gln His Leu Cys Gly Ser His Leu Val
            20   25   30
Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Ser Pro
        35   40   45
Lys Ala Arg Arg Asp Ala Glu His Pro Leu Val Asn Gly Pro Leu His
    50   55   60
Gly Glu Val Gly Asp Leu Pro Phe Gln Gln Glu Glu Phe Glu Lys Val
65   70   75   80
Lys Arg Gly Ile Val Glu Gln Cys Cys His Asn Thr Cys Ser Leu Tyr
                85   90   95
Gln Leu Glu Asn Tyr Cys Asn
            100

<210> 141
<211> 106
<212> PRT
<213> Xenopus laevis

<220>
<223> Preproinsulin 1

<400> 141
Met Ala Leu Trp Met Gln Cys Leu Pro Leu Val Leu Val Leu Phe Phe
 1   5   10   15
Ser Thr Pro Asn Thr Glu Ala Leu Val Asn Gln His Leu Cys Gly Ser
            20   25   30
His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Asp Arg Gly Phe Phe
        35   40   45
Tyr Tyr Pro Lys Val Lys Arg Asp Met Glu Gln Ala Leu Val Ser Gly
    50   55   60
Pro Gln Asp Asn Glu Leu Asp Gly Met Gln Leu Gln Pro Gln Glu Tyr
65   70   75   80
Gln Lys Met Lys Arg Gly Ile Val Glu Gln Cys Cys His Ser Thr Cys
                85   90   95
Ser Leu Phe Gln Leu Glu Ser Tyr Cys Asn
            100   105

<210> 142
<211> 106
<212> PRT
<213> Xenopus laevis

<220>
<223> Preproinsulin 2

<400> 142
Met Ala Leu Trp Met Gln Cys Leu Pro Leu Val Leu Val Leu Leu Phe
 1   5   10   15
Ser Thr Pro Asn Thr Glu Ala Leu Ala Asn Gln His Leu Cys Gly Ser
            20   25   30
His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Asp Arg Gly Phe Phe
        35   40   45
Tyr Tyr Pro Lys Ile Lys Arg Asp Ile Glu Gln Ala Gln Val Asn Gly
    50   55   60
Pro Gln Asp Asn Glu Leu Asp Gly Met Gln Phe Gln Pro Gln Glu Tyr
65   70   75   80
Gln Lys Met Lys Arg Gly Ile Val Glu Gln Cys Cys His Ser Thr Cys
                85   90   95
Ser Leu Phe Gln Leu Glu Asn Tyr Cys Asn
            100   105

<210> 143
<211> 106
<212> PRT
<213> Xenopus tropicalis

<220>
<223> Preproinsulin

<400> 143
Met Ala Leu Trp Met Gln Cys Leu Pro Leu Val Leu Val Leu Leu Phe
 1   5   10   15
Ser Thr Pro Asn Thr Glu Ala Leu Ala Asn Gln His Leu Cys Gly Ser
            20   25   30
His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Asp Arg Gly Phe Phe
        35   40   45
Tyr Tyr Pro Lys Ile Lys Arg Asp Ile Glu Gln Ala Met Val Asn Gly
    50   55   60
Pro Gln Asp Asn Glu Leu Asp Gly Met Gln Leu Gln Pro Gln Glu Tyr
65   70   75   80
Gln Lys Met Lys Arg Gly Ile Val Glu Gln Cys Cys His Ser Thr Cys
                85   90   95
Ser Leu Phe Gln Leu Glu Ser Tyr Cys Asn
            100   105

<210> 144
<211> 106
<212> PRT
<213> Rana pipiens

<220>
<223> Preproinsulin

<400> 144
Met Ala Leu Trp Ile Gln Cys Leu His Ile Ala Val Leu Leu Ser Leu
 1   5   10   15
Phe Thr Pro Thr Leu Lys Thr Phe Asp Asn Gln Tyr Leu Cys Gly Ser
            20   25   30
His Leu Val Glu Ala Leu Tyr Met Val Cys Gly Asp Arg Gly Phe Phe
        35   40   45
Tyr Ser Pro Arg Ser Arg Arg Asp Leu Glu Gln Pro Leu Val Asn Gly
    50   55   60

Leu Gln Gly Ser Glu Leu Asp Glu Met Gln Val Gln Ser Gln Ala Phe
65 70 75 80
Gln Lys Arg Lys Pro Gly Ile Val Glu Gln Cys Cys His Asn Thr Cys
85 90 95
Ser Leu Tyr Asp Leu Glu Asn Tyr Cys Asn
100 105

<210> 145
<211> 81
<212> PRT
<213> Anas platyrhynchos

<220>
<223> Preproinsulin

<220>
<221> UNSURE
<222> 31
<223> Xaa=Basic residue assumed by homology

<220>
<221> UNSURE
<222> 32
<223> Xaa=Basic residue assumed by homology

<220>
<221> UNSURE
<222> 59
<223> Xaa=Basic residue assumed by homology

<220>
<221> UNSURE
<222> 60
<223> Xaa=Basic residue assumed by homology

<400> 145
Ala Ala Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
1 5 10 15
Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Ser Pro Lys Thr Xaa Xaa
20 25 30
Asp Val Glu Gln Pro Leu Val Asn Gly Pro Leu His Gly Glu Val Gly
35 40 45
Glu Leu Pro Phe Gln His Glu Glu Tyr Gln Xaa Xaa Gly Ile Val Glu
50 55 60
Gln Cys Cys Glu Asn Pro Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys
65 70 75 80
Asn

<210> 146
<211> 51
<212> PRT
<213> Capra hircus

<220>
<223> Preproinsulin

<400> 146
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
1 5 10 15
Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Ala Gly Ile
20 25 30
Val Glu Gln Cys Cys Ala Gly Val Cys Ser Leu Tyr Gln Leu Glu Asn
35 40 45
Tyr Cys Asn
50

<210> 147
<211> 30
<212> PRT
<213> Homo sapiens

<220>
<223> Aspart insulin

<220>
<223> Insulin B chain analog

<400> 147
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
 1   5   10   15
Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Asp Lys Thr
            20   25   30

