PROSTAGLANDIN E2 DUAL VARIABLE DOMAIN IMMUNOGLOBULINS AND USES THEREOF

Reference to Related Applications

This application is a non-provisional application claiming priority to U.S. Provisional Application Ser. No. 61/134,284, filed July 8, 2008, and U.S. Provisional Application Ser. No. 61/191,711, filed September 11, 2008, the contents of which are hereby incorporated by reference.

Field of the Invention

The present invention relates to multivalent and multispecific binding proteins, at least one variable domain specific for prostaglandin E2 (PGE2), methods of making, and specifically to their uses in the, diagnosis, prevention and/or treatment of acute and chronic inflammation, autoimmune diseases, cancer, pain, bone, neuronal and other diseases.

Background of the Invention

Engineered proteins, such as multispecific antibodies capable of binding two or more antigens are known in the art. Such multispecific binding proteins can be generated using cell fusion, chemical conjugation, or recombinant DNA techniques.

Bispecific antibodies have been produced using quadroma technology (see Milstein, C. and A.C. Cuello (1983) Nature 305(5934):537-40) based on the somatic fusion of two different hybridoma cell lines expressing murine monoclonal antibodies (mAbs) with the desired specificities of the bispecific antibody. Because of the random pairing of two different immunoglobulin (Ig) heavy and light chains within the resulting hybrid–hybridoma (or quadroma) cell line, up to ten different Ig species are generated, of which only one is the functional bispecific antibody. The presence of mis-paired by-products, and significantly reduced production yields, means sophisticated purification procedures are required.

Bispecific antibodies can also be produced by chemical conjugation of two different mAbs (see Staerz, U.D., et al. (1985) Nature 314(6012): 628-31). This approach does not yield homogeneous preparation. Other approaches have used chemical

conjugation of two different mAbs or smaller antibody fragments (see Brennan, M., et al. (1985) Science 229(4708): 81-3).

Another method used to produce bispecific antibodies is the coupling of two parental antibodies with a hetero-bifunctional crosslinker, but the resulting bispecific antibodies suffer from significant molecular heterogeneity because reaction of the crosslinker with the parental antibodies is not site-directed. To obtain more homogeneous preparations of bispecific antibodies two different Fab fragments have been chemically crosslinked at their hinge cysteine residues in a site-directed manner (see Glennie, M.J., et al. (1987) J. Immunol. 139(7): 2367-75). But this method results in Fab'2 fragments, not full IgG molecule.

A wide variety of other recombinant bispecific antibody formats have been developed (see Kriangkum, J., et al. (2001) Biomol. Eng. 18(2): 31-40). Amongst them tandem single-chain Fv molecules and diabodies, and various derivatives thereof, are the most widely used. Routinely, construction of these molecules starts from two single-chain Fv (scFv) fragments that recognize different antigens (see Economides, A.N., et al. (2003) Nat. Med. 9(1): 47-52). Tandem scFv molecules (taFv) represent a straightforward format simply connecting the two scFv molecules with an additional peptide linker. The two scFv fragments present in these tandem scFv molecules form separate folding entities. Various linkers can be used to connect the two scFv fragments and linkers with a length of up to 63 residues (see Nakanishi, K., et al. (2001) Ann. Rev. Immunol. 19: 423-74). Although the parental scFv fragments can normally be expressed in soluble form in bacteria, it is, however, often observed that tandem scFv molecules form insoluble aggregates in bacteria. Hence, refolding protocols or the use of mammalian expression systems are routinely applied to produce soluble tandem scFv molecules. In a recent study, *in vivo* expression by transgenic rabbits and cattle of a tandem scFv directed against CD28 and a melanoma-associated proteoglycan was reported (see Gracie, J.A., et al. (1999) J. Clin. Invest. 104(10): 1393-401). In this construct, the two scFv molecules were connected by a CH1 linker and serum concentrations of up to 100 mg/L of the bispecific antibody were found. Various strategies including variations of the domain order or using middle linkers with varying length or flexibility were employed to allow soluble expression in bacteria. A few studies have now reported expression of soluble tandem scFv molecules in bacteria (see Leung,

B.P., et al. (2000) J. Immunol. 164(12): 6495-502; Ito, A., et al. (2003) J. Immunol. 170(9): 4802-9; Karni, A., et al. (2002) J. Neuroimmunol. 125(1-2): 134-40) using either a very short Ala3 linker or long glycine/serine-rich linkers. In another recent study, phage display of a tandem scFv repertoire containing randomized middle linkers with a length of 3 or 6 residues was employed to enrich for those molecules that are produced in soluble and active form in bacteria. This approach resulted in the isolation of a tandem scFv molecule with a 6 amino acid residue linker (see Arndt, M. and J. Krauss (2003) Methods Mol. Biol. 207: 305-21). It is unclear whether this linker sequence represents a general solution to the soluble expression of tandem scFv molecules. Nevertheless, this study demonstrated that phage display of tandem scFv molecules in combination with directed mutagenesis is a powerful tool to enrich for these molecules, which can be expressed in bacteria in an active form.

Bispecific diabodies (Db) utilize the diabody format for expression. Diabodies are produced from scFv fragments by reducing the length of the linker connecting the VH and VL domain to approximately 5 residues (see Peipp, M. and T. Valerius (2002) Biochem. Soc. Trans. 30(4): 507-11). This reduction of linker size facilitates dimerization of two polypeptide chains by crossover pairing of the VH and VL domains. Bispecific diabodies are produced by expressing, two polypeptide chains with, either the structure VHA-VLB and VHB-VLA (VH-VL configuration), or VLA-VHB and VLB-VHA (VL-VH configuration) within the same cell. A large variety of different bispecific diabodies have been produced in the past and most of them are expressed in soluble form in bacteria. However, a recent comparative study demonstrates that the orientation of the variable domains can influence expression and formation of active binding sites (see Mack, M. et al.(1995) Proc. Natl. Acad. Sci. U S A 92(15): 7021-5). Nevertheless, soluble expression in bacteria represents an important advantage over tandem scFv molecules. However, since two different polypeptide chains are expressed within a single cell inactive homodimers can be produced together with active heterodimers. This necessitates the implementation of additional purification steps in order to obtain homogenous preparations of bispecific diabodies. One approach to force the generation of bispecific diabodies is the production of knob-into-hole diabodies (see Holliger, P., T. Prospero, and G. Winter (1993) Proc. Natl. Acad. Sci. U S A 90(14): 6444-8.18). This approach was demonstrated for a bispecific diabody directed against HER2 and CD3. A

large knob was introduced in the VH domain by exchanging Val37 with Phe and Leu45 with Trp and a complementary hole was produced in the VL domain by mutating Phe98 to Met and Tyr87 to Ala, either in the anti- HER2 or the anti-CD3 variable domains. By using this approach the production of bispecific diabodies could be increased from 72% by the parental diabody to over 90% by the knob-into-hole diabody. Importantly, production yields only slightly decreased as a result of these mutations. However, a reduction in antigen-binding activity was observed for several constructs. Thus, this rather elaborate approach requires the analysis of various constructs in order to identify those mutations that produce heterodimeric molecule with unaltered binding activity. In addition, such an approach requires mutational modification of the immunoglobulin sequence at the constant region, thus creating a non-native and non-natural form of the antibody sequence, which may result in increased immunogenicity, poor *in vivo* stability, as well as undesirable pharmacokinetics.

Single-chain diabodies (scDb) represent an alternative strategy for improving the formation of bispecific diabody-like molecules (see Holliger, P. and G. Winter (1997) Cancer Immunol. Immunother. 45(3-4): 128-30; Wu, A.M., et al. (1996) Immunotechnology 2(1): p. 21-36). Bispecific single-chain diabodies are produced by connecting the two diabody-forming polypeptide chains with an additional middle linker with a length of approximately 15 amino acid residues. Consequently, all molecules with a molecular weight corresponding to monomeric single-chain diabodies (50-60 kDa) are bispecific. Several studies have demonstrated that bispecific single chain diabodies are expressed in bacteria in soluble and active form with the majority of purified molecules present as monomers (see Holliger, P. and G. Winter (1997) Cancer Immunol. Immunother. 45(3-4): 128-30; Wu, A.M., et al. (1996) Immunotechnol. 2(1): 21-36; Pluckthun, A. and P. Pack (1997) Immunotechnol. 3(2): 83-105; Ridgway, J.B., et al. (1996) Protein Engin. 9(7): 617-21). Thus, single-chain diabodies combine the advantages of tandem scFvs (all monomers are bispecific) and diabodies (soluble expression in bacteria).

More recently diabodies have been fused to Fc to generate more Ig-like molecules, named di-diabodies (see Lu, D., et al. (2004) J. Biol. Chem. 279(4): 2856-65). In addition, multivalent antibody construct comprising two Fab repeats in the heavy chain

of an IgG and capable of binding four antigen molecules has been described (see WO 0177342A1, and Miller, K., et al. (2003) J. Immunol. 170(9): 4854-61).

There is a need in the art for improved multivalent binding proteins capable of binding two or more antigens. U.S. Patent Application Serial No. 11/507,050 provides a novel family of binding proteins capable of binding two or more antigens with high affinity, which are called dual variable domain immunoglobulins (DVD-Ig™). The present invention provides further novel binding proteins capable of binding two or more antigens.

Summary of the Invention

This invention pertains to multivalent binding proteins capable of binding two or more antigens. The present invention provides a novel family of binding proteins capable of binding two or more antigens with high affinity.

In one embodiment the invention provides a binding protein comprising a polypeptide chain, wherein the polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first variable domain, VD2 is a second variable domain, C is a constant domain, X1 represents an amino acid or polypeptide, X2 represents an Fc region and n is 0 or 1. In an embodiment the VD1 and VD2 in the binding protein are heavy chain variable domains. In another embodiment, the heavy chain variable domain is selected from the group consisting of a murine heavy chain variable domain, a human heavy chain variable domain, a CDR grafted heavy chain variable domain, and a humanized heavy chain variable domain. In yet another, embodiment VD1 and VD2 are capable of binding the same antigen. In another embodiment VD1 and VD2 are capable of binding different antigens. In still another embodiment, C is a heavy chain constant domain. For example, X1 is a linker with the proviso that X1 is not CH1. For example, X1 is a linker selected from the group consisting of AKTTPKLEEGEFSEAR (SEQ ID NO: 1); AKTTPKLEEGEFSEARV (SEQ ID NO: 2); AKTTPKLGG (SEQ ID NO: 3); SAKTTPKLGG (SEQ ID NO: 4); SAKTTP (SEQ ID NO: 5); RADAAP (SEQ ID NO: 6); RADAAPTVS (SEQ ID NO: 7); RADAAAAGGPGS (SEQ ID NO: 8); RADAAAA$(G_4S)_4$ (SEQ ID NO: 9); SAKTTPKLEEGEFSEARV (SEQ ID NO: 10); ADAAP (SEQ ID NO: 11); ADAAPTVSIFPP (SEQ ID NO: 12); TVAAP (SEQ ID NO: 13); TVAAPSVFIFPP (SEQ ID NO: 14); QPKAAP (SEQ ID NO: 15);

QPKAAPSVTLFPP (SEQ ID NO: 16); AKTTPP (SEQ ID NO: 17); AKTTPPSVTPLAP (SEQ ID NO: 18); AKTTAP (SEQ ID NO: 19); AKTTAPSVYPLAP (SEQ ID NO: 20); ASTKGP (SEQ ID NO: 21); ASTKGPSVFPLAP (SEQ ID NO: 22), GGGGSGGGGSGGGGS (SEQ ID NO: 23); GENKVEYAPALMALS (SEQ ID NO: 24); GPAKELTPLKEAKVS (SEQ ID NO: 25); and GHEAAAVMQVQYPAS (SEQ ID NO: 26). In an embodiment, X2 is an Fc region. In another embodiment, X2 is a variant Fc region.

In an embodiment the binding protein disclosed herein comprises a polypeptide chain, wherein the polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 is an Fc region.

In an embodiment, VD1 and VD2 in the binding protein are light chain variable domains. In an embodiment, the light chain variable domain is selected from the group consisting of a murine light chain variable domain, a human light chain variable domain, a CDR grafted light chain variable domain, and a humanized light chain variable domain. In one embodiment VD1 and VD2 are capable of binding the same antigen. In another embodiment VD1 and VD2 are capable of binding different antigens. In an embodiment, C is a light chain constant domain. In another embodiment, X1 is a linker with the proviso that X1 is not CL1. In an embodiment, X1 is a linker selected from the group consisting of AKTTPKLEEGEFSEAR (SEQ ID NO: 1); AKTTPKLEEGEFSEARV (SEQ ID NO: 2); AKTTPKLGG (SEQ ID NO: 3); SAKTTPKLGG (SEQ ID NO: 4); SAKTTP (SEQ ID NO: 5); RADAAP (SEQ ID NO: 6); RADAAPTVS (SEQ ID NO: 7); RADAAAAGGPGS (SEQ ID NO: 8); RADAAAA$(G_4S)_4$ (SEQ ID NO: 9), SAKTTPKLEEGEFSEARV (SEQ ID NO: 10); ADAAP (SEQ ID NO: 11); ADAAPTVSIFPP (SEQ ID NO: 12); TVAAP (SEQ ID NO: 13); TVAAPSVFIFPP (SEQ ID NO: 14); QPKAAP (SEQ ID NO: 15); QPKAAPSVTLFPP (SEQ ID NO: 16); AKTTPP (SEQ ID NO: 17); AKTTPPSVTPLAP (SEQ ID NO: 18); AKTTAP (SEQ ID NO: 19); AKTTAPSVYPLAP (SEQ ID NO: 20); ASTKGP (SEQ ID NO: 21); ASTKGPSVFPLAP (SEQ ID NO: 22), GGGGSGGGGSGGGGS (SEQ ID NO: 23); GENKVEYAPALMALS (SEQ ID NO: 24); GPAKELTPLKEAKVS (SEQ ID NO: 25);

and GHEAAAVMQVQYPAS (SEQ ID NO: 26). In an embodiment, the binding protein does not comprise X2.