<210> 148
<211> 30
<212> PRT
<213> Homo sapiens

<220>
<223> Lispro insulin

<220>
<223> Insulin B chain analog

<400> 148
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
 1   5   10   15
Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Lys Pro Thr
            20   25   30

<210> 149
<211> 30
<212> PRT
<213> Homo sapiens

<220>
<223> Glulisine insulin

<220>
<223> Insulin B chain analog

<400> 149
Phe Val Lys Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
 1   5   10   15
Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Glu Thr
            20   25   30

<210> 150
<211> 21
<212> PRT
<213> Homo sapiens

<220>
<223> Glargine insulin

<220>
<223> Insulin A chain analog

<400> 150

Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu
 1   5   10   15
Glu Asn Tyr Cys Gly
            20

<210> 151
<211> 32
<212> PRT
<213> Homo sapiens

<220>
<223> Glargine insulin

<220>
<223> Insulin B chain analog

<400> 151
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
 1   5   10   15
Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr Arg Arg
            20   25   30

<210> 152
<211> 53
<212> PRT
<213> Homo sapiens

<220>
<223> Detemir insulin

<220>
<223> Proinsulin analog

<220>
<221> CHAIN
<222> (33)...(53)
<223> A Chain

<220>
<221> CHAIN
<222> (1)...(29)
<223> B chain

<400> 152
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
 1   5   10   15
Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Ala Ala Lys
            20   25   30
Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu
        35   40   45
Glu Asn Tyr Cys Asn
    50

<210> 153
<211> 30
<212> PRT
<213> Homo sapiens

<220>
<223> Insulin B chain analog

<220>
<221> VARIANT
<222> 28
<223> Xaa=Pro, Lys, Leu, Val or Ala

<220>
<221> VARIANT
<222> 29
<223> Xaa=Lys or Pro

<400> 153
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
 1   5   10   15
Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Xaa Xaa Thr
            20   25   30

<210> 154
<211> 27
<212> PRT
<213> Homo sapiens

<220>
<223> Insulin B chain analog (des 28-30)

<400> 154
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
 1   5   10   15
Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr
            20   25

<210> 155
<211> 29
<212> PRT
<213> Homo sapiens

<220>
<223> Insulin B chain analog (des 27)

<400> 155
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
 1   5   10   15
Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Pro Lys Thr
            20   25

<210> 156
<211> 21
<212> PRT
<213> Homo sapiens

<220>
<223> Insulin A chain analog

<220>
<221> VARIANT
<222> 21
<223> Xaa=Ala, Asn, Asp, Gln, Glu, Gly, Thr or Ser

<400> 156
Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu
 1   5   10   15
Glu Asn Tyr Cys Xaa
            20

<210> 157
<211> 31
<212> PRT
<213> Homo sapiens

<220>
<223> Insulin B chain analog

<220>
<221> VARIANT
<222> 1
<223> Xaa=Phe, Asp or none

<220>
<221> VARIANT
<222> 2
<223> Xaa=Val or none

<220>
<221> VARIANT
<222> 3
<223> Xaa=Asn or Asp

<220>
<221> VARIANT
<222> 9
<223> Xaa=Ser or Asp

<220>
<221> VARIANT
<222> 10
<223> Xaa=His or Asp

<220>
<221> VARIANT
<222> 28
<223> Xaa=any amino acid

<220>
<221> VARIANT
<222> 29
<223> Xaa=L-Pro, D-Pro, D-hydroxypro, L-hydroxypro, L-(N-methyllysine), D-Lysine, L-(N-methyl arginine) or D-arginine

<220>
<221> VARIANT
<222> 30
<223> Xaa=Ala or Thr

<220>
<221> VARIANT
<222> 31
<223> Xaa=Arg, Arg-Arg, Lys, Lys-Lys, Arg-Lys, Lys-Arg or none

<400> 157
Xaa Xaa Asn Gln His Leu Cys Gly Xaa Xaa Leu Val Glu Ala Leu Tyr
 1   5   10   15
Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Xaa Xaa Xaa Xaa
            20   25   30

<210> 158
<211> 21
<212> PRT
<213> Homo sapiens

<220>
<223> Insulin A chain analog

<220>
<221> VARIANT
<222> 8
<223> Xaa=His, Phe, Gly, Gln, Glu, Ser, Asn, Asp, or Pro

<220>
<221> VARIANT
<222> 9
<223> Xaa=Gly, Asp, Glu, Thr, His, Gln, Asn, Ala or Pro

<220>
<221> VARIANT
<222> 10
<223> Xaa=Leu, Pro, Val, His, Ala, Glu, Asp, Thr, Gln or Asn

<220>
<221> VARIANT
<222> 13
<223> Xaa=Pro, Val, Arg, His, Ala, Glu, Asp, Thr, Gly, Gln or Asn

<220>
<221> VARIANT
<222> 21
<223> Xaa=Asp or Glu

<400> 158
Gly Ile Val Glu Gln Cys Cys Xaa Xaa Xaa Cys Ser Xaa Tyr Gln Leu
 1   5   10   15
Glu Asn Tyr Cys Xaa
            20

<210> 159
<211> 30
<212> PRT
<213> Homo sapiens

<220>
<223> Insulin B chain analog

<220>
<221> VARIANT
<222> 1
<223> Xaa=Phe, Glu, Asp, Thr or Ser

<220>
<221> VARIANT
<222> 2
<223> Xaa=Val, Arg, His, Ala, Glu, Asp, Thr, Pro, Gly, Gln, Ser or Asn

<220>
<221> VARIANT
<222> 5
<223> Xaa=His, Ala, Glu, Aps, Thr, Ser, Gln, or Asn

<220>
<221> VARIANT
<222> 9
<223> Xaa=Ser, Leu, Asp, Pro, Glu, Ile, Val, His, Thr, Gln, Asn, Met, Tyr,
Trp or Phe

<220>
<221> VARIANT
<222> 10
<223> Xaa=His, Asn, Leu, Asp, Arg, Glu, Ser, Thr, Ile or Gln

<220>
<221> VARIANT
<222> 12
<223> Xaa=Val, Asn, His, Ile or Tyr

<220>
<221> VARIANT
<222> 14
<223> Xaa=Ala, Glu, Asp, Asn, Gln, Ser, Thr, Gly

<220>
<221> VARIANT
<222> 16
<223> Xaa=Tyr, Asp, Glu, Gln, Asn, Ser, Thr, His or Arg

<220>
<221> VARIANT
<222> 17
<223> Xaa=Leu, Ser, Thr, Asn, Gln, Glu, Asp or His

<220>
<221> VARIANT
<222> 18
<223> Xaa=Val, Ser, Thr, Asn, Gln or His

<220>
<221> VARIANT
<222> 20
<223> Xaa=Gly, Gln, Ser, Thr, Asn, Asp, Glu, His or Arg

<220>
<221> VARIANT
<222> 26
<223> Xaa=Tyr, Ala, Asp, His or Glu

<220>
<221> VARIANT
<222> 27
<223> Xaa=Thr, Asp, His or Glu

<220>
<221> VARIANT
<222> 28
<223> Xaa=Pro, Asp or Glu

<400> 159
Xaa Xaa Asn Gln Xaa Leu Cys Gly Xaa Xaa Leu Xaa Glu Xaa Leu Xaa
 1   5   10   15
Xaa Xaa Cys Xaa Glu Arg Gly Phe Phe Xaa Xaa Xaa Lys Thr
            20   25   30

<210> 160
<211> 21
<212> PRT
<213> Homo sapiens

<220>
<223> Insulin A chain analog

<220>
<221> VARIANT
<222> 21
<223> Xaa=Asn, Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Met, Ser, Thr,
Cys, Tyr, Asp or Glu

<400> 160
Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu
 1   5   10   15
Glu Asn Tyr Cys Xaa
            20

<210> 161
<211> 30
<212> PRT
<213> Homo sapiens

<220>
<223> Insulin B chain analog

<220>
<221> VARIANT
<222> 1
<223> Xaa=Phe or none

<220>
<221> VARIANT
<222> 29
<223> Xaa=Ly, Ala, Thr or Ser

<220>
<221> VARIANT
<222> 30
<223> Xaa=Thr, Arg, Lys, Hyl, Orn or Cit

<400> 161
Xaa Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
 1   5   10   15
Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Xaa Xaa
            20   25   30

<210> 162
<211> 21
<212> PRT
<213> Homo sapiens

<220>
<223> Insulin A chain analog

<220>
<221> VARIANT
<222> 21
<223> Xaa=Asn, Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Met, Ser, Thr,
Cys, Tyr, Asp, Glu, His or Gln

<400> 162
Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu
 1   5   10   15
Glu Asn Tyr Cys Xaa
            20

<210> 163
<211> 30
<212> PRT
<213> Homo sapiens

<220>
<223> Insulin B chain analog

<220>
<221> VARIANT
<222> 1
<223> Xaa=Phe or none

<220>
<221> VARIANT
<222> 3

<223> Xaa=Asn, Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Met, Ser, Thr, Cys, Tyr, Asp, Glu, His or Gln

<220>
<221> VARIANT
<222> 30
<223> Xaa=Asn, Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Met, Ser, Thr, Cys, Tyr, Asp, Glu, His or Gln

<400> 163
Xaa Val Xaa Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
 1   5   10   15
Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Xaa
            20   25   30

<210> 164
<211> 21
<212> PRT
<213> Homo sapiens

<220>
<223> Insulin A chain analog

<220>
<221> VARIANT
<222> 8
<223> Xaa=Ala or Thr

<220>
<221> VARIANT
<222> 10
<223> Xaa=Val or Ile

<220>
<221> VARIANT
<222> 21
<223> Xaa=Asn, Asp, Gly, Ser, Thr or Ala

<400> 164
Gly Ile Val Glu Gln Cys Cys Xaa Ser Xaa Cys Ser Leu Tyr Gln Leu
 1   5   10   15
Glu Asn Tyr Cys Xaa
            20

<210> 165
<211> 30
<212> PRT
<213> Homo sapiens

<220>
<223> Insulin B chain analog

<220>
<221> VARIANT
<222> 1
<223> Xaa=Phe or none

<220>
<221> VARIANT
<222> 1
<223> Xaa=Thr or none

<400> 165
Xaa Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
 1   5   10   15

Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Xaa
            20   25   30

<210> 166
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker Peptide Sequence

<400> 166
Arg Pro Pro Pro Pro Cys
 1   5

<210> 167
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker Peptide Sequence

<400> 167
Ser Ser Pro Pro Pro Pro Cys
 1   5

<210> 168
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker Peptide Sequence

<400> 168
Gly Gly Gly Gly Ser
 1   5

<210> 169
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker Peptide Sequence

<220>
<221> REPEAT
<222> (1)...(5)
<223> Repeating linker peptide

<400> 169
Gly Gly Gly Gly Ser
 1   5

<210> 170
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker Peptide Sequence

<400> 170
Gly Lys Ser Ser Gly Ser Gly Ser Glu Ser Lys Ser
 1   5   10

<210> 171
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker Peptide Sequence

<400> 171
Gly Ser Thr Ser Gly Ser Gly Lys Ser Ser Glu Gly Lys Gly
 1   5   10

<210> 172
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker Peptide Sequence

<400> 172
Gly Ser Thr Ser Gly Ser Gly Lys Ser Ser Glu Gly Ser Gly Ser Thr
 1   5   10   15
Lys Gly

<210> 173
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker Peptide Sequence

<400> 173
Gly Ser Thr Ser Gly Ser Gly Lys Ser Ser Glu Gly Lys Gly
 1   5   10

<210> 174
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker Peptide Sequence

<400> 174
Gly Ser Thr Ser Gly Ser Gly Lys Pro Gly Ser Gly Glu Gly Ser Thr
 1   5   10   15
Lys Gly

<210> 175
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker Peptide Sequence