In an embodiment, both the variable heavy and variable light chain comprise the same linker. In another embodiment, the variable heavy and variable light chain comprise different linkers. In another embodiment, both the variable heavy and variable light chain comprise a short (about 6 amino acids) linker. In another embodiment, both the variable heavy and variable light chain comprise a long (greater than 6 amino acids) linker. In another embodiment, the variable heavy chain comprises a short linker and the variable light chain comprises a long linker. In another embodiment, the variable heavy chain comprises a long linker and the variable light chain comprises a short linker.

In an embodiment the binding protein disclosed herein comprises a polypeptide chain, wherein the polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 does not comprise an Fc region.

In another embodiment the invention provides a binding protein comprising two polypeptide chains, wherein the first polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 is an Fc region; and the second polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 does not comprise an Fc region. In a particular embodiment, the Dual Variable Domain (DVD) binding protein comprises four polypeptide chains wherein the first two polypeptide chains comprises VD1-(X1)n-VD2-C-(X2)n, respectively wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 is an Fc region; and the second two polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n respectively, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 does not

comprise an Fc region. Such a Dual Variable Domain (DVD) protein has four antigen binding sites.

In another embodiment the binding proteins disclosed herein are capable of binding one or more targets. In an embodiment, the target is selected from the group consisting of cytokines, cell surface proteins, enzymes and receptors. In another embodiment, the binding protein is capable of modulating a biological function of one or more targets. In another embodiment, the binding protein is capable of neutralizing one or more targets. The binding protein of the invention is capable of binding cytokines selected from the group consisting of lymphokines, monokines, polypeptide hormones, receptors, or tumor markers. For example, the DVD-Ig of the invention is capable of binding two or more of the following: murine or human Tumor Necrosis Factor alpha (TNF-α), Prostaglandin E2 (PGE2), Amyloid beta (Abeta) (seq. 1), Abeta (seq. 2), Abeta (seq. 3), Interleukin 1β (IL-1β), Insulin-like Growth Factor Receptor (IGFR), Interleukin 17A (IL-17A), Interleukin 6 (IL-6), Interleukin 6 receptor (IL-6R), Interleukin 15 (IL-15), Interleukin 18 (IL-18), Nerve Growth Factor (NGF), Epidermal Growth Factor Receptor (EGFR) (seq. 1), EGFR (seq. 2), vascular endothelial growth factor (VEGF), and S1P (see also Table 2 and Example 2). In a specific embodiment the binding protein is capable of binding pairs of targets selected from the group consisting of mouse or human TNF and PGE2, NGF and PGE2, IL-17A and PGE2, IL-1b and PGE2, IL-6 and PGE2, IL-6R and PGE2, VEGF and PGE2, Abeta (seq. 1) and PGE2, Abeta (seq. 2) and PGE2, Abeta (seq. 3) and PGE2, IL-18 and PGE2, PGE2 and PGE2, IL-15 and PGE2, S1P and PGE2, EGFR (seq. 1) and PGE2, EGFR (seq. 2) and PGE2, and IGFR and PGE2 (see Examples).

In an embodiment, the binding protein capable of binding murine TNF and PGE2 comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 86, 92, 94, 96, 98, 100, 102 104, 106, and 108; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 89, 93, 95, 97, 99, 101, 103, 105, 107, and 109. In an embodiment, the binding protein capable of binding murine TNF and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 86 and a DVD light chain amino acid sequence of SEQ ID NO: 89. In another embodiment, the binding protein capable of binding murine TNF and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 92 and a DVD

light chain amino acid sequence of SEQ ID NO: 93. In another embodiment, the binding protein capable of binding murine TNF and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 94 and a DVD light chain amino acid sequence of SEQ ID NO: 95. In another embodiment, the binding protein capable of binding murine TNF and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 96 and a DVD light chain amino acid sequence of SEQ ID NO: 97. In another embodiment, the binding protein capable of binding murine TNF and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 98 and a DVD light chain amino acid sequence of SEQ ID NO: 99. In another embodiment, the binding protein capable of binding murine TNF and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 100 and a DVD light chain amino acid sequence of SEQ ID NO: 101. In another embodiment, the binding protein capable of binding murine TNF and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 102 and a DVD light chain amino acid sequence of SEQ ID NO: 103. In another embodiment, the binding protein capable of binding murine TNF and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 104 and a DVD light chain amino acid sequence of SEQ ID NO: 105. In another embodiment, the binding protein capable of binding murine TNF and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 106 and a DVD light chain amino acid sequence of SEQ ID NO: 107. In another embodiment, the binding protein capable of binding murine TNF and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 108 and a DVD light chain amino acid sequence of SEQ ID NO: 109.

In an embodiment, the binding protein capable of binding human TNF and PGE2 comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 114, 116, 118, and 120; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 115, 117, 119, and 121. In an embodiment, the binding protein capable of binding human TNF and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 114 and a DVD light chain amino acid sequence of SEQ ID NO: 115. In another embodiment, the binding protein capable of binding human TNF and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 116 and a DVD light chain amino acid sequence of SEQ ID NO: 117. In another embodiment, the binding protein capable of binding human TNF and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 118 and a

DVD light chain amino acid sequence of SEQ ID NO: 119. In another embodiment, the binding protein capable of binding human TNF and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 120 and a DVD light chain amino acid sequence of SEQ ID NO: 121.

In an embodiment, the binding protein capable of binding VEGF and PGE2 comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 128 and SEQ ID NO. 130; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 129 and SEQ ID NO. 131. In an embodiment, the binding protein capable of binding VEGF and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 128 and a DVD light chain amino acid sequence of SEQ ID NO: 129. In another embodiment, the binding protein capable of binding VEGF and PGE2 has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 130 and a DVD light chain amino acid sequence of SEQ ID NO: 131.

In an embodiment, the binding protein capable of binding NGF and PGE2 comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 132 and SEQ ID NO. 134; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 133 and SEQ ID NO. 135. In an embodiment, the binding protein capable of binding NGF and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 132 and a DVD light chain amino acid sequence of SEQ ID NO: 133. In another embodiment, the binding protein capable of binding NGF and PGE2 has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 134 and a DVD light chain amino acid sequence of SEQ ID NO: 135.

In an embodiment, the binding protein capable of binding IL-17A and PGE2 comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 136 and SEQ ID NO. 138; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 137 and SEQ ID NO. 139. In an embodiment, the binding protein capable of binding IL-17A and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 136 and a DVD light chain amino acid sequence of SEQ ID NO: 137. In another embodiment, the binding protein capable

of binding IL-17A and PGE2 has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 138 and a DVD light chain amino acid sequence of SEQ ID NO: 139.

In an embodiment, the binding protein capable of binding IL-1b and PGE2 comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 140 and SEQ ID NO. 142; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 141 and SEQ ID NO. 143. In an embodiment, the binding protein capable of binding IL-1b and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 140 and a DVD light chain amino acid sequence of SEQ ID NO: 141. In another embodiment, the binding protein capable of binding IL-1b and PGE2 has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 142 and a DVD light chain amino acid sequence of SEQ ID NO: 143.

In an embodiment, the binding protein capable of binding IL-6 and PGE2 comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 144 and SEQ ID NO. 146; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 145 and SEQ ID NO. 147. In an embodiment, the binding protein capable of binding IL-6 and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 144 and a DVD light chain amino acid sequence of SEQ ID NO: 145. In another embodiment, the binding protein capable of binding IL-6 and PGE2 has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 146 and a DVD light chain amino acid sequence of SEQ ID NO: 147.

In an embodiment, the binding protein capable of binding Abeta (seq. 1) and PGE2 comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 148 and SEQ ID NO. 150; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 149 and SEQ ID NO. 151. In an embodiment, the binding protein capable of binding Abeta (seq. 1) and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 148 and a DVD light chain amino acid sequence of SEQ ID NO: 149. In another embodiment, the binding protein capable of binding Abeta (seq. 1) and PGE2 has a reverse orientation and

comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 150 and a DVD light chain amino acid sequence of SEQ ID NO: 151.

In an embodiment, the binding protein capable of binding Abeta (seq. 2) and PGE2 comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 152 and SEQ ID NO. 154; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 153 and SEQ ID NO. 155. In an embodiment, the binding protein capable of binding Abeta (seq. 2) and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 152 and a DVD light chain amino acid sequence of SEQ ID NO: 153. In another embodiment, the binding protein capable of binding Abeta (seq. 2) and PGE2 has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 154 and a DVD light chain amino acid sequence of SEQ ID NO: 155.

In an embodiment, the binding protein capable of binding Abeta (seq. 3) and PGE2 comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 156 and SEQ ID NO. 158; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 157 and SEQ ID NO. 159. In an embodiment, the binding protein capable of binding Abeta (seq. 3) and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 156 and a DVD light chain amino acid sequence of SEQ ID NO: 157. In another embodiment, the binding protein capable of binding Abeta (seq. 3) and PGE2 has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 158 and a DVD light chain amino acid sequence of SEQ ID NO: 159.

In an embodiment, the binding protein capable of binding IL-18 and PGE2 comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 160 and SEQ ID NO. 162; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 161 and SEQ ID NO. 163. In an embodiment, the binding protein capable of binding IL-18 and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 160 and a DVD light chain amino acid sequence of SEQ ID NO: 161. In another embodiment, the binding protein capable of binding IL-18 and PGE2 has a reverse orientation and comprises a DVD heavy chain

amino acid sequence of SEQ ID NO. 162 and a DVD light chain amino acid sequence of SEQ ID NO: 163.

In an embodiment, the binding protein capable of binding IL-15 and PGE2 comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 164 and SEQ ID NO. 166; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 165 and SEQ ID NO. 167. In an embodiment, the binding protein capable of binding IL-15 and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 164 and a DVD light chain amino acid sequence of SEQ ID NO: 165. In another embodiment, the binding protein capable of binding IL-15 and PGE2 has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 166 and a DVD light chain amino acid sequence of SEQ ID NO: 167.

In an embodiment, the binding protein capable of binding S1P and PGE2 comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 168 and SEQ ID NO. 170; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 169 and SEQ ID NO. 171. In an embodiment, the binding protein capable of binding S1P and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 168 and a DVD light chain amino acid sequence of SEQ ID NO: 169. In another embodiment, the binding protein capable of binding S1P and PGE2 has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 170 and a DVD light chain amino acid sequence of SEQ ID NO: 171.

In an embodiment, the binding protein capable of binding IL-6R and PGE2 comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 172 and SEQ ID NO. 174; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 173 and SEQ ID NO. 175. In an embodiment, the binding protein capable of binding IL-6R and PGE2 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 172 and a DVD light chain amino acid sequence of SEQ ID NO: 173. In another embodiment, the binding protein capable of binding IL-6R and PGE2 has a reverse orientation and comprises a DVD heavy chain

amino acid sequence of SEQ ID NO. 174 and a DVD light chain amino acid sequence of SEQ ID NO: 175.

In another embodiment, the DVD heavy chain amino acid sequence comprises at least one VH region selected from the group consisting of SEQ ID NO. 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 87, 90, 110, and 112; and a DVD light chain amino acid sequence comprising at least one VL region selected from the group consisting of SEQ ID NO. 39, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 88, 91, 111, and 113.

In another embodiment the invention provides a binding protein comprising a polypeptide chain, wherein the polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein; VD1 is a first heavy chain variable domain obtained from a first parent antibody or antigen binding portion thereof; VD2 is a second heavy chain variable domain obtained from a second parent antibody or antigen binding portion thereof; C is a heavy chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein the (X1)n is either present or absent; and (X2)n is an Fc region, wherein the (X2)n is either present or absent. In an embodiment, the Fc region is absent from the binding protein.

In another embodiment, the invention provides a binding protein comprising a polypeptide chain, wherein the polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein, VD1 is a first light chain variable domain obtained from a first parent antibody or antigen binding portion thereof; VD2 is a second light chain variable domain obtained from a second parent antibody or antigen binding portion thereof; C is a light chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein the (X1)n is either present or absent; and (X2)n does not comprise an Fc region, wherein the (X2)n is either present or absent. In an embodiment, (X2)n is absent from the binding protein.

In another embodiment the binding protein of the invention comprises first and second polypeptide chains, wherein the first polypeptide chain comprises a first VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first heavy chain variable domain obtained from a first parent antibody or antigen binding portion thereof; VD2 is a second heavy chain variable domain obtained from a second parent antibody or antigen binding portion thereof; C is a heavy chain constant domain; (X1)n is a linker with the proviso that it is

not CH1, wherein the (X1)n is either present or absent; and (X2)n is an Fc region, wherein the (X2)n is either present or absent; and wherein the second polypeptide chain comprises a second VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first light chain variable domain obtained from a first parent antibody or antigen binding portion thereof; VD2 is a second light chain variable domain obtained from a second parent antibody or antigen binding portion thereof; C is a light chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein the (X1)n is either present or absent; and (X2)n does not comprise an Fc region, wherein the (X2)n is either present or absent. In another embodiment, the binding protein comprises two first polypeptide chains and two second polypeptide chains. In yet another embodiment, (X2)n is absent from the second polypeptide. In still another embodiment, the Fc region, if present in the first polypeptide is selected from the group consisting of native sequence Fc region and a variant sequence Fc region. In still another embodiment, the Fc region is selected from the group consisting of an Fc region from an IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgE, and IgD.

In another embodiment the binding protein of the invention is a DVD-Ig capable of binding two antigens comprising four polypeptide chains, wherein, first and third polypeptide chains comprise VD1-(X1)n-VD2-C-(X2)n, wherein,VD1 is a first heavy chain variable domain obtained from a first parent antibody or antigen binding portion thereof; VD2 is a second heavy chain variable domain obtained from a second parent antibody or antigen binding portion thereof; C is a heavy chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein the (X1)n is either present or absent; and (X2)n is an Fc region, wherein the (X2)n is either present or absent; and wherein second and fourth polypeptide chains comprise VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first light chain variable domain obtained from a first parent antibody or antigen binding portion thereof; VD2 is a second light chain variable domain obtained from a second parent antibody or antigen binding portion thereof; C is a light chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein the (X1)n is either present or absent; and (X2)n does not comprise an Fc region, wherein the (X2)n is either present or absent.