<400> 175
Glu Gly Lys Ser Ser Gly Ser Gly Ser Glu Ser Lys Glu Phe
 1   5   10

<210> 176
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker Peptide Sequence

<400> 176
Ser Arg Ser Ser Gly
 1   5

<210> 177
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker Peptide Sequence

<400> 177
Ser Gly Ser Ser Cys
 1   5

<210> 178
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker Peptide Sequence

<400> 178
Ala Ala Lys
 1

<210> 179
<211> 2
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker Peptide Sequence

<400> 179
Val Met
 1

<210> 180
<211> 2
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker Peptide Sequence

<400> 180
Ala Met

<210> 181
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker Peptide Sequence

<220>
<221> REPEAT
<222> (3)...(5)
<223> 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 repeats

<220>
<221> VARIANT
<222> 4
<223> Xaa=Gly, Gly-Gly or Gly-Gly-Gly

<400> 181
Ala Met Gly Xaa Ser Ala Met
 1   5

210> 182
<211> 30
<212> PRT
<213> Homo sapiens

<220>
<223> HMR-1 153 B-chain

<400> 182
Phe Val Lys Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
 1   5   10   15
Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Ile Lys Thr
            20   25   30

<210> 183
<211> 21
<212> PRT
<213> Homo sapiens

<220>
<223> HMR-1 1423 A-chain

<400> 183
Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu
 1   5   10   15
Glu Asn Tyr Cys Gly
            20

<210> 184
<211> 32
<212> PRT
<213> Homo sapiens

<220>
<223> HMR-1 1423 B-chain

<400> 184
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
 1   5   10   15
Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr His His

20 25 30

<210> 185
<211> 510
<212> PRT
<213> Pan troglodytes

<220>
<223> Chimpanzee PH20

<400> 185
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
1 5 10 15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
20 25 30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
35 40 45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
50 55 60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65 70 75 80
Ile Asn Val Thr Gly Gln Asp Val Thr Ile Phe Tyr Val Asp Arg Leu
85 90 95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
100 105 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
115 120 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
130 135 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145 150 155 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
165 170 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
180 185 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
195 200 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
210 215 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225 230 235 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
245 250 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
260 265 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Gln Glu Ala Ile Arg Val
275 280 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Val Tyr Thr
290 295 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305 310 315 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
325 330 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
340 345 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
355 360 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
370 375 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385 390 395 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
405 410 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
420 425 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
435 440 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys

```
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Ser Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Asp
                485                 490                 495
Leu Cys Asp Leu Tyr Leu Val Pro Thr Ser Tyr Leu Ile Leu
            500                 505                 510


<210> 186
<211> 512
<212> PRT
<213> Macaca mulatta

<220>
<223> Rhesus Monkey PH20

<400> 186
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Ile Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asn Glu Pro Leu Asp Met Ser Leu Phe Thr Leu Met Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ile Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Leu Thr Thr Gly Val Thr Val His Gly
            100                 105                 110
Gly Ile Pro Gln Lys Val Ser Leu Gln Asp His Leu Asp Lys Ser Lys
        115                 120                 125
Gln Asp Ile Leu Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Pro Gln Ala Thr Asp Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Met Leu Glu Thr Ile Lys Leu Gly Arg
        195                 200                 205
Ser Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Arg Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asp
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Val Val
            260                 265                 270
Val Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Asn Pro Leu Pro Val Phe Val Tyr Ala
    290                 295                 300
Arg Leu Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Arg Glu Glu
305                 310                 315                 320
Leu Val Ser Thr Leu Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Ser Leu Ser Ile Thr Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Thr Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asp Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
```

```
Asn Pro Asp Asn Phe Asp Ile Arg Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val His Gly Lys Pro Thr Val Glu Asp Leu Glu Glu Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Thr Asn Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Ser Leu Lys Pro Pro Val Glu Thr Glu Gly Ser Pro Pro
465                 470                 475                 480
Ile Phe Tyr Asn Thr Ser Ser Ser Thr Val Ser Thr Thr Met Phe Ile
                485                 490                 495
Trp Arg Leu Glu Val Trp Asp Gln Gly Ile Ser Arg Ile Gly Phe Phe
            500                 505                 510


<210> 187
<211> 505
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-505

<400> 187
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1                5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
```

```
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser Ile Leu Phe Leu Ile Ile Ser Ser
            500                 505


<210> 188
<211> 503
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-503

<400> 188
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1                  5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
```

```
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser Ile Leu Phe Leu Ile Ile
            500
```

<210> 189
<211> 501
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-501

<400> 189

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1                5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
```

225 230 235 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
245 250 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
260 265 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
275 280 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
290 295 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305 310 315 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
325 330 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
340 345 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
355 360 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
370 375 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385 390 395 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
405 410 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
420 425 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
435 440 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
450 455 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465 470 475 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
485 490 495
Ser Ile Leu Phe Leu
500

<210> 190
<211> 499
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-499

<400> 190
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
1 5 10 15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
20 25 30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
35 40 45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
50 55 60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65 70 75 80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
85 90 95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
100 105 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
115 120 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
130 135 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145 150 155 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
165 170 175

```
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser Ile Leu
```

<210> 191
<211> 497
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-497

<400> 191

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
```

```
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser
```

<210> 192
<211> 495
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-495

<400> 192

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
```

```
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile
                485                 490                 495


<210> 193
<211> 493
<212> PRT

<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-493

<400> 193
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
```

```
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met
                485                 490
```

<210> 194
<211> 491
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-491

<400> 194
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1   5   10   15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20   25   30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35   40   45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50   55   60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65   70   75   80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85   90   95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100   105   110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115   120   125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130   135   140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145   150   155   160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165   170   175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180   185   190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195   200   205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210   215   220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225   230   235   240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245   250   255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260   265   270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275   280   285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290   295   300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305   310   315   320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325   330   335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340   345   350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355   360   365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370   375   380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385   390   395   400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405   410   415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420   425   430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435   440   445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450   455   460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465   470   475   480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala
                485   490

<210> 195
<211> 489
<212> PRT

<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-489

<400> 195

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu
                485
```