In another aspect, the invention provides a humanized binding protein comprising a polypeptide chain, wherein the polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first humanized variable domain, VD2 is a second

humanized variable domain, C is a constant domain, X1 represents an amino acid or polypeptide, X2 represents an Fc region and n is 0 or 1, wherein the binding protein binds prostaglandin E2 and tumor necrosis factor alpha.

In an embodiment, C is a heavy chain constant domain, such as, for example, C is a human heavy chain constant domain of an antibody class selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgA, IgA1, IgA2, IgD, IgM, IgE, IgY, and IgG mutants. C may be a wild type or mutant heavy chain constant domain. For example, C comprises the sequence of SEQ ID NO: 122 or 123, or functional variant or mutant thereof.

In another embodiment, C is a light chain kappa constant domain. For example, C is a human light chain kappa constant domain of an antibody class selected from the group consisting of IgG, IgG1, IgG2, IgG3, IgG4, IgA, IgA1, IgA2, IgD, IgM, IgE, IgY, and mutants thereof. C may be a wild type or mutant light chain kappa or lambda constant domain. For example, C comprises the sequence of SEQ ID NO: 124 or 125, or functional variant or mutant thereof.

In another aspect, the invention provides a binding protein comprising a polypeptide chain, wherein the polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 does not comprise an Fc region, wherein the binding protein binds prostaglandin E2 and tumor necrosis factor alpha.

In another aspect, the invention provides a binding protein comprising first and second polypeptide chains, wherein the first polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 is an Fc region; and the second polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 does not comprise an Fc region, wherein the binding protein binds prostaglandin E2 and tumor necrosis factor alpha.

In yet another aspect, the invention provides a binding protein comprising four polypeptide chains, wherein two polypeptide chains comprise VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 is an Fc region; and two polypeptide chains comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 does not comprise an Fc region, wherein the binding protein binds prostaglandin E2 and tumor necrosis factor alpha. .

The invention provides a method of making a DVD-Ig binding protein by preselecting the parent antibodies. In an embodiment, the method of making a Dual Variable Domain Immunoglobulin capable of binding two antigens comprising the steps of a) obtaining a first parent antibody or antigen binding portion thereof, capable of binding a first antigen; b) obtaining a second parent antibody or antigen binding portion thereof, capable of binding a second antigen; c) constructing first and third polypeptide chains comprising VD1-(X1)n-VD2-C-(X2)n, wherein, VD1 is a first heavy chain variable domain obtained from the first parent antibody or antigen binding portion thereof; VD2 is a second heavy chain variable domain obtained from the second parent antibody or antigen binding portion thereof; C is a heavy chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein the (X1)n is either present or absent; and (X2)n is an Fc region, wherein the (X2)n is either present or absent; d) constructing second and fourth polypeptide chains comprising VD1-(X1)n-VD2-C-(X2)n, wherein, VD1 is a first light chain variable domain obtained from the first parent antibody or antigen binding portion thereof; VD2 is a second light chain variable domain obtained from the second parent antibody or antigen binding thereof; C is a light chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein the (X1)n is either present or absent; and (X2)n does not comprise an Fc region, wherein the (X2)n is either present or absent; e) expressing the first, second, third and fourth polypeptide chains; such that a Dual Variable Domain Immunoglobulin capable of binding the first and the second antigen is generated.

In still another embodiment, the invention provides a method of generating a Dual Variable Domain Immunoglobulin capable of binding two antigens with desired

properties comprising the steps of a) obtaining a first parent antibody or antigen binding portion thereof, capable of binding a first antigen and possessing at least one desired property exhibited by the Dual Variable Domain Immunoglobulin; b) obtaining a second parent antibody or antigen binding portion thereof, capable of binding a second antigen and possessing at least one desired property exhibited by the Dual Variable Domain Immunoglobulin; c) constructing first and third polypeptide chains comprising VD1-(X1)n-VD2-C-(X2)n, wherein; VD1 is a first heavy chain variable domain obtained from the first parent antibody or antigen binding portion thereof; VD2 is a second heavy chain variable domain obtained from the second parent antibody or antigen binding portion thereof; C is a heavy chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein the (X1)n is either present or absent; and (X2)n is an Fc region, wherein the (X2)n is either present or absent; d) constructing second and fourth polypeptide chains comprising VD1-(X1)n-VD2-C-(X2)n, wherein; VD1 is a first light chain variable domain obtained from the first parent antibody or antigen binding portion thereof; VD2 is a second light chain variable domain obtained from the second parent antibody or antigen binding portion thereof; C is a light chain constant domain; (X1)n is a linker with the proviso that it is not CH1, wherein the (X1)n is either present or absent; and (X2)n does not comprise an Fc region, wherein the (X2)n is either present or absent; e) expressing the first, second, third and fourth polypeptide chains; such that a Dual Variable Domain Immunoglobulin capable of binding the first and the second antigen with desired properties is generated.

In one embodiment, the VDI of the first and second polypeptide chains disclosed herein are obtained from the same parent antibody or antigen binding portion thereof. In another embodiment, the VDI of the first and second polypeptide chains disclosed herein are obtained from different parent antibodies or antigen binding portions thereof. In another embodiment, the VD2 of the first and second polypeptide chains disclosed herein are obtained from the same parent antibody or antigen binding portion thereof. In another embodiment, the VD2 of the first and second polypeptide chains disclosed herein are obtained from different parent antibodies or antigen binding portions thereof.

In one embodiment the first parent antibody or antigen binding portion thereof, and the second parent antibody or antigen binding portion thereof, are the same antibody.

In another embodiment the first parent antibody or antigen binding portion thereof, and the second parent antibody or antigen binding portion thereof, are different antibodies.

In one embodiment the first parent antibody or antigen binding portion thereof, binds a first antigen and the second parent antibody or antigen binding portion thereof, binds a second antigen. In a particular embodiment, the first and second antigens are the same antigen. In another embodiment, the parent antibodies bind different epitopes on the same antigen. In another embodiment the first and second antigens are different antigens. In another embodiment, the first parent antibody or antigen binding portion thereof, binds the first antigen with a potency different from the potency with which the second parent antibody or antigen binding portion thereof, binds the second antigen. In yet another embodiment, the first parent antibody or antigen binding portion thereof, binds the first antigen with an affinity different from the affinity with which the second parent antibody or antigen binding portion thereof, binds the second antigen.

In another embodiment the first parent antibody or antigen binding portion thereof, and the second parent antibody or antigen binding portion thereof, are selected from the group consisting of, human antibody, CDR grafted antibody, and humanized antibody. In an embodiment, the antigen binding portions are selected from the group consisting of a Fab fragment, a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the VH and CH1 domains; a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, a dAb fragment, an isolated complementarity determining region (CDR), a single chain antibody, and diabodies.

In another embodiment the binding protein of the invention possesses at least one desired property exhibited by the first parent antibody or antigen binding portion thereof, or the second parent antibody or antigen binding portion thereof. Alternatively, the first parent antibody or antigen binding portion thereof and the second parent antibody or antigen binding portion thereof possess at least one desired property exhibited by the Dual Variable Domain Immunoglobulin. In an embodiment, the desired property is selected from one or more antibody parameters. In another embodiment, the antibody parameters are selected from the group consisting of antigen specificity, affinity to antigen, potency, biological function, epitope recognition, stability, solubility, production

efficiency, immunogenicity, pharmacokinetics, bioavailability, tissue cross reactivity, and orthologous antigen binding.In an embodiment the binding protein is multivalent. In another embodiment, the binding protein is multispecific. The multivalent and or multispecific binding proteins described herein have desirable properties particularly from a therapeutic standpoint. For instance, the multivalent and or multispecific binding protein may (1) be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind; (2) be an agonist antibody; and/or (3) induce cell death and/or apoptosis of a cell expressing an antigen which the multivalent antibody is capable of binding to. The "parent antibody" which provides at least one antigen binding specificity of the multivalent and or multispecific binding proteins may be one which is internalized (and/or catabolized) by a cell expressing an antigen to which the antibody binds; and/or may be an agonist, cell death-inducing, and/or apoptosis-inducing antibody, and the multivalent and or multispecific binding protein as described herein may display improvement(s) in one or more of these properties. Moreover, the parent antibody may lack any one or more of these properties, but may be endowed with them when constructed as a multivalent binding protein as described herein.

In another embodiment the binding protein of the invention has an on rate constant (Kon) to the one or more targets selected from the group consisting of: at least about $10^2M^{-1}s^{-1}$; at least about $10^3M^{-1}s^{-1}$; at least about $10^4M^{-1}s^{-1}$; at least about $10^5M^{-1}s^{-1}$; and at least about $10^6M^{-1}s^{-1}$, as measured by surface plasmon resonance. In an embodiment, the binding protein of the invention has an on rate constant (Kon) to one or more targets between $10^2M^{-1}s^{-1}$ and $10^3M^{-1}s^{-1}$; between $10^3M^{-1}s^{-1}$ and $10^4M^{-1}s^{-1}$; between $10^4M^{-1}s^{-1}$ and $10^5M^{-1}s^{-1}$; or between $10^5M^{-1}s^{-1}$ and $10^6M^{-1}s^{-1}$, as measured by surface plasmon resonance.

In another embodiment the binding protein has an off rate constant (Koff) for one or more targets selected from the group consisting of: at most about $10^{-3}s^{-1}$; at most about $10^{-4}s^{-1}$; at most about $10^{-5}s^{-1}$; and at most about $10^{-6}s^{-1}$, as measured by surface plasmon resonance. In an embodiment, the binding protein of the invention has an off rate constant (Koff) to one or more targets of $10^{-3}s^{-1}$ to $10^{-4}s^{-1}$; of $10^{-4}s^{-1}$ to $10^{-5}s^{-1}$; or of $10^{-5}s^{-1}$ to $10^{-6}s^{-1}$, as measured by surface plasmon resonance.

In another embodiment the binding protein has a dissociation constant (K_D) to one or more targets selected from the group consisting of: at most about 10^{-7} M; at most about 10^{-8} M; at most about 10^{-9} M; at most about 10^{-10} M; at most about 10^{-11} M; at most about 10^{-12} M; and at most 10^{-13} M. In an embodiment, the binding protein of the invention has a dissociation constant (K_D) to its targets of 10^{-7} M to 10^{-8} M; of 10^{-8} M to 10^{-9} M; of 10^{-9} M to 10^{-10} M; of 10^{-10} to 10^{-11} M; of 10^{-11} M to 10^{-12} M; or of 10^{-12} to M 10^{-13} M.

In another embodiment, the binding protein described herein is a conjugate further comprising an agent selected from the group consisting of an immunoadhesion molecule, an imaging agent, a therapeutic agent, and a cytotoxic agent. In an embodiment, the imaging agent is selected from the group consisting of a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, and biotin. In another embodiment, the imaging agent is a radiolabel selected from the group consisting of: 3H, 14C, 35S, 90Y, 99Tc, 111In, 125I, 131I, 177Lu, 166Ho, and 153Sm. In yet another embodiment, the therapeutic or cytotoxic agent is selected from the group consisting of an anti-metabolite, an alkylating agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, toxin, and an apoptotic agent.

In another embodiment, the binding protein described herein is a crystallized binding protein and exists as a crystal. In an embodiment, the crystal is a carrier-free pharmaceutical controlled release crystal. In yet another embodiment, the crystallized binding protein has a greater half life in vivo than the soluble counterpart of said binding protein. In still another embodiment, the crystallized binding protein retains biological activity.

In another embodiment, the binding protein described herein is glycosylated. For example, the glycosylation is a human glycosylation pattern.

Another aspect of the invention pertains to an isolated nucleic acid encoding any one of the binding proteins disclosed herein. A further embodiment provides a vector comprising the isolated nucleic acid disclosed herein wherein the vector is selected from the group consisting of pcDNA; pTT (Durocher et al., *Nucleic Acids Research* 2002, Vol 30, No.2); pTT3 (pTT with additional multiple cloning site; pEFBOS (Mizushima, S. and Nagata, S., (1990) *Nucleic acids Research* Vol 18, No. 17); pBV; pJV; pcDNA3.1 TOPO,

pEF6 TOPO and pBJ. In an embodiment, the vector is a vector disclosed in US Patent Application Serial No. 61/021,282.

In another aspect a host cell is transformed with the vector disclosed herein. In an embodiment, the host cell is a prokaryotic cell. In another embodiment, the host cell is E.Coli. In a related embodiment the host cell is a eukaryotic cell. In another embodiment, the eukaryotic cell is selected from the group consisting of a protist cell, an animal cell, an avian cell, a plant cell and a fungal cell. In yet another embodiment, the host cell is a mammalian cell including, but not limited to, CHO, COS; NS0, SP2, PER.C6 or a fungal cell such as Saccharomyces cerevisiae; or an insect cell such as Sf9.

In an embodiment, two or more DVD-Igs, e.g., with different specificities, are produced in a single recombinant host cell. For example, the expression of a mixture of antibodies has been called Oligoclonics™,(Merus B.V., The Netherlands) U.S. Patent Nos. 7,262,028; 7,429,486.

Another aspect of the invention provides a method of producing a binding protein disclosed herein comprising culturing any one of the host cells also disclosed herein in a culture medium under conditions sufficient to produce the binding protein. In an embodiment, 50%-75% of the binding protein produced by this method is a dual specific tetravalent binding protein. In a particular embodiment, 75%-90% of the binding protein produced by this method is a dual specific tetravalent binding protein. In a particular embodiment, 90%-95% of the binding protein produced is a dual specific tetravalent binding protein.

One embodiment provides a composition for the release of a binding protein wherein the composition comprises a formulation that in turn comprises a crystallized binding protein, as disclosed herein, and an ingredient, and at least one polymeric carrier. For example, the polymeric carrier is a polymer selected from one or more of the group consisting of: poly (acrylic acid), poly (cyanoacrylates), poly (amino acids), poly (anhydrides), poly (depsipeptide), poly (esters), poly (lactic acid), poly (lactic-co-glycolic acid) or PLGA, poly (b-hydroxybutryate), poly (caprolactone), poly (dioxanone); poly (ethylene glycol), poly ((hydroxypropyl) methacrylamide, poly [(organo)phosphazene], poly (ortho esters), poly (vinyl alcohol), poly (vinylpyrrolidone), maleic anhydride- alkyl vinyl ether copolymers, pluronic polyols, albumin, alginate, cellulose and cellulose

derivatives, collagen, fibrin, gelatin, hyaluronic acid, oligosaccharides, glycaminoglycans, sulfated polysaccharides, blends and copolymers thereof. For example, the ingredient is selected from the group consisting of albumin, sucrose, trehalose, lactitol, gelatin, hydroxypropyl-β- cyclodextrin, methoxypolyethylene glycol and polyethylene glycol. Another embodiment provides a method for treating a mammal comprising the step of administering to the mammal an effective amount of the composition disclosed herein.

The invention also provides a pharmaceutical composition comprising a binding protein, as disclosed herein and a pharmaceutically acceptable carrier. In a further embodiment the pharmaceutical composition comprises at least one additional therapeutic agent for treating a disorder. For example, the additional agent is selected from the group consisting of: a therapeutic agent, an imaging agent, a cytotoxic agent, an angiogenesis inhibitor (including but not limited to an anti-VEGF antibody or a VEGF-trap), a kinase inhibitor (including but not limited to a KDR and a TIE-2 inhibitor), a co-stimulation molecule blocker (including but not limited to anti-B7.1, anti-B7.2, CTLA4-Ig, anti-CD20), an adhesion molecule blocker (including but not limited to an anti-LFA-1 antibody, an anti-E/L selectin antibody, a small molecule inhibitor), an anti-cytokine antibody or functional fragment thereof (including but not limited to an anti-IL-18, an anti-TNF, and an anti-IL-6/cytokine receptor antibody), methotrexate, cyclosporin, rapamycin, FK506, a detectable label or reporter, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteriod, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, and a cytokine antagonist.

In another aspect, the invention provides a method for treating a human subject suffering from a disorder in which the target, or targets, capable of being bound by the binding protein disclosed herein is detrimental, comprising administering to the human subject a binding protein disclosed herein such that the activity of the target, or targets in the human subject is inhibited and one of more symptoms is alleviated or treatment is

achieved. For example, the disorder is selected from the group comprising arthritis, osteoarthritis, juvenile chronic arthritis, septic arthritis, Lyme arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, inflammatory bowel disease, insulin dependent diabetes mellitus, thyroiditis, asthma, allergic diseases, psoriasis, dermatitis scleroderma, graft versus host disease, organ transplant rejection, acute or chronic immune disease associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, Wegener's granulomatosis, Henoch-Schoenlein purpurea, microscopic vasculitis of the kidneys, chronic active hepatitis, uveitis, septic shock, toxic shock syndrome, sepsis syndrome, cachexia, infectious diseases, parasitic diseases, acquired immunodeficiency syndrome, acute transverse myelitis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary cirrhosis, hemolytic anemia, malignancies, heart failure, myocardial infarction, Addison's disease, sporadic polyglandular deficiency type I and polyglandular deficiency type II, Schmidt's syndrome, adult (acute) respiratory distress syndrome, alopecia, alopecia areata, seronegative arthopathy, arthropathy, Reiter's disease, psoriatic arthropathy, ulcerative colitic arthropathy, enteropathic synovitis, chlamydia, yersinia and salmonella associated arthropathy, spondyloarthopathy, atheromatous disease/arteriosclerosis, atopic allergy, autoimmune bullous disease, pemphigus vulgaris, pemphigus foliaceus, pemphigoid, linear IgA disease, autoimmune haemolytic anaemia, Coombs positive haemolytic anaemia, acquired pernicious anaemia, juvenile pernicious anaemia, myalgic encephalitis/Royal Free Disease, chronic mucocutaneous candidiasis, giant cell arteritis, primary sclerosing hepatitis, cryptogenic autoimmune hepatitis, Acquired Immunodeficiency Disease Syndrome, Acquired Immunodeficiency Related Diseases, Hepatitis B, Hepatitis C, common varied immunodeficiency (common variable hypogammaglobulinaemia), dilated cardiomyopathy, female infertility, ovarian failure, premature ovarian failure, fibrotic lung disease, cryptogenic fibrosing alveolitis, post-inflammatory interstitial lung disease, interstitial pneumonitis, connective tissue disease associated interstitial lung disease, mixed connective tissue disease associated lung disease, systemic sclerosis associated interstitial lung disease, rheumatoid arthritis associated interstitial lung disease, systemic lupus erythematosus associated lung disease, dermatomyositis/polymyositis associated lung disease, Sjögren's disease associated lung

disease, ankylosing spondylitis associated lung disease, vasculitic diffuse lung disease, haemosiderosis associated lung disease, drug-induced interstitial lung disease, fibrosis, radiation fibrosis, bronchiolitis obliterans, chronic eosinophilic pneumonia, lymphocytic infiltrative lung disease, postinfectious interstitial lung disease, gouty arthritis, autoimmune hepatitis, type-1 autoimmune hepatitis (classical autoimmune or lupoid hepatitis), type-2 autoimmune hepatitis (anti-LKM antibody hepatitis), autoimmune mediated hypoglycaemia, type B insulin resistance with acanthosis nigricans, hypoparathyroidism, acute immune disease associated with organ transplantation, chronic immune disease associated with organ transplantation, osteoarthrosis, primary sclerosing cholangitis, psoriasis type 1, psoriasis type 2, idiopathic leucopaenia, autoimmune neutropaenia, renal disease NOS, glomerulonephritides, microscopic vasulitis of the kidneys, lyme disease, discoid lupus erythematosus, male infertility idiopathic or NOS, sperm autoimmunity, multiple sclerosis (all subtypes), sympathetic ophthalmia, pulmonary hypertension secondary to connective tissue disease, Goodpasture's syndrome, pulmonary manifestation of polyarteritis nodosa, acute rheumatic fever, rheumatoid spondylitis, Still's disease, systemic sclerosis, Sjörgren's syndrome, Takayasu's disease/arteritis, autoimmune thrombocytopaenia, idiopathic thrombocytopaenia, autoimmune thyroid disease, hyperthyroidism, goitrous autoimmune hypothyroidism (Hashimoto's disease), atrophic autoimmune hypothyroidism, primary myxoedema, phacogenic uveitis, primary vasculitis, vitiligo acute liver disease, chronic liver diseases, alcoholic cirrhosis, alcohol-induced liver injury, choleosatatis, idiosyncratic liver disease, Drug-Induced hepatitis, Non-alcoholic Steatohepatitis, allergy and asthma, group B streptococci (GBS) infection, mental disorders (*e.g.*, depression and schizophrenia), Th2 Type and Th1 Type mediated diseases, acute and chronic pain (different forms of pain), and cancers such as lung, breast, stomach, bladder, colon, pancreas, ovarian, prostate and rectal cancer and hematopoietic malignancies (leukemia and lymphoma), Abetalipoprotemia, Acrocyanosis, acute and chronic parasitic or infectious processes, acute leukemia, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), acute or chronic bacterial infection, acute pancreatitis, acute renal failure, adenocarcinomas, aerial ectopic beats, AIDS dementia complex, alcohol-induced hepatitis, allergic conjunctivitis, allergic contact dermatitis, allergic rhinitis, allograft rejection, alpha-1- antitrypsin deficiency, amyotrophic lateral sclerosis, anemia, angina pectoris, anterior horn cell degeneration, anti cd3 therapy, antiphospholipid syndrome,

anti-receptor hypersensitivity reactions, aortic and peripheral aneuryisms, aortic dissection, arterial hypertension, arteriosclerosis, arteriovenous fistula, ataxia, atrial fibrillation (sustained or paroxysmal), atrial flutter, atrioventricular block, B cell lymphoma, bone graft rejection, bone marrow transplant (BMT) rejection, bundle branch block, Burkitt's lymphoma, Burns, cardiac arrhythmias, cardiac stun syndrome, cardiac tumors, cardiomyopathy, cardiopulmonary bypass inflammation response, cartilage transplant rejection, cerebellar cortical degenerations, cerebellar disorders, chaotic or multifocal atrial tachycardia, chemotherapy associated disorders, chronic myelocytic leukemia (CML), chronic alcoholism, chronic inflammatory pathologies, chronic lymphocytic leukemia (CLL), chronic obstructive pulmonary disease (COPD), chronic salicylate intoxication, colorectal carcinoma, congestive heart failure, conjunctivitis, contact dermatitis, cor pulmonale, coronary artery disease, Creutzfeldt-Jakob disease, culture negative sepsis, cystic fibrosis, cytokine therapy associated disorders, Dementia pugilistica, demyelinating diseases, dengue hemorrhagic fever, dermatitis, dermatologic conditions, diabetes, diabetes mellitus, diabetic ateriosclerotic disease, Diffuse Lewy body disease, dilated congestive cardiomyopathy, disorders of the basal ganglia, Down's Syndrome in middle age, drug- induced movement disorders induced by drugs which block CNS dopamine receptors, drug sensitivity, eczema, encephalomyelitis, endocarditis, endocrinopathy, epiglottitis, epstein-barr virus infection, erythromelalgia, extrapyramidal and cerebellar disorders, familial hematophagocytic lymphohistiocytosis, fetal thymus implant rejection, Friedreich's ataxia, functional peripheral arterial disorders, fungal sepsis, gas gangrene, gastric ulcer, glomerular nephritis, graft rejection of any organ or tissue, gram negative sepsis, gram positive sepsis, granulomas due to intracellular organisms, hairy cell leukemia, Hallerrorden-Spatz disease, hashimoto's thyroiditis, hay fever, heart transplant rejection, hemachromatosis, hemodialysis, hemolytic uremic syndrome/thrombolytic thrombocytopenic purpura, hemorrhage, hepatitis (A), His bundle arrythmias, HIV infection/HIV neuropathy, Hodgkin's disease, hyperkinetic movement disorders, hypersensitity reactions, hypersensitivity pneumonitis, hypertension, hypokinetic movement disorders, hypothalamic-pituitary-adrenal axis evaluation, idiopathic Addison's disease, idiopathic pulmonary fibrosis, antibody mediated cytotoxicity, Asthenia, infantile spinal muscular atrophy, inflammation of the aorta, influenza a, ionizing radiation exposure, iridocyclitis/uveitis/optic neuritis, ischemia- reperfusion injury, ischemic stroke, juvenile rheumatoid arthritis, juvenile

spinal muscular atrophy, Kaposi's sarcoma, kidney transplant rejection, legionella, leishmaniasis, leprosy, lesions of the corticospinal system, lipedema, liver transplant rejection, lymphederma, malaria, malignamt Lymphoma, malignant histiocytosis, malignant melanoma, meningitis, meningococcemia, metabolic/idiopathic diseases, migraine headache, mitochondrial multi.system disorder, mixed connective tissue disease, monoclonal gammopathy, multiple myeloma, multiple systems degenerations (Mencel Dejerine- Thomas Shi-Drager and Machado-Joseph), myasthenia gravis, mycobacterium avium intracellulare, mycobacterium tuberculosis, myelodyplastic syndrome, myocardial infarction, myocardial ischemic disorders, nasopharyngeal carcinoma, neonatal chronic lung disease, nephritis, nephrosis, neurodegenerative diseases, neurogenic I muscular atrophies, neutropenic fever, non- hodgkins lymphoma, occlusion of the abdominal aorta and its branches, occlusive arterial disorders, okt3 therapy, orchitis/epidydimitis, orchitis/vasectomy reversal procedures, organomegaly, osteoporosis, pancreas transplant rejection, pancreatic carcinoma, paraneoplastic syndrome/hypercalcemia of malignancy, parathyroid transplant rejection, pelvic inflammatory disease, perennial rhinitis, pericardial disease, peripheral atherlosclerotic disease, peripheral vascular disorders, peritonitis, pernicious anemia, pneumocystis carinii pneumonia, pneumonia, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes syndrome), post perfusion syndrome, post pump syndrome, post-MI cardiotomy syndrome, preeclampsia, Progressive supranucleo Palsy, primary pulmonary hypertension, radiation therapy, Raynaud's phenomenon and disease, Raynoud's disease, Refsum's disease, regular narrow QRS tachycardia, renovascular hypertension, reperfusion injury, restrictive cardiomyopathy, sarcomas, scleroderma, senile chorea, Senile Dementia of Lewy body type, seronegative arthropathies, shock, sickle cell anemia, skin allograft rejection, skin changes syndrome, small bowel transplant rejection, solid tumors, specific arrythmias, spinal ataxia, spinocerebellar degenerations, streptococcal myositis, structural lesions of the cerebellum, Subacute sclerosing panencephalitis, Syncope, syphilis of the cardiovascular system, systemic anaphalaxis, systemic inflammatory response syndrome, systemic onset juvenile rheumatoid arthritis, T-cell or FAB ALL, Telangiectasia, thromboangitis obliterans, thrombocytopenia, toxicity, transplants, trauma/hemorrhage, type III hypersensitivity reactions, type IV hypersensitivity, unstable angina, uremia, urosepsis, urticaria, valvular heart diseases, varicose veins, ,vasculitis, venous diseases, venous thrombosis, ventricular fibrillation,