<210> 196
<211> 490
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-490

<400> 196
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1   5   10   15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20   25   30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35   40   45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50   55   60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65   70   75   80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85   90   95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100   105   110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115   120   125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130   135   140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145   150   155   160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165   170   175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180   185   190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195   200   205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210   215   220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225   230   235   240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245   250   255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260   265   270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275   280   285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290   295   300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305   310   315   320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325   330   335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340   345   350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355   360   365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370   375   380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385   390   395   400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405   410   415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420   425   430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435   440   445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys

```
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser
                485                 490
```

<210> 197
<211> 487
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-487

<400> 197

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
```

Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
420 425 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
435 440 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
450 455 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465 470 475 480
Phe Tyr Asn Ala Ser Pro Ser
485

<210> 198
<211> 485
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-485

<400> 198
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
1 5 10 15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
20 25 30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
35 40 45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
50 55 60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65 70 75 80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
85 90 95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
100 105 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
115 120 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
130 135 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145 150 155 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
165 170 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
180 185 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
195 200 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
210 215 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225 230 235 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
245 250 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
260 265 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
275 280 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
290 295 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305 310 315 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
325 330 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
340 345 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
355 360 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln

370 375 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385 390 395 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
405 410 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
420 425 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
435 440 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
450 455 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465 470 475 480
Phe Tyr Asn Ala Ser
485

<210> 199
<211> 484
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-484

<400> 199
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
1 5 10 15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
20 25 30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
35 40 45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
50 55 60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65 70 75 80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
85 90 95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
100 105 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
115 120 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
130 135 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145 150 155 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
165 170 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
180 185 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
195 200 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
210 215 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225 230 235 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
245 250 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
260 265 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
275 280 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
290 295 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305 310 315 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
325 330 335

```
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala
```

<210> 200
<211> 476
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-476

<400> 200

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
```

```
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu
465                 470                 475


<210> 201
<211> 475
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-475

<400> 201
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1                  5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
```

275 280 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
290 295 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305 310 315 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
325 330 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
340 345 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
355 360 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
370 375 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385 390 395 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
405 410 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
420 425 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
435 440 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
450 455 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr
465 470 475

<210> 202
<211> 474
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-474

<400> 202
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
1 5 10 15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
20 25 30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
35 40 45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
50 55 60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65 70 75 80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
85 90 95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
100 105 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
115 120 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
130 135 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145 150 155 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
165 170 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
180 185 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
195 200 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
210 215 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225 230 235 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
245 250 255

```
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu
465                 470


<210> 203
<211> 473
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-473

<400> 203
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1                  5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
```

```
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
            245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
        260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
    275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
            325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
        340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
    355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
            405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
        420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
    435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met
465                 470


<210> 204
<211> 472
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-472

<400> 204
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1                5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
            85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
        100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
    115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
            165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
        180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
    195                 200                 205
```

```
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro
465                 470
```

<210> 205
<211> 471
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-471

<400> 205

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
```

```
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro
465                 470


<210> 206
<211> 470
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-470

<400> 206
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1                5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
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```
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys
465                 470


<210> 207
<211> 469
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-469

<400> 207
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1                5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
```

130 135 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145 150 155 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
165 170 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
180 185 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
195 200 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
210 215 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225 230 235 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
245 250 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
260 265 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
275 280 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
290 295 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305 310 315 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
325 330 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
340 345 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
355 360 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
370 375 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385 390 395 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
405 410 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
420 425 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
435 440 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
450 455 460
Ile Asp Ala Phe Leu
465

<210> 208
<211> 468
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-468

<400> 208
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
1 5 10 15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
20 25 30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
35 40 45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
50 55 60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65 70 75 80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
85 90 95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
100 105 110

```
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe
465


<210> 209
<211> 466
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-466

<400> 209
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
```

```
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp
465


<210> 210
<211> 465
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-465

<400> 210
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1                5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
```

```
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile
465
```

<210> 211
<211> 464
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-464

<400> 211

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
```

```
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460


<210> 212
<211> 462
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-462

<400> 212
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
```

```
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly
    450                 455                 460


<210> 213
<211> 460
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-460

<400> 213
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
```

```
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala
    450                 455                 460


<210> 214
<211> 458
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-458

<400> 214
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
```

```
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys
    450                 455


<210> 215
<211> 456
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-456

<400> 215
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
```

```
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp
    450                 455
```

<210> 216
<211> 454
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-454

<400> 216

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala
    450
```

<210> 217
<211> 452
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-452

<400> 217
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1   5   10   15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20   25   30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35   40   45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50   55   60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65   70   75   80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85   90   95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100   105   110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115   120   125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130   135   140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145   150   155   160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165   170   175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180   185   190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195   200   205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210   215   220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225   230   235   240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245   250   255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260   265   270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275   280   285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290   295   300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305   310   315   320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325   330   335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340   345   350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355   360   365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370   375   380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385   390   395   400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405   410   415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420   425   430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435   440   445
Val Lys Asp Thr
    450

<210> 218
<211> 450
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-450

<400> 218
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1   5   10   15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20   25   30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35   40   45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50   55   60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65   70   75   80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85   90   95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100   105   110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115   120   125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130   135   140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145   150   155   160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165   170   175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180   185   190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195   200   205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210   215   220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225   230   235   240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245   250   255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260   265   270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275   280   285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290   295   300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305   310   315   320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325   330   335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340   345   350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355   360   365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370   375   380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385   390   395   400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405   410   415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420   425   430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435   440   445
Val Lys
    450

<210> 219
<211> 508
<212> PRT
<213> Homo sapiens

<220>

<223> HuPH20 precursor 1-508

<400> 219

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Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser Ile Leu Phe Leu Ile Ile Ser Ser Val Ala Ser
            500                 505
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<210> 220
<211> 506
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-506

<400> 220
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1   5   10   15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20   25   30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35   40   45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50   55   60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65   70   75   80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85   90   95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100   105   110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115   120   125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130   135   140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145   150   155   160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165   170   175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180   185   190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195   200   205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210   215   220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225   230   235   240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245   250   255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260   265   270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275   280   285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290   295   300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305   310   315   320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325   330   335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340   345   350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355   360   365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370   375   380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385   390   395   400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405   410   415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420   425   430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435   440   445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450   455   460

Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465 470 475 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
485 490 495
Ser Ile Leu Phe Leu Ile Ile Ser Ser Val
500 505

<210> 221
<211> 504
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-504

<400> 221
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
1 5 10 15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
20 25 30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
35 40 45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
50 55 60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65 70 75 80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
85 90 95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
100 105 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
115 120 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
130 135 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145 150 155 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
165 170 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
180 185 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
195 200 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
210 215 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225 230 235 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
245 250 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
260 265 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
275 280 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
290 295 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305 310 315 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
325 330 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
340 345 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
355 360 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
370 375 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385 390 395 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr

405 410 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
420 425 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
435 440 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
450 455 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465 470 475 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
485 490 495
Ser Ile Leu Phe Leu Ile Ile Ser
500

<210> 222
<211> 502
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-502

<400> 222
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
1 5 10 15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
20 25 30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
35 40 45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
50 55 60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65 70 75 80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
85 90 95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
100 105 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
115 120 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
130 135 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145 150 155 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
165 170 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
180 185 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
195 200 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
210 215 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225 230 235 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
245 250 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
260 265 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
275 280 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
290 295 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305 310 315 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
325 330 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
340 345 350