viral and fungal infections, vital encephalitis/aseptic meningitis, vital-associated hemaphagocytic syndrome, Wernicke- Korsakoff syndrome, Wilson's disease, xenograft rejection of any organ or tissue, acute coronary syndromes, acute idiopathic polyneuritis, acute inflammatory demyelinating polyradiculoneuropathy, acute ischemia, adult Still's disease, alopecia areata, anaphylaxis, anti-phospholipid antibody syndrome, aplastic anemia, arteriosclerosis, atopic eczema, atopic dermatitis, autoimmune dermatitis, autoimmune disorder associated with streptococcus infection, autoimmune enteropathy, autoimmune hearing loss, autoimmune lymphoproliferative syndrome (ALPS), autoimmune myocarditis, autoimmune premature ovarian failure, blepharitis, bronchiectasis, bullous pemphigoid, cardiovascular disease, catastrophic antiphospholipid syndrome, celiac disease, cervical spondylosis, chronic ischemia, cicatricial pemphigoid, clinically isolated syndrome (cis) with risk for multiple sclerosis, conjunctivitis, childhood onset psychiatric disorder, chronic obstructive pulmonary disease (COPD), dacryocystitis, dermatomyositis, diabetic retinopathy, diabetes mellitus, disk herniation, disk prolaps, drug induced immune hemolytic anemia, endocarditis, endometriosis, endophthalmitis, episcleritis, erythema multiforme, erythema multiforme major, gestational pemphigoid, Guillain-Barré syndrome (GBS), hay fever, Hughes syndrome, idiopathic Parkinson's disease, idiopathic interstitial pneumonia, IgE-mediated allergy, immune hemolytic anemia, inclusion body myositis, infectious ocular inflammatory disease, inflammatory demyelinating disease, inflammatory heart disease, inflammatory kidney disease, IPF/UIP, iritis, keratitis, keratojuntivitis sicca, Kussmaul disease or Kussmaul-Meier disease, Landry's paralysis, Langerhan's cell histiocytosis, livedo reticularis, macular degeneration, microscopic polyangiitis, morbus bechterev, motor neuron disorders, mucous membrane pemphigoid, multiple organ failure, myasthenia gravis, myelodysplastic syndrome, myocarditis, nerve root disorders, neuropathy, non-A non-B hepatitis, optic neuritis, osteolysis, ovarian cancer, pauciarticular JRA, peripheral artery occlusive disease (PAOD), peripheral vascular disease (PVD), peripheral artery, disease (PAD), phlebitis, polyarteritis nodosa (or periarteritis nodosa), polychondritis, polymyalgia rheumatica, poliosis, polyarticular JRA, polyendocrine deficiency syndrome, polymyositis, polymyalgia rheumatica (PMR), post-pump syndrome, primary Parkinsonism, prostate and rectal cancer and hematopoietic malignancies (leukemia and lymphoma), prostatitis, pure red cell aplasia, primary adrenal insufficiency, recurrent neuromyelitis optica, restenosis, rheumatic heart disease, sapho (synovitis, acne,

pustulosis, hyperostosis, and osteitis), scleroderma, secondary amyloidosis, shock lung, scleritis, sciatica, secondary adrenal insufficiency, silicone associated connective tissue disease, sneddon-wilkinson dermatosis, spondilitis ankylosans, Stevens-Johnson syndrome (SJS), systemic inflammatory response syndrome, temporal arteritis, toxoplasmic retinitis, toxic epidermal necrolysis, transverse myelitis, TRAPS (tumor necrosis factor receptor, type 1 allergic reaction, type II diabetes, urticaria, usual interstitial pneumonia (UIP), vasculitis, vernal conjunctivitis, viral retinitis, Vogt-Koyanagi-Harada syndrome (VKH syndrome), wet macular degeneration, wound healing, yersinia and salmonella associated arthropathy.

In an embodiment, diseases that can be treated or diagnosed with the compositions and methods of the invention include, but are not limited to, primary and metastatic cancers, including carcinomas of breast, colon, rectum, lung, oropharynx, hypopharynx, esophagus, stomach, pancreas, liver, gallbladder and bile ducts, small intestine, urinary tract (including kidney, bladder and urothelium), female genital tract (including cervix, uterus, and ovaries as well as choriocarcinoma and gestational trophoblastic disease), male genital tract (including prostate, seminal vesicles, testes and germ cell tumors), endocrine glands (including the thyroid, adrenal, and pituitary glands), and skin, as well as hemangiomas, melanomas, sarcomas (including those arising from bone and soft tissues as well as Kaposi's sarcoma), tumors of the brain, nerves, eyes, and meninges (including astrocytomas, gliomas, glioblastomas, retinoblastomas, neuromas, neuroblastomas, Schwannomas, and meningiomas), solid tumors arising from hematopoietic malignancies such as leukemias, and lymphomas (both Hodgkin's and non-Hodgkin's lymphomas).

The DVD-Igs of the invention may also treat one or more of the following diseases: Acute coronary syndromes, Acute Idiopathic Polyneuritis, Acute Inflammatory Demyelinating Polyradiculoneuropathy, Acute ischemia, Adult Still's Disease, Alopecia areata, Anaphylaxis, Anti-Phospholipid Antibody Syndrome, Aplastic anemia, Arteriosclerosis, Atopic eczema, Atopic dermatitis, Autoimmune dermatitis, Autoimmune disorder associated with Streptococcus infection, Autoimmune hearingloss, Autoimmune Lymphoproliferative Syndrome (ALPS), Autoimmune myocarditis, autoimmune thrombocytopenia (AITP), Blepharitis, Bronchiectasis, Bullous pemphigoid, Cardiovascular Disease, Catastrophic Antiphospholipid Syndrome, Celiac Disease,

Cervical Spondylosis, Chronic ischemia, Cicatricial pemphigoid, Clinically isolated Syndrome (CIS) with Risk for Multiple Sclerosis, Conjunctivitis, Childhood Onset Psychiatric Disorder, Chronic obstructive pulmonary disease (COPD), Dacryocystitis, dermatomyositis, Diabetic retinopathy, Diabetes mellitus, Disk herniation, Disk prolaps, Drug induced immune hemolytic anemia, Endocarditis, Endometriosis, endophthalmitis, , Episcleritis, Erythema multiforme, erythema multiforme major, Gestational pemphigoid, Guillain-Barré Syndrome (GBS), Hay Fever, Hughes Syndrome , Idiopathic Parkinson's Disease, idiopathic interstitial pneumonia, IgE-mediated Allergy, Immune hemolytic anemia, Inclusion Body Myositis, Infectious ocular inflammatory disease, Inflammatory demyelinating disease, Inflammatory heart disease, Inflammatory kidney disease, IPF/UIP, Iritis, Keratitis, Keratojuntivitis sicca, Kussmaul disease or Kussmaul-Meier Disease, Landry's Paralysis, Langerhan's Cell Histiocytosis, Livedo reticularis, Macular Degeneration, malignancies, Microscopic Polyangiitis, Morbus Bechterev, Motor Neuron Disorders, Mucous membrane pemphigoid, Multiple Organ failure, Myasthenia Gravis, Myelodysplastic Syndrome, Myocarditis, Nerve Root Disorders, Neuropathy, Non-A Non-B Hepatitis, Optic Neuritis, Osteolysis, Ovarian cancer, Pauciarticular JRA, peripheral artery occlusive disease (PAOD), peripheral vascular disease (PVD), peripheral artery disease (PAD), Phlebitis, Polyarteritis nodosa (or periarteritis nodosa), Polychondritis, Polymyalgia Rheumatica, Poliosis, Polyarticular JRA, Polyendocrine Deficiency Syndrome, Polymyositis, polymyalgia rheumatica (PMR), Post-Pump Syndrome, primary parkinsonism, prostate and rectal cancer and hematopoietic malignancies (leukemia and lymphoma), Prostatitis, Pure red cell aplasia, Primary Adrenal Insufficiency, Recurrent Neuromyelitis Optica, Restenosis, Rheumatic heart disease, SAPHO (synovitis, acne, pustulosis, hyperostosis, and osteitis), Scleroderma, Secondary Amyloidosis, Shock lung, Scleritis, Sciatica, Secondary Adrenal Insufficiency, Silicone associated connective tissue disease, Sneddon-Wilkinson Dermatosis, spondilitis ankylosans, Stevens-Johnson Syndrome (SJS), Systemic inflammatory response syndrome, Temporal arteritis, toxoplasmic retinitis, toxic epidermal necrolysis, Transverse myelitis, TRAPS (Tumor Necrosis Factor Receptor, Type 1 allergic reaction, Type II Diabetes, Urticaria, Usual interstitial pneumonia (UIP), Vasculitis, Vernal conjunctivitis, viral retinitis, Vogt-Koyanagi-Harada syndrome (VKH syndrome), Wet macular degeneration, and Wound healing.

In an embodiment, the antibodies of the invention or antigen-binding portions thereof, are used to treat cancer or in the prevention of metastases from the tumors described herein either when used alone or in combination with radiotherapy and/or other chemotherapeutic agents.

In another aspect the invention provides a method of treating a patient suffering from a disorder comprising the step of administering any one of the binding proteins disclosed herein before, concurrent, or after the administration of a second agent, as discussed herein. In a particular embodiment the second agent is selected from the group consisting of budenoside, epidermal growth factor, corticosteroids, cyclosporin, sulfasalazine, aminosalicylates, 6-mercaptopurine, azathioprine, metronidazole, lipoxygenase inhibitors, mesalamine, olsalazine, balsalazide, antioxidants, thromboxane inhibitors, IL-1 receptor antagonists, anti-IL-1β mAbs, anti-IL-6 or IL-6 receptor mAbs, growth factors, elastase inhibitors, pyridinyl-imidazole compounds, antibodies or agonists of TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-12, IL-13, IL-15, IL-16, IL-18, IL-23, EMAP-II, GM-CSF, FGF, and PDGF, antibodies of CD2, CD3, CD4, CD8, CD-19, CD25, CD28, CD30, CD40, CD45, CD69, CD90 or their ligands, methotrexate, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, ibuprofen, corticosteroids, prednisolone, phosphodiesterase inhibitors, adensosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, IRAK, NIK, IKK, p38, MAP kinase inhibitors, IL-1β converting enzyme inhibitors, TNFαconverting enzyme inhibitors, T-cell signalling inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors, soluble p55 TNF receptor, soluble p75 TNF receptor, sIL-1RI, sIL-1RII, sIL-6R, antiinflammatory cytokines, IL-4, IL-10, IL-11, IL-13 and TGFβ.

In a particular embodiment the pharmaceutical compositions disclosed herein are administered to the patient by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, and transdermal.

One aspect of the invention provides at least one anti-idiotype antibody to at least one binding protein of the present invention. The anti-idiotype antibody includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule such as, but not limited to, at least one complementarily determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework region, or any portion thereof, that can be incorporated into a binding protein of the present invention.

Brief Description of the Drawings

The foregoing and other objects, features and advantages of the present invention, as well as the invention itself, will be more fully understood from the following description of preferred embodiments when read together with the accompanying drawings, in which:

Figure 1A is a schematic representation of Dual Variable Domain (DVD)-Ig constructs and shows the strategy for generation of a DVD-Ig from two parent antibodies;

Figure 1B, is a schematic representation of a TNF/PGE2 DVD-Ig, without (DVD1-Ig) and with (DVD2-Ig) linkers, and two parental mono-specific antibodies: anti-TNF D2E7 (α) and anti-PGE2 2B5-8.0 (β).

Figure 2 shows ELISA data of binding of 2B5-7.0, 2B5-8.0, 2B5-9.0, and 2B5-10.0 to PGE2-biotin.

Figure 3 shows EP4 bioassay data of binding of 2B5-8.0 relative to a 2B5 and IgG1.

Figure 4 shows the effect of anti-TNF mAb 8C11 and anti-PGE2 mAb 2B5, alone and in combination, on mean arthritic score in mice injected twice weekly over a 15 day period.

Figure 5 shows temperature-induced unfolding of Adalimumab (IgG1). Three unfolding transitions can be shown, demonstrating unfolding of 3 domains as typical for antibodies. Tm values are 56.9 °C, 67.4 °C, and 76.75 °C (pH 4.3 buffer).

Figure 6 shows temperature-induced unfolding of D2E7-SL-Hu2B5.7 DVD-Ig. Four unfolding transitions can be shown, demonstrating unfolding of 4 domains. Tm values are 58.8°C, 68.6°C, 75.0°C, and 83.4°C (pH 6.0 buffer).

Figure 7 shows temperature-induced unfolding of Adalimumab (IgG1). Three unfolding transitions can be shown, demonstrating unfolding of 3 domains as typical for antibodies. Tm values are 68.3 °C, 75.4 °C, and 83.1 °C (pH 6.1 buffer).

Detailed Description of the Invention

This invention pertains to multivalent and/or multispecific binding proteins capable of binding two or more antigens. Specifically, the invention relates to dual variable domain immunoglobulins (DVD-Ig), and pharmaceutical compositions thereof, as well as nucleic acids, recombinant expression vectors and host cells for making such DVD-Igs. Methods of using the DVD-Igs of the invention to detect specific antigens, either in vitro or in vivo are also encompassed by the invention.

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. The meaning and scope of the terms should be clear, however, in the event of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. In this application, the use of "or" means "and/or" unless stated otherwise. Furthermore, the use of the term "including", as well as other forms, such as "includes" and "included", is not limiting. Also, terms such as "element" or "component" encompass both elements and components comprising one unit and elements and components that comprise more than one subunit unless specifically stated otherwise.

Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art. The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as

described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

That the present invention may be more readily understood, select terms are defined below.

The term "polypeptide" as used herein, refers to any polymeric chain of amino acids. The terms "peptide" and "protein" are used interchangeably with the term polypeptide and also refer to a polymeric chain of amino acids. The term "polypeptide" encompasses native or artificial proteins, protein fragments and polypeptide analogs of a protein sequence. A polypeptide may be monomeric or polymeric. Use of "polypeptide" herein is intended to encompass polypeptide and fragments and variants (including fragments of variants) thereof, unless otherwise contradicted by context. For an antigenic polypeptide, a fragment of polypeptide optionally contains at least one contiguous or nonlinear epitope of polypeptide. The precise boundaries of the at least one epitope fragment can be confirmed using ordinary skill in the art. The fragment comprises at least about 5 contiguous amino acids, such as at least about 10 contiguous amino acids, at least about 15 contiguous amino acids, or at least about 20 contiguous amino acids. A variant of polypeptide is as described herein.

The term "isolated protein" or "isolated polypeptide" is a protein or polypeptide that by virtue of its origin or source of derivation is not associated with naturally associated components that accompany it in its native state; is substantially free of other proteins from the same species; is expressed by a cell from a different species; or does not occur in nature. Thus, a polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be "isolated" from its naturally associated components. A protein may also be rendered substantially

free of naturally associated components by isolation, using protein purification techniques well known in the art.

The term "recovering" as used herein, refers to the process of rendering a chemical species such as a polypeptide substantially free of naturally associated components by isolation, e.g., using protein purification techniques well known in the art.

"Biological activity" as used herein, refers to any one or more inherent biological properties of a molecule. (whether present naturally as found in vivo, or provided or enabled by recombinant means). Biological properties include but are not limited to binding receptor; induction of cell proliferation, inhibiting cell growth, inductions of other cytokines, induction of apoptosis, and enzymatic activity. Biological activity also includes activity of an Ig molecule.

The terms "specific binding" or "specifically binding", as used herein, in reference to the interaction of an antibody, a protein, or a peptide with a second chemical species, mean that the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the chemical species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody is specific for epitope "A", the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled "A" and the antibody, will reduce the amount of labeled A bound to the antibody.

The term "antibody", as used herein, broadly refers to any immunoglobulin (Ig) molecule comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains, or any functional fragment, mutant, variant, or derivation thereof, which retains the essential epitope binding features of an Ig molecule. Such mutant, variant, or derivative antibody formats are known in the art. Nonlimiting embodiments of which are discussed below.

In a full-length antibody, each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of

one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG 1, IgG2, IgG 3, IgG4, IgA1 and IgA2) or subclass.

The term "Fc region" is used to define the C-terminal region of an immunoglobulin heavy chain, which may be generated by papain digestion of an intact antibody. The Fc region may be a native sequence Fc region or a variant Fc region. The Fc region of an immunoglobulin generally comprises two constant domains, a CH2 domain and a CH3 domain, and optionally comprises a CH4 domain. Replacements of amino acid residues in the Fc portion to alter antibody effector function are known in the art (Winter, *et al.* US Patent Nos 5,648,260 and 5,624,821). The Fc portion of an antibody mediates several important effector functions e.g.,cytokine induction, ADCC, phagocytosis, complement dependent cytotoxicity (CDC) and half-life/ clearance rate of antibody and antigen-antibody complexes. In some cases these effector functions are desirable for therapeutic antibody but in other cases might be unnecessary or even deleterious, depending on the therapeutic objectives. Certain human IgG isotypes, particularly IgG1 and IgG3, mediate ADCC and CDC via binding to FcγRs and complement C1q, respectively. Neonatal Fc receptors (FcRn) are the critical components determining the circulating half-life of antibodies. In still another embodiment at least one amino acid residue is replaced in the constant region of the antibody, for example the Fc region of the antibody, such that effector functions of the antibody are altered. The dimerization of two identical heavy chains of an immunoglobulin is mediated by the dimerization of CH3 domains and is stabilized by the disulfide bonds within the hinge region (Huber et al. Nature; 264: 415-20; Thies et al 1999 J Mol Biol; 293: 67-79.). Mutation of cysteine residues within the hinge regions to prevent heavy chain-heavy chain disulfide bonds will destabilize dimeration of CH3 domains. Residues responsible for CH3 dimerization have been identified (Dall'Acqua 1998 Biochemistry 37: 9266-73.). Therefore, it is possible to generate a monovalent half-Ig. Interestingly, these monovalent half Ig molecules have been found in nature for both IgG and IgA subclasses

(Seligman 1978 Ann Immunol 129: 855-70;Biewenga et al 1983 Clin Exp Immunol 51: 395-400). The stoichiometry of FcRn: Ig Fc region has been determined to be 2:1 (West et al .2000 Biochemistry 39: 9698-708), and half Fc is sufficient for mediating FcRn binding (Kim et al 1994 Eur J Immunol; 24: 542-548.). Mutations to disrupt the dimerization of CH3 domain may not have greater adverse effect on its FcRn binding as the residues important for CH3 dimerization are located on the inner interface of CH3 b sheet structure, whereas the region responsible for FcRn binding is located on the outside interface of CH2-CH3 domains. However the half Ig molecule may have certain advantage in tissue penetration due to its smaller size than that of a regular antibody. In one embodiment at least one amino acid residue is replaced in the constant region of the binding protein of the invention, for example the Fc region, such that the dimerization of the heavy chains is disrupted, resulting in half DVD Ig molecules. The anti-inflammatory activity of IgG is completely dependent on sialylation of the N-linked glycan of the IgG Fc fragment. The precise glycan requirements for anti-inflammatory activity has been determined, such that an appropriate IgG1 Fc fragment can be created, thereby generating a fully recombinant, sialylated IgG1 Fc with greatly enhanced potency (Anthony, R.M., et al. (2008) Science 320:373-376).

The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen. It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Such antibody embodiments may also be bispecific, dual specific, or multi-specific formats; specifically binding to two or more different antigens. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546, Winter et al., PCT publication WO 90/05144 A1), which comprises a single variable domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using

recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see *e.g.*, Holliger, P., *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R.J., *et al.* (1994) *Structure* 2:1121-1123). Such antibody binding portions are known in the art (Kontermann and Dubel eds., Antibody Engineering (2001) Springer-Verlag. New York. 790 pp. (ISBN 3-540-41354-5). In addition single chain antibodies also include "linear antibodies" comprising a pair of tandem Fv segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions (Zapata et al. Protein Eng. 8(10):1057-1062 (1995); and US Patent No. 5,641,870).

The term "multivalent binding protein" is used throughout this specification to denote a binding protein comprising two or more antigen binding sites. In an embodiment, the multivalent binding protein is engineered to have the three or more antigen binding sites, and is generally not a naturally occurring antibody. The term "multispecific binding protein" refers to a binding protein capable of binding two or more related or unrelated targets. Dual variable domain (DVD) binding proteins of the invention comprise two or more antigen binding sites and are tetravalent or multivalent binding proteins. DVDs may be monospecific, i.e., capable of binding one antigen or multispecific, i.e. capable of binding two or more antigens. DVD binding proteins comprising two heavy chain DVD polypeptides and two light chain DVD polypeptides are referred to as DVD-Ig. Each half of a DVD-Ig comprises a heavy chain DVD polypeptide, and a light chain DVD polypeptide, and two antigen binding sites. Each binding site comprises a heavy chain variable domain and a light chain variable domain with a total of 6 CDRs involved in antigen binding per antigen binding site.

The term "bispecific antibody", as used herein, refers to full-length antibodies that are generated by quadroma technology (see Milstein, C. and A.C. Cuello, Nature, 1983. 305(5934): p. 537-40), by chemical conjugation of two different monoclonal antibodies (see Staerz, U.D., et al., Nature, 1985. 314(6012): p. 628-31), or by knob-into-hole or similar approaches which introduces mutations in the Fc region (see Holliger, P., T. Prospero, and G. Winter, Proc Natl Acad Sci U S A, 1993. 90(14): p. 6444-8.18), resulting in multiple different immunoglobulin species of which only one is the functional bispecific antibody. By molecular function, a bispecific antibody binds one antigen (or epitope) on one of its two binding arms (one pair of HC/LC), and binds a different antigen (or epitope) on its second arm (a different pair of HC/LC). By this definition, a bispecific antibody has two distinct antigen binding arms (in both specificity and CDR sequences), and is monovalent for each antigen it binds to.

The term "dual-specific antibody", as used herein, refers to full-length antibodies that can bind two different antigens (or epitopes) in each of its two binding arms (a pair of HC/LC) (see PCT publication WO 02/02773). Accordingly a dual-specific binding protein has two identical antigen binding arms, with identical specificity and identical CDR sequences, and is bivalent for each antigen it binds to.

A "functional antigen binding site" of a binding protein is one that is capable of binding a target antigen. The antigen binding affinity of the antigen binding site is not necessarily as strong as the parent antibody from which the antigen binding site is derived, but the ability to bind antigen must be measurable using any one of a variety of methods known for evaluating antibody binding to an antigen. Moreover, the antigen binding affinity of each of the antigen binding sites of a multivalent antibody herein need not be quantitatively the same.

The term "cytokine" is a generic term for proteins released by one cell population, which act on another cell population as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH);

hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-alpha and - beta; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-alpha; platelet-growth factor; placental growth factor, transforming growth factors (TGFs) such as TGF- alpha and TGF-beta; insulin-like growth factor-1 and -11; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-alpha, -beta and -gamma colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-18, IL-21, IL-22, IL-23, IL-33; a tumor necrosis factor such as TNF-alpha or TNF-beta; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

The term "linker" is used to denote polypeptides comprising two or more amino acid residues joined by peptide bonds and are used to link one or more antigen binding portions. Such linker polypeptides are well known in the art (see *e.g.*, Holliger, P., *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R.J., *et al.* (1994) *Structure* 2:1121-1123). Exemplary linkers include, but are not limited to, AKTTPKLEEGEFSEAR (SEQ ID NO: 1); AKTTPKLEEGEFSEARV (SEQ ID NO: 2); AKTTPKLGG (SEQ ID NO: 3); SAKTTPKLGG (SEQ ID NO: 4); SAKTTP (SEQ ID NO: 5); RADAAP (SEQ ID NO: 6); RADAAPTVS (SEQ ID NO: 7); RADAAAAGGPGS (SEQ ID NO: 8); RADAAAA$(G_4S)_4$ (SEQ ID NO: 9), SAKTTPKLEEGEFSEARV (SEQ ID NO: 10); ADAAP (SEQ ID NO: 11); ADAAPTVSIFPP (SEQ ID NO: 12); TVAAP (SEQ ID NO: 13); TVAAPSVFIFPP (SEQ ID NO: 14); QPKAAP (SEQ ID NO: 15); QPKAAPSVTLFPP (SEQ ID NO: 16); AKTTPP (SEQ ID NO: 17); AKTTPPSVTPLAP (SEQ ID NO: 18); AKTTAP (SEQ ID NO: 19); AKTTAPSVYPLAP (SEQ ID NO: 20); ASTKGP (SEQ ID NO: 21); ASTKGPSVFPLAP (SEQ ID NO: 22), GGGGSGGGGSGGGGS (SEQ ID NO: 23); GENKVEYAPALMALS (SEQ ID NO: 24); GPAKELTPLKEAKVS (SEQ ID NO: 25); and GHEAAAVMQVQYPAS (SEQ ID NO: 26).

An "immunoglobulin constant domain" refers to a heavy or light chain constant domain. Human IgG heavy chain and light chain constant domain amino acid sequences are known in the art.

The term "monoclonal antibody" or "mAb" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigen. Furthermore, in contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each mAb is directed against a single determinant on the antigen. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method.

The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further in Section II C, below), antibodies isolated from a recombinant, combinatorial human antibody library (Hoogenboom H.R. (1997) TIB Tech. 15:62-70; Azzazy H., and Highsmith W.E. (2002) Clin. Biochem. 35:425-445; Gavilondo J.V., and Larrick J.W. (2002) BioTechniques 29:128-145; Hoogenboom H., and Chames P. (2000) Immunology Today 21:371-378), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see, Taylor, L. D., et al. (1992) Nucl. Acids Res. 20:6287-6295; Kellermann S-A. and Green L.L.

(2002) Current Opinion in Biotechnology 13:593-597; Little M. et al. (2000) Immunology Today 21:364-370) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire in vivo.

An "affinity matured" antibody is an antibody with one or more alterations in one or more CDRs thereof which result an improvement in the affinity of the antibody for antigen, compared to a parent antibody which does not possess those alteration(s). Exemplary affinity matured antibodies will have nanomolar or even picomolar affinities for the target antigen. Affinity matured antibodies are produced by procedures known in the art. Marks et al. BidlTechnology 10:779-783 (1992) describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of CDR and/or framework residues is described by: Barbas et al. Proc Nat. Acad. Sci, USA 91:3809-3813 (1994); Schier et al. Gene 169:147- 155 (1995); Yelton et al. J. Immunol. 155:1994-2004 (1995); Jackson et al., J. Immunol. 154(7):3310-9 (1995); Hawkins et al, J. Mol. Biol. 226:889-896 (1992) and selective mutation at selective mutagenesis positions, contact or hypermutation positions with an activity enhancing amino acid residue as described in US patent US 6914128B1.

The term "chimeric antibody" refers to antibodies which comprise heavy and light chain variable region sequences from one species and constant region sequences from another species, such as antibodies having murine heavy and light chain variable regions linked to human constant regions.

The term "CDR-grafted antibody" refers to antibodies which comprise heavy and light chain variable region sequences from one species but in which the sequences of one or more of the CDR regions of VH and/or VL are replaced with CDR sequences of

another species, such as antibodies having murine heavy and light chain variable regions in which one or more of the murine CDRs (e.g., CDR3) has been replaced with human CDR sequences.

The term "humanized antibody" refers to antibodies which comprise heavy and light chain variable region sequences from a non-human species (e.g., a mouse) but in which at least a portion of the VH and/or VL sequence has been altered to be more "human-like", i.e., more similar to human germline variable sequences. One type of humanized antibody is a CDR-grafted antibody, in which human CDR sequences are introduced into non-human VH and VL sequences to replace the corresponding nonhuman CDR sequences. Also "humanized antibody"is an antibody or a variant, derivative, analog or fragment thereof which immunospecifically binds to an antigen of interest and which comprises a framework (FR) region having substantially the amino acid sequence of a human antibody and a complementary determining region (CDR) having substantially the amino acid sequence of a non-human antibody. As used herein, the term "substantially" in the context of a CDR refers to a CDR having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of a non-human antibody CDR. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', F(ab') 2, FabC, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (i.e., donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. In an embodiment, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. In some embodiments, a humanized antibody contains both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. In some embodiments, a humanized antibody only contains a humanized light chain. In some embodiments, a humanized antibody only contains a humanized heavy chain. In specific embodiments, a humanized antibody only contains a humanized variable domain of a light chain and/or humanized heavy chain.

The terms "Kabat numbering", "Kabat definitions" and "Kabat labeling" are used interchangeably herein. These terms, which are recognized in the art, refer to a system of

numbering amino acid residues which are more variable (*i.e.* hypervariable) than other amino acid residues in the heavy and light chain variable regions of an antibody, or an antigen binding portion thereof (Kabat *et al.* (1971) *Ann. NY Acad, Sci.* 190:382-391 and, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). For the heavy chain variable region, the hypervariable region ranges from amino acid positions 31 to 35 for CDR1, amino acid positions 50 to 65 for CDR2, and amino acid positions 95 to 102 for CDR3. For the light chain variable region, the hypervariable region ranges from amino acid positions 24 to 34 for CDR1, amino acid positions 50 to 56 for CDR2, and amino acid positions 89 to 97 for CDR3.