```
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser Ile Leu Phe Leu Ile
            500
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<210> 223
<211> 500
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-500

<400> 223

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Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
```

```
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser Ile Leu Phe
            500


<210> 224
<211> 498
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-498

<400> 224
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
```

Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
245 250 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
260 265 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
275 280 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
290 295 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305 310 315 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
325 330 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
340 345 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
355 360 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
370 375 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385 390 395 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
405 410 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
420 425 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
435 440 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
450 455 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465 470 475 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
485 490 495
Ser Ile

<210> 225
<211> 496
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-496

<400> 225
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
1 5 10 15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
20 25 30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
35 40 45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
50 55 60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65 70 75 80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
85 90 95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
100 105 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
115 120 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
130 135 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145 150 155 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
165 170 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
180 185 190

```
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495


<210> 226
<211> 494
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-494

<400> 226
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1                5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
```

```
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe
                485                 490


<210> 227
<211> 492
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-492

<400> 227
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
```

```
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr
                485                 490
```

<210> 228
<211> 488
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-488

<400> 228

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
```

```
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr
                485
```

<210> 229
<211> 486
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-486

<400> 229

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
```

```
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro
                485
```

<210> 230
<211> 472
<212> PRT
<213> Homo sapiens

<220>
<223> mature HuPH20 36-507

<400> 230
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1   5   10   15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20   25   30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35   40   45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50   55   60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65   70   75   80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85   90   95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100   105   110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115   120   125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130   135   140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145   150   155   160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165   170   175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180   185   190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195   200   205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210   215   220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225   230   235   240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245   250   255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260   265   270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275   280   285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290   295   300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305   310   315   320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325   330   335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340   345   350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355   360   365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370   375   380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385   390   395   400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405   410   415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420   425   430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435   440   445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
    450   455   460
Phe Leu Ile Ile Ser Ser Val Ala
465   470

<210> 231
<211> 470
<212> PRT
<213> Homo sapiens

<220>
<223> mature HuPH20 36-505

<400> 231
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1   5   10   15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20   25   30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35   40   45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50   55   60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65   70   75   80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85   90   95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100   105   110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115   120   125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Asn Val Gln Leu
    130   135   140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145   150   155   160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165   170   175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180   185   190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195   200   205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210   215   220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225   230   235   240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245   250   255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260   265   270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275   280   285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290   295   300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305   310   315   320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325   330   335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340   345   350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355   360   365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370   375   380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385   390   395   400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405   410   415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420   425   430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435   440   445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
    450   455   460
Phe Leu Ile Ile Ser Ser
465   470

<210> 232
<211> 468

<212> PRT
<213> Homo sapiens

<220>
<223> mature HuPH20 36-503

<400> 232

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
    450                 455                 460
Phe Leu Ile Ile
465
```

<210> 233
<211> 466
<212> PRT
<213> Homo sapiens

<220>
<223> mature HuPH20 36-501

<400> 233
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1   5   10   15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20   25   30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35   40   45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50   55   60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65   70   75   80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85   90   95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100   105   110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115   120   125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130   135   140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145   150   155   160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165   170   175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180   185   190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195   200   205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210   215   220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225   230   235   240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245   250   255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260   265   270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275   280   285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290   295   300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305   310   315   320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325   330   335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340   345   350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355   360   365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370   375   380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385   390   395   400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405   410   415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420   425   430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435   440   445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
    450   455   460

Phe Leu
465

<210> 234
<211> 464
<212> PRT
<213> Homo sapiens

<220>
<223> mature HuPH20 36-499

<400> 234

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
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435 440 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
450 455 460

<210> 235
<211> 462
<212> PRT
<213> Homo sapiens

<220>
<223> esHuPH20 36-497

<400> 235
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
1 5 10 15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
20 25 30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
35 40 45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
50 55 60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65 70 75 80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
85 90 95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
100 105 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
115 120 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
130 135 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145 150 155 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
165 170 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
180 185 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
195 200 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
210 215 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225 230 235 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
245 250 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
260 265 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
275 280 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
290 295 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305 310 315 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
325 330 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
340 345 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
355 360 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
370 375 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385 390 395 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
405 410 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
420 425 430

Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
435 440 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser
450 455 460

<210> 236
<211> 460
<212> PRT
<213> Homo sapiens

<220>
<223> esHuPH20 36-495

<400> 236
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
1 5 10 15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
20 25 30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
35 40 45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
50 55 60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65 70 75 80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
85 90 95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
100 105 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
115 120 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
130 135 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145 150 155 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
165 170 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
180 185 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
195 200 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
210 215 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225 230 235 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
245 250 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
260 265 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
275 280 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
290 295 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305 310 315 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
325 330 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
340 345 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
355 360 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
370 375 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385 390 395 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
405 410 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala

420 425 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
435 440 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile
450 455 460

<210> 237
<211> 458
<212> PRT
<213> Homo sapiens

<220>
<223> esHuPH20 36-493

<400> 237
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
1 5 10 15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
20 25 30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
35 40 45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
50 55 60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65 70 75 80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
85 90 95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
100 105 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
115 120 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
130 135 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145 150 155 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
165 170 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
180 185 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
195 200 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
210 215 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225 230 235 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
245 250 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
260 265 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
275 280 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
290 295 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305 310 315 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
325 330 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
340 345 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
355 360 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
370 375 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385 390 395 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
405 410 415

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Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met
    450                 455


<210> 238
<211> 456
<212> PRT
<213> Homo sapiens

<220>
<223> esHuPH20 36-491

<400> 238
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
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405 410 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
420 425 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
435 440 445
Ala Ser Pro Ser Thr Leu Ser Ala
450 455

<210> 239
<211> 454
<212> PRT
<213> Homo sapiens

<220>
<223> mature HuPH20 36-489

<400> 239
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
1 5 10 15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
20 25 30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
35 40 45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
50 55 60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65 70 75 80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
85 90 95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
100 105 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
115 120 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
130 135 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145 150 155 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
165 170 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
180 185 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
195 200 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
210 215 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225 230 235 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
245 250 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
260 265 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
275 280 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
290 295 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305 310 315 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
325 330 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
340 345 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
355 360 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
370 375 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385 390 395 400

Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
405 410 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
420 425 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
435 440 445
Ala Ser Pro Ser Thr Leu
450

<210> 240
<211> 455
<212> PRT
<213> Homo sapiens

<220>
<223> mature HuPH20 36-490

<400> 240
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
1 5 10 15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
20 25 30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
35 40 45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
50 55 60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65 70 75 80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
85 90 95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
100 105 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
115 120 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
130 135 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145 150 155 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
165 170 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
180 185 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
195 200 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
210 215 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225 230 235 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
245 250 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
260 265 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
275 280 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
290 295 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305 310 315 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
325 330 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
340 345 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
355 360 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
370 375 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys

385 390 395 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
405 410 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
420 425 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
435 440 445
Ala Ser Pro Ser Thr Leu Ser
450 455

<210> 241
<211> 452
<212> PRT
<213> Homo sapiens

<220>
<223> mature HuPH20 36-487

<400> 241
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
1 5 10 15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
20 25 30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
35 40 45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
50 55 60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65 70 75 80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
85 90 95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
100 105 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
115 120 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
130 135 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145 150 155 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
165 170 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
180 185 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
195 200 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
210 215 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225 230 235 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
245 250 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
260 265 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
275 280 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
290 295 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305 310 315 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
325 330 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
340 345 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
355 360 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
370 375 380

Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385 390 395 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
405 410 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
420 425 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
435 440 445
Ala Ser Pro Ser
450

<210> 242
<211> 450
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-485

<400> 242
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
1 5 10 15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
20 25 30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
35 40 45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
50 55 60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65 70 75 80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
85 90 95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
100 105 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
115 120 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
130 135 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145 150 155 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
165 170 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
180 185 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
195 200 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
210 215 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225 230 235 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
245 250 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
260 265 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
275 280 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
290 295 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305 310 315 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
325 330 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
340 345 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
355 360 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly

370 375 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385 390 395 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
405 410 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
420 425 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
435 440 445
Ala Ser
450

<210> 243
<211> 449
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-484

<400> 243
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
1 5 10 15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
20 25 30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
35 40 45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
50 55 60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65 70 75 80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
85 90 95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
100 105 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
115 120 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
130 135 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145 150 155 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
165 170 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
180 185 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
195 200 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
210 215 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225 230 235 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
245 250 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
260 265 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
275 280 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
290 295 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305 310 315 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
325 330 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
340 345 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
355 360 365

```
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala
```

<210> 244
<211> 441
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-476

<400> 244

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Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
```

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Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu
        435                 440


<210> 245
<211> 440
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-475

<400> 245
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
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    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr
        435                 440


<210> 246
<211> 439
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-474

<400> 246
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
```

Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385 390 395 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
405 410 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
420 425 430
Phe Leu Lys Pro Pro Met Glu
435

<210> 247
<211> 438
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-473

<400> 247
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
1 5 10 15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
20 25 30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
35 40 45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
50 55 60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65 70 75 80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
85 90 95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
100 105 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
115 120 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
130 135 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145 150 155 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
165 170 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
180 185 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
195 200 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
210 215 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225 230 235 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
245 250 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
260 265 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
275 280 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
290 295 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305 310 315 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
325 330 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
340 345 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
355 360 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
370 375 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys

385 390 395 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
405 410 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
420 425 430
Phe Leu Lys Pro Pro Met
435

<210> 248
<211> 437
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-472

<400> 248
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
1 5 10 15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
20 25 30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
35 40 45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
50 55 60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65 70 75 80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
85 90 95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
100 105 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
115 120 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
130 135 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145 150 155 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
165 170 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
180 185 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
195 200 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
210 215 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225 230 235 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
245 250 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
260 265 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
275 280 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
290 295 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305 310 315 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
325 330 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
340 345 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
355 360 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
370 375 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385 390 395 400

Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
405 410 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
420 425 430
Phe Leu Lys Pro Pro
435

<210> 249
<211> 436
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-471

<400> 249
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
1 5 10 15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
20 25 30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
35 40 45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
50 55 60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65 70 75 80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
85 90 95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
100 105 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
115 120 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
130 135 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145 150 155 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
165 170 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
180 185 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
195 200 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
210 215 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225 230 235 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
245 250 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
260 265 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
275 280 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
290 295 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305 310 315 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
325 330 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
340 345 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
355 360 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
370 375 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385 390 395 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp

405 410 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
420 425 430
Phe Leu Lys Pro
435