As used herein, the term "CDR" refers to the complementarity determining region within antibody variable sequences. There are three CDRs in each of the variable regions of the heavy chain and the light chain, which are designated CDR1, CDR2 and CDR3, for each of the variable regions. The term "CDR set" as used herein refers to a group of three CDRs that occur in a single variable region capable of binding the antigen. The exact boundaries of these CDRs have been defined differently according to different systems. The system described by Kabat (Kabat et al., Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987) and (1991)) not only provides an unambiguous residue numbering system applicable to any variable region of an antibody, but also provides precise residue boundaries defining the three CDRs. These CDRs may be referred to as Kabat CDRs. Chothia and coworkers (Chothia &Lesk, J. Mol. Biol. 196:901-917 (1987) and Chothia et al., Nature 342:877-883 (1989)) found that certain sub- portions within Kabat CDRs adopt nearly identical peptide backbone conformations, despite having great diversity at the level of amino acid sequence. These sub-portions were designated as L1, L2 and L3 or H1, H2 and H3 where the "L" and the "H" designates the light chain and the heavy chains regions, respectively. These regions may be referred to as Chothia CDRs, which have boundaries that overlap with Kabat CDRs. Other boundaries defining CDRs overlapping with the Kabat CDRs have been described by Padlan (FASEB J. 9:133-139 (1995)) and MacCallum (J Mol Biol 262(5):732-45 (1996)). Still other CDR boundary definitions may not strictly follow one of the herein systems, but will nonetheless overlap with the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact

antigen binding. The methods used herein may utilize CDRs defined according to any of these systems, although certain embodiments use Kabat or Chothia defined CDRs.

As used herein, the term "framework" or "framework sequence" refers to the remaining sequences of a variable region minus the CDRs. Because the exact definition of a CDR sequence can be determined by different systems, the meaning of a framework sequence is subject to correspondingly different interpretations. The six CDRs (CDR-L1, -L2, and -L3 of light chain and CDR-H1, -H2, and -H3 of heavy chain) also divide the framework regions on the light chain and the heavy chain into four sub-regions (FR1, FR2, FR3 and FR4) on each chain, in which CDR1 is positioned between FR1 and FR2, CDR2 between FR2 and FR3, and CDR3 between FR3 and FR4. Without specifying the particular sub-regions as FR1, FR2, FR3 or FR4, a framework region, as referred by others, represents the combined FR's within the variable region of a single, naturally occurring immunoglobulin chain. As used herein, a FR represents one of the four sub-regions, and FRs represents two or more of the four sub- regions constituting a framework region.

As used herein, the term "germline antibody gene" or "gene fragment" refers to an immunoglobulin sequence encoded by non- lymphoid cells that have not undergone the maturation process that leads to genetic rearrangement and mutation for expression of a particular immunoglobulin. (See, e.g., Shapiro et al., Crit. Rev. Immunol. 22(3): 183-200 (2002); Marchalonis et al., Adv Exp Med Biol. 484:13-30 (2001)). One of the advantages provided by various embodiments of the present invention stems from the recognition that germline antibody genes are more likely than mature antibody genes to conserve essential amino acid sequence structures characteristic of individuals in the species, hence less likely to be recognized as from a foreign source when used therapeutically in that species.

As used herein, the term "neutralizing" refers to counteracting the biological activity of an antigen when a binding protein specifically binds the antigen. In an embodiment, the neutralizing binding protein binds the cytokine and reduces its biologically activity by at least about 20%, 40%, 60%, 80%, 85% or more.

The term "activity" includes activities such as the binding specificity and affinity of a DVD-Ig for two or more antigens.

The term "epitope" includes any polypeptide determinant capable of specific binding to an immunoglobulin or T-cell receptor. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three dimensional structural characteristics, and/or specific charge characteristics. An epitope is a region of an antigen that is bound by an antibody. In certain embodiments, an antibody specifically binds an antigen when it recognizes its target antigen in a complex mixture of proteins and/or macromolecules. Antibodies "bind to the same epitope" if the antibodies cross-compete (one prevents the binding or modulating effect of the other). In addition structural definitions of epitopes (overlapping, similar, identical) are informative, but functional definitions are often more relevant as they encompass structural (binding) and functional (modulation, competition) parameters.

The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore® system (BIAcore International AB, a GE Healthcare company, Uppsala, Sweden and Piscataway, NJ). For further descriptions, see Jönsson, U., *et al.* (1993) *Ann. Biol. Clin.* 51:19-26; Jönsson, U., *et al.* (1991) *Biotechniques* 11:620-627; Johnsson, B., *et al.* (1995) *J. Mol. Recognit.* 8:125-131; and Johnnson, B., *et al.* (1991) *Anal. Biochem.* 198:268-277.

The term "K_{on}", as used herein, is intended to refer to the on rate constant for association of a binding protein (e.g., an antibody) to the antigen to form the, e.g., antibody/antigen complex as is known in the art. The "Kon" also is known by the terms "association rate constant", or "ka", as used interchangeably herein. This value indicating the binding rate of an antibody to its target antigen or the rate of complex formation between an antibody and antigen also is shown by the equation below:

Antibody ("Ab") + Antigen ("Ag")'Ab-Ag.

The term "Koff", as used herein, is intended to refer to the off rate constant for dissociation, or "dissociation rate constant", of a binding protein (e.g., an antibody) from the, e.g., antibody/antigen complex as is known in the art. This value indicates the

dissociation rate of an antibody from its target antigen or separation of Ab-Ag complex over time into free antibody and antigen as shown by the equation below:

$$Ab + Ag \rightarrow Ab\text{-}Ag.$$

The term "KD" as used herein, is intended to refer to the "equilibrium dissociation constant", and refers to the value obtained in a titration measurement at equilibrium, or by dividing the dissociation rate constant (koff) by the association rate constant (kon). The association rate constant, the dissociation rate constant and the equilibrium dissociation constant are used to represent the binding affinity of an antibody to an antigen. Methods for determining association and dissociation rate constants are well known in the art. Using fluorescence–based techniques offers high sensitivity and the ability to examine samples in physiological buffers at equilibrium. Other experimental approaches and instruments such as a BIAcore® (biomolecular interaction analysis) assay can be used (e.g., instrument available from BIAcore International AB, a GE Healthcare company, Uppsala, Sweden). Additionally, a KinExA® (Kinetic Exclusion Assay) assay, available from Sapidyne Instruments (Boise, Idaho) can also be used.

"Label" and "detectable label" mean a moiety attached to a specific binding partner, such as an antibody or an analyte, e.g., to render the reaction between members of a specific binding pair, such as an antibody and an analyte, detectable, and the specific binding partner, e.g., antibody or analyte, so labeled is referred to as "detectably labeled." Thus, the term "labeled binding protein" as used herein, refers to a protein with a label incorporated that provides for the identification of the binding protein. In an embodiment, the label is a detectable marker that can produce a signal that is detectable by visual or instrumental means, e.g., incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (e.g., 3H, 14C, 35S, 90Y, 99Tc, 111In, 125I, 131I, 177Lu, 166Ho, or 153Sm); chromogens, fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, luciferase, alkaline phosphatase); chemiluminescent markers;

biotinyl groups; predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags); and magnetic agents, such as gadolinium chelates. Representative examples of labels commonly employed for immunassays include moieties that produce light, e.g., acridinium compounds, and moieties that produce fluorescence, e.g., fluorescein. Other labels are described herein. In this regard, the moiety itself may not be detectably labeled but may become detectable upon reaction with yet another moiety. Use of "detectably labeled" is intended to encompass the latter type of detectable labeling.

The term "conjugate" refers to a binding protein, such as an antibody, chemically linked to a second chemical moiety, such as a therapeutic or cytotoxic agent. The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials. In an embodiment, the therapeutic or cytotoxic agents include, but are not limited to, pertussis toxin, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. When employed in the context of an immunoassay, the conjugate antibody may be a detectably labeled antibody used as the detection antibody.

The terms "crystal" and "crystallized" as used herein, refer to a binding protein (e.g., an antibody), or antigen binding portion thereof, that exists in the form of a crystal. Crystals are one form of the solid state of matter, which is distinct from other forms such as the amorphous solid state or the liquid crystalline state. Crystals are composed of regular, repeating, three-dimensional arrays of atoms, ions, molecules (e.g., proteins such as antibodies), or molecular assemblies (e.g., antigen/antibody complexes). These three-dimensional arrays are arranged according to specific mathematical relationships that are well-understood in the field. The fundamental unit, or building block, that is repeated in a crystal is called the asymmetric unit. Repetition of the asymmetric unit in an arrangement that conforms to a given, well-defined crystallographic symmetry provides the "unit cell" of the crystal. Repetition of the unit

cell by regular translations in all three dimensions provides the crystal. See Giege, R. and Ducruix, A. Barrett, Crystallization of Nucleic Acids and Proteins, a Practical Approach, 2nd ea., pp. 20 1-16, Oxford University Press, New York, New York, (1999)."

The term "polynucleotide" means a polymeric form of two or more nucleotides, either ribonucleotides or deoxvnucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

The term "isolated polynucleotide" shall mean a polynucleotide (e.g., of genomic, cDNA, or synthetic origin, or some combination thereof) that, by virtue of its origin , the "isolated polynucleotide" is not associated with all or a portion of a polynucleotide with which the "isolated polynucleotide" is found in nature; is operably linked to a polynucleotide that it is not linked to in nature; or does not occur in nature as part of a larger sequence.

The term "vector", is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. "Operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in *trans* or at a distance to control the gene of interest. The term "expression control sequence" as used herein refers to polynucleotide sequences that are necessary to effect the expression and processing of coding sequences to which they are ligated. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

"Transformation", refers to any process by which exogenous DNA enters a host cell. Transformation may occur under natural or artificial conditions using various methods well known in the art. Transformation may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method is selected based on the host cell being transformed and may include, but is not limited to, viral infection, electroporation, lipofection, and particle bombardment. Such "transformed" cells include stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome. They also include cells which transiently express the inserted DNA or RNA for limited periods of time.

The term "recombinant host cell" (or simply "host cell"), is intended to refer to a cell into which exogenous DNA has been introduced. In an embodiment, the host cell comprises two more more (e.g., multiple) nucleic acids encoding antibodies, such as the host cells described in US Patent No. 7,262,028, for example. It should be understood that such terms are intended to refer not only to the particular subject cell, but also to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. In an embodiment, host cells include prokaryotic and eukaryotic cells selected from any of the Kingdoms of life. In another embodiment, eukaryotic cells include protist, fungal, plant and animal cells. In another embodiment, host cells include but are not limited to the prokaryotic cell line E.Coli; mammalian cell lines CHO, HEK 293, COS, NS0, SP2 and PER.C6; the insect cell line Sf9; and the fungal cell Saccharomyces cerevisiae.

Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See e.g., Sambrook et al. Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)).

"Transgenic organism", as known in the art, refers to an organism having cells that contain a transgene, wherein the transgene introduced into the organism (or an ancestor of the organism) expresses a polypeptide not naturally expressed in the organism. A "transgene" is a DNA construct, which is stably and operably integrated into the genome of a cell from which a transgenic organism develops, directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic organism.

The term "regulate"and "modulate" are used interchangeably, and, as used herein, refers to a change or an alteration in the activity of a molecule of interest (e.g., the biological activity of a cytokine). Modulation may be an increase or a decrease in the magnitude of a certain activity or function of the molecule of interest. Exemplary activities and functions of a molecule include, but are not limited to, binding characteristics, enzymatic activity, cell receptor activation, and signal transduction.

Correspondingly, the term "modulator" is a compound capable of changing or altering an activity or function of a molecule of interest (e.g., the biological activity of a cytokine). For example, a modulator may cause an increase or decrease in the magnitude of a certain activity or function of a molecule compared to the magnitude of the activity or function observed in the absence of the modulator. In certain embodiments, a modulator is an inhibitor, which decreases the magnitude of at least one activity or function of a molecule. Exemplary inhibitors include, but are not limited to, proteins, peptides, antibodies, peptibodies, carbohydrates or small organic molecules. Peptibodies are described, e.g., in WO01/83525.

The term "agonist", refers to a modulator that, when contacted with a molecule of interest, causes an increase in the magnitude of a certain activity or function of the molecule compared to the magnitude of the activity or function observed in the absence of the agonist. Particular agonists of interest may include, but are not limited to, polypeptides, nucleic acids, carbohydrates, or any other molecules that bind to the antigen.

The term "antagonist" or "inhibitor", refer to a modulator that, when contacted with a molecule of interest causes a decrease in the magnitude of a certain activity or function of the molecule compared to the magnitude of the activity or function observed in the absence of the antagonist. Particular antagonists of interest include those that block or modulate the biological or immunological activity of of the antigen. Antagonists and inhibitors of antigens may include, but are not limited to, proteins, nucleic acids, carbohydrates, or any other molecules, which bind to the antigen.

As used herein, the term "effective amount" refers to the amount of a therapy which is sufficient to reduce or ameliorate the severity and/or duration of a disorder or one or more symptoms thereof, prevent the advancement of a disorder, cause regression of

a disorder, prevent the recurrence, development, onset or progression of one or more symptoms associated with a disorder, detect a disorder, or enhance or improve the prophylactic or therapeutic effect(s) of another therapy (e.g., prophylactic or therapeutic agent).

"Patient" and "subject" may be used interchangeably herein to refer to an animal, such as a mammal, including a primate (for example, a human, a monkey, and a chimpanzee), a non-primate (for example, a cow, a pig, a camel, a llama, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, a mouse, a whale), a bird (e.g., a duck or a goose), and a shark. Preferably, the patient or subject is a human, such as a human being treated or assessed for a disease, disorder or condition, a human at risk for a disease, disorder or condition, a human having a disease, disorder or condition, and/or human being treated for a disease, disorder or condition.

The term "sample", as used herein, is used in its broadest sense. A "biological sample", as used herein, includes, but is not limited to, any quantity of a substance from a living thing or formerly living thing. Such living things include, but are not limited to, humans, mice, rats, monkeys, dogs, rabbits and other animals. Such substances include, but are not limited to, blood, (e.g., whole blood), plasma, serum, urine, amniotic fluid, synovial fluid, endothelial cells, leukocytes, monocytes, other cells, organs, tissues, bone marrow, lymph nodes and spleen.