<210> 250
<211> 435
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-470

<400> 250
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
1 5 10 15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
20 25 30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
35 40 45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
50 55 60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65 70 75 80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
85 90 95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
100 105 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
115 120 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
130 135 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145 150 155 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
165 170 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
180 185 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
195 200 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
210 215 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225 230 235 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
245 250 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
260 265 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
275 280 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
290 295 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305 310 315 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
325 330 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
340 345 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
355 360 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
370 375 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385 390 395 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
405 410 415

Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
420 425 430

Phe Leu Lys
435

<210> 251
<211> 434
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-469

<400> 251
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
1 5 10 15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
20 25 30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
35 40 45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
50 55 60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65 70 75 80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
85 90 95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
100 105 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
115 120 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
130 135 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145 150 155 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
165 170 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
180 185 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
195 200 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
210 215 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225 230 235 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
245 250 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
260 265 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
275 280 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
290 295 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305 310 315 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
325 330 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
340 345 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
355 360 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
370 375 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385 390 395 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
405 410 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala

420 425 430
Phe Leu

<210> 252
<211> 433
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-468

<400> 252
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
1 5 10 15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
20 25 30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
35 40 45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
50 55 60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65 70 75 80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
85 90 95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
100 105 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
115 120 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
130 135 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145 150 155 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
165 170 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
180 185 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
195 200 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
210 215 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225 230 235 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
245 250 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
260 265 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
275 280 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
290 295 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305 310 315 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
325 330 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
340 345 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
355 360 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
370 375 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385 390 395 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
405 410 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
420 425 430
Phe

<210> 253
<211> 431
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-466

<400> 253
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1   5   10   15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20   25   30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35   40   45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50   55   60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65   70   75   80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85   90   95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100   105   110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115   120   125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130   135   140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145   150   155   160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165   170   175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180   185   190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195   200   205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210   215   220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225   230   235   240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245   250   255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260   265   270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275   280   285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290   295   300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305   310   315   320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325   330   335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340   345   350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355   360   365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370   375   380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385   390   395   400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405   410   415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp
            420   425   430

<210> 254

<211> 430
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-465

<400> 254
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Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile
            420                 425                 430
```

<210> 255
<211> 429
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-464

<400> 255
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1   5   10   15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20   25   30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35   40   45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50   55   60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65   70   75   80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85   90   95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100   105   110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115   120   125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Asn Val Gln Leu
    130   135   140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145   150   155   160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165   170   175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180   185   190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195   200   205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210   215   220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225   230   235   240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245   250   255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260   265   270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275   280   285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290   295   300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305   310   315   320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325   330   335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340   345   350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355   360   365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370   375   380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385   390   395   400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405   410   415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
            420   425

<210> 256
<211> 427
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-462

<400> 256
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1   5   10   15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20   25   30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35   40   45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50   55   60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65   70   75   80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85   90   95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100   105   110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115   120   125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130   135   140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145   150   155   160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165   170   175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180   185   190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195   200   205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210   215   220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225   230   235   240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245   250   255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260   265   270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275   280   285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290   295   300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305   310   315   320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325   330   335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340   345   350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355   360   365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370   375   380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385   390   395   400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405   410   415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly
            420   425

<210> 257
<211> 425
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-460

<400> 257
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp

```
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala
            420                 425


<210> 258
<211> 423
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-458

<400> 258
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
```

```
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys
            420


<210> 259
<211> 421
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-456

<400> 259
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1                5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
```

```
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp
            420
```

<210> 260
<211> 419
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-454

<400> 260

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
```

```
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala
```

<210> 261
<211> 417
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-452

<400> 261

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
```

```
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr
```

<210> 262
<211> 415
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-450

<400> 262

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
```

130 135 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145 150 155 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
165 170 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
180 185 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
195 200 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
210 215 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225 230 235 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
245 250 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
260 265 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
275 280 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
290 295 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305 310 315 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
325 330 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
340 345 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
355 360 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
370 375 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385 390 395 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys
405 410 415

<210> 263
<211> 473
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-508

<400> 263
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
1 5 10 15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
20 25 30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
35 40 45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
50 55 60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65 70 75 80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
85 90 95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
100 105 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
115 120 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
130 135 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145 150 155 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
165 170 175

```
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
    450                 455                 460
Phe Leu Ile Ile Ser Ser Val Ala Ser
465                 470


<210> 264
<211> 471
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-506

<400> 264
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
```

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145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
    450                 455                 460
Phe Leu Ile Ile Ser Ser Val
465                 470


<210> 265
<211> 469
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-504

<400> 265
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
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Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
    450                 455                 460
Phe Leu Ile Ile Ser
465
```

<210> 266
<211> 467
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-502

<400> 266

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
```

```
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
    450                 455                 460
Phe Leu Ile
465


<210> 267
<211> 465
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-500

<400> 267
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
```

```
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
    450                 455                 460
Phe
465
```

<210> 268
<211> 463
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-498

<400> 268

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
```

50 55 60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65 70 75 80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
85 90 95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
100 105 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
115 120 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
130 135 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145 150 155 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
165 170 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
180 185 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
195 200 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
210 215 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225 230 235 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
245 250 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
260 265 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
275 280 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
290 295 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305 310 315 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
325 330 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
340 345 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
355 360 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
370 375 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385 390 395 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
405 410 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
420 425 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
435 440 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile
450 455 460

<210> 269
<211> 461
<212> PRT
<213> Homo sapiens

<220>
<223> esHuPH20 36-496

<400> 269
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
1 5 10 15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
20 25 30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
35 40 45

```
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
    450                 455                 460


<210> 270
<211> 459
<212> PRT
<213> Homo sapiens

<220>
<223> esHuPH20 36-494

<400> 270
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
```

```
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe
    450                 455


<210> 271
<211> 457
<212> PRT
<213> Homo sapiens

<220>
<223> esHuPH20 36-492

<400> 271
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
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Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr
    450                 455


<210> 272
<211> 453
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-488

<400> 272
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
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```
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr
    450
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<210> 273
<211> 451
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-486

<400> 273

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Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
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Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro
    450
```