"Component," "components," and "at least one component," refer generally to a capture antibody, a detection or conjugate antibody, a control, a calibrator, a series of calibrators, a sensitivity panel, a container, a buffer, a diluent, a salt, an enzyme, a co-factor for an enzyme, a detection reagent, a pretreatment reagent/solution, a substrate (e.g., as a solution), a stop solution, and the like that can be included in a kit for assay of a test sample, such as a patient urine, serum or plasma sample, in accordance with the methods described herein and other methods known in the art. Thus, in the context of the present disclosure, "at least one component," "component," and "components" can include a polypeptide or other analyte as above, such as a composition comprising an analyte such as polypeptide, which is optionally immobilized on a solid support, such as by binding to an anti-analyte (e.g., anti-polypeptide) antibody. Some components can be in solution or lyophilized for reconstitution for use in an assay.

"Control" refers to a composition known to not analyte ("negative control") or to contain analyte ("positive control"). A positive control can comprise a known concentration of analyte. "Control," "positive control," and "calibrator" may be used interchangeably herein to refer to a composition comprising a known concentration of analyte. A "positive control" can be used to establish assay performance characteristics and is a useful indicator of the integrity of reagents (e.g., analytes).

"Predetermined cutoff" and "predetermined level" refer generally to an assay cutoff value that is used to assess diagnostic/prognostic/therapeutic efficacy results by comparing the assay results against the predetermined cutoff/level, where the predetermined cutoff/level already has been linked or associated with various clinical parameters (e.g., severity of disease, progression/nonprogression/improvement, etc.). While the present disclosure may provide exemplary predetermined levels, it is well-known that cutoff values may vary depending on the nature of the immunoassay (e.g., antibodies employed, etc.). It further is well within the ordinary skill of one in the art to adapt the disclosure herein for other immunoassays to obtain immunoassay-specific cutoff values for those other immunoassays based on this disclosure. Whereas the precise value of the predetermined cutoff/level may vary between assays, correlations as described herein (if any) should be generally applicable.

"Pretreatment reagent," e.g., lysis, precipitation and/or solubilization reagent, as used in a diagnostic assay as described herein is one that lyses any cells and/or solubilizes any analyte that is/are present in a test sample. Pretreatment is not necessary for all samples, as described further herein. Among other things, solubilizing the analyte (e.g., polypeptide of interest) may entail release of the analyte from any endogenous binding proteins present in the sample. A pretreatment reagent may be homogeneous (not requiring a separation step) or heterogeneous (requiring a separation step). With use of a heterogeneous pretreatment reagent there is removal of any precipitated analyte binding proteins from the test sample prior to proceeding to the next step of the assay.

"Quality control reagents" in the context of immunoassays and kits described herein, include, but are not limited to, calibrators, controls, and sensitivity panels. A "calibrator" or "standard" typically is used (e.g., one or more, such as a plurality) in order to establish calibration (standard) curves for interpolation of the concentration of an

analyte, such as an antibody or an analyte. Alternatively, a single calibrator, which is near a predetermined positive/negative cutoff, can be used. Multiple calibrators (i.e., more than one calibrator or a varying amount of calibrator(s)) can be used in conjunction so as to comprise a "sensitivity panel."

"Risk" refers to the possibility or probability of a particular event occurring either presently or at some point in the future. "Risk stratification" refers to an array of known clinical risk factors that allows physicians to classify patients into a low, moderate, high or highest risk of developing a particular disease, disorder or condition.

"Specific" and "specificity" in the context of an interaction between members of a specific binding pair (e.g., an antigen (or fragment thereof) and an antibody (or antigenically reactive fragment thereof)) refer to the selective reactivity of the interaction. The phrase "specifically binds to" and analogous phrases refer to the ability of antibodies (or antigenically reactive fragments thereof) to bind specifically to analyte (or a fragment thereof) and not bind specifically to other entities.

"Specific binding partner" is a member of a specific binding pair. A specific binding pair comprises two different molecules, which specifically bind to each other through chemical or physical means. Therefore, in addition to antigen and antibody specific binding pairs of common immunoassays, other specific binding pairs can include biotin and avidin (or streptavidin), carbohydrates and lectins, complementary nucleotide sequences, effector and receptor molecules, cofactors and enzymes, enzyme inhibitors and enzymes, and the like. Furthermore, specific binding pairs can include members that are analogs of the original specific binding members, for example, an analyte-analog. Immunoreactive specific binding members include antigens, antigen fragments, and antibodies, including monoclonal and polyclonal antibodies as well as complexes, fragments, and variants (including fragments of variants) thereof, whether isolated or recombinantly produced.

"Variant" as used herein means a polypeptide that differs from a given polypeptide (e.g., IL-18, BNP, NGAL or HIV polypeptide or anti-polypeptide antibody) in amino acid sequence by the addition (e.g., insertion), deletion, or conservative substitution of amino acids, but that retains the biological activity of the given polypeptide (e.g., a variant IL-18 can compete with anti-IL-18 antibody for binding to IL-

18). A conservative substitution of an amino acid, i.e., replacing an amino acid with a different amino acid of similar properties (e.g., hydrophilicity and degree and distribution of charged regions) is recognized in the art as typically involving a minor change. These minor changes can be identified, in part, by considering the hydropathic index of amino acids, as understood in the art (see, e.g., Kyte et al., J. Mol. Biol. 157: 105-132 (1982)). The hydropathic index of an amino acid is based on a consideration of its hydrophobicity and charge. It is known in the art that amino acids of similar hydropathic indexes can be substituted and still retain protein function. In one aspect, amino acids having hydropathic indexes of ± 2 are substituted. The hydrophilicity of amino acids also can be used to reveal substitutions that would result in proteins retaining biological function. A consideration of the hydrophilicity of amino acids in the context of a peptide permits calculation of the greatest local average hydrophilicity of that peptide, a useful measure that has been reported to correlate well with antigenicity and immunogenicity (see, e.g., U.S. Pat. No. 4,554,101, which is incorporated herein by reference). Substitution of amino acids having similar hydrophilicity values can result in peptides retaining biological activity, for example immunogenicity, as is understood in the art. In one aspect, substitutions are performed with amino acids having hydrophilicity values within ± 2 of each other. Both the hydrophobicity index and the hydrophilicity value of amino acids are influenced by the particular side chain of that amino acid. Consistent with that observation, amino acid substitutions that are compatible with biological function are understood to depend on the relative similarity of the amino acids, and particularly the side chains of those amino acids, as revealed by the hydrophobicity, hydrophilicity, charge, size, and other properties. "Variant" also can be used to describe a polypeptide or fragment thereof that has been differentially processed, such as by proteolysis, phosphorylation, or other post-translational modification, yet retains its biological activity or antigen reactivity, e.g., the ability to bind to IL-18. Use of "variant" herein is intended to encompass fragments of a variant unless otherwise contradicted by context.

I. Generation Of DVD Binding Protein

The invention pertains to Dual Variable Domain (DVD) binding proteins capable of binding one or more targets and methods of making the same. In an embodiment, the binding protein comprises a polypeptide chain, wherein the polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first variable domain, VD2 is a second

variable domain, C is a constant domain, X1 represents an amino acid or polypeptide, X2 represents an Fc region and n is 0 or 1. The binding protein of the invention can be generated using various techniques. The invention provides expression vectors, host cell and methods of generating the binding protein.

1.A: Generation Of Parent Monoclonal Antibodies

The variable domains of the DVD binding protein can be obtained from parent antibodies, including polyclonal and mAbs capable of binding antigens of interest. These antibodies may be naturally occurring or may be generated by recombinant technology.

MAbs can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, mAbs can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al. , Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Hybridomas are selected, cloned and further screened for desirable characteristics, including robust hybridoma growth, high antibody production and desirable antibody characteristics, as discussed in Example 1below. Hybridomas may be cultured and expanded *in vivo* in syngeneic animals, in animals that lack an immune system, e.g., nude mice, or in cell culture *in vitro*. Methods of selecting, cloning and expanding hybridomas are well known to those of ordinary skill in the art. In a particular embodiment, the hybridomas are mouse hybridomas. In another embodiment, the hybridomas are produced in a non-human, non-mouse species such as rats, sheep, pigs, goats, cattle or horses. In another embodiment, the hybridomas are human hybridomas, in which a human non-secretory myeloma is fused with a human cell expressing an antibody capable of binding a specific antigen.

Recombinant mAbs are also generated from single, isolated lymphocytes using a procedure referred to in the art as the selected lymphocyte antibody method (SLAM), as described in U.S. Patent No. 5,627,052, PCT Publication WO 92/02551 and Babcock, J.S.

et al. (1996) *Proc. Natl. Acad. Sci. USA* 93:7843-7848. In this method, single cells secreting antibodies of interest, *e.g.*, lymphocytes derived from an immunized animal, are identified, and, heavy- and light-chain variable region cDNAs are rescued from the cells by reverse transcriptase-PCR and these variable regions can then be expressed, in the context of appropriate immunoglobulin constant regions (*e.g.*, human constant regions), in mammalian host cells, such as COS or CHO cells. The host cells transfected with the amplified immunoglobulin sequences, derived from *in vivo* selected lymphocytes, can then undergo further analysis and selection *in vitro*, for example by panning the transfected cells to isolate cells expressing antibodies to the antigen of interest. The amplified immunoglobulin sequences further can be manipulated *in vitro*, such as by *in vitro* affinity maturation methods such as those described in PCT Publication WO 97/29131 and PCT Publication WO 00/56772.

Monoclonal antibodies are also produced by immunizing a non-human animal comprising some, or all, of the human immunoglobulin locus with an antigen of interest. In an embodiment, the non-human animal is a XENOMOUSE transgenic mouse, an engineered mouse strain that comprises large fragments of the human immunoglobulin loci and is deficient in mouse antibody production. *See, e.g.*, Green et al. *Nature Genetics* 7:13-21 (1994) and United States Patents Nos. 5,916,771, 5,939,598, 5,985,615, 5,998,209, 6,075,181, 6,091,001, 6,114,598 and 6,130,364. See also WO 91/10741, published July 25,1991, WO 94/02602, published February 3, 1994, WO 96/34096 and WO 96/33735, both published October 31, 1996, WO 98/16654, published April 23, 1998, WO 98/24893, published June 11, 1998, WO 98/50433, published November 12, 1998, WO 99/45031, published September 10, 1999, WO 99/53049, published October 21, 1999, WO 00 09560, published February 24, 2000 and WO 00/037504, published June 29, 2000. The XENOMOUSE transgenic mouse produces an adult-like human repertoire of fully human antibodies, and generates antigen-specific human monoclonal antibodies. The XENOMOUSE transgenic mouse contains approximately 80% of the human antibody repertoire through introduction of megabase sized, germline configuration YAC fragments of the human heavy chain loci and x light chain loci. See Mendez et al., *Nature Genetics* 15:146-156 (1997), Green and Jakobovits *J. Exp. Med.* 188:483-495 (1998).

In vitro methods also can be used to make the parent antibodies, wherein an antibody library is screened to identify an antibody having the desired binding specificity. Methods for such screening of recombinant antibody libraries are well known in the art and include methods described in, for example, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* PCT Publication No. WO 92/18619; Dower *et al.* PCT Publication No. WO 91/17271; Winter *et al.* PCT Publication No. WO 92/20791; Markland *et al.* PCT Publication No. WO 92/15679; Breitling *et al.* PCT Publication No. WO 93/01288; McCafferty *et al.* PCT Publication No. WO 92/01047; Garrard *et al.* PCT Publication No. WO 92/09690; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum Antibod Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; McCafferty *et al.*, *Nature* (1990) 348:552-554; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J Mol Biol* 226:889-896; Clackson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *PNAS* 89:3576-3580; Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc Acid Res* 19:4133-4137; and Barbas *et al.* (1991) *PNAS* 88:7978-7982, US patent application publication 20030186374, and PCT Publication No. WO 97/29131.

Parent antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles that carry the polynucleotide sequences encoding them. In a particular, such phage can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (e. g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Pat. Nos.

5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780, 225; 5,658,727; 5,733,743 and 5,969,108.

After phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies including human antibodies or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., BioTechniques 12(6):864-869 (1992); and Sawai et al., AJRI 34:26-34 (1995); and Better et al., Science 240:1041-1043 (1988). Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Pat. 4,946,778 and 5,258, 498; Huston et al., Methods in Enzymology 203:46-88 (1991); Shu et al., PNAS 90:7995-7999 (1993); and Skerra et al., Science 240:1038-1040 (1988).

Alternative to screening of recombinant antibody libraries by phage display, other methodologies known in the art for screening large combinatorial libraries can be applied to the identification of parent antibodies. One type of alternative expression system is one in which the recombinant antibody library is expressed as RNA-protein fusions, as described in PCT Publication No. WO 98/31700 by Szostak and Roberts, and in Roberts, R.W. and Szostak, J.W. (1997) *Proc. Natl. Acad. Sci. USA* 94:12297-12302. In this system, a covalent fusion is created between an mRNA and the peptide or protein that it encodes by *in vitro* translation of synthetic mRNAs that carry puromycin, a peptidyl acceptor antibiotic, at their 3' end. Thus, a specific mRNA can be enriched from a complex mixture of mRNAs (*e.g.*, a combinatorial library) based on the properties of the encoded peptide or protein, *e.g.*, antibody, or portion thereof, such as binding of the antibody, or portion thereof, to the dual specificity antigen. Nucleic acid sequences encoding antibodies, or portions thereof, recovered from screening of such libraries can be expressed by recombinant means as described herein (*e.g.*, in mammalian host cells) and, moreover, can be subjected to further affinity maturation by either additional rounds of screening of mRNA-peptide fusions in which mutations have been introduced into the originally selected sequence(s), or by other methods for affinity maturation *in vitro* of recombinant antibodies, as described herein.

In another approach the parent antibodies can also be generated using yeast display methods known in the art. In yeast display methods, genetic methods are used to tether antibody domains to the yeast cell wall and display them on the surface of yeast. In particular, such yeast can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Examples of yeast display methods that can be used to make the parent antibodies include those disclosed in Wittrup, et al. U.S. Patent No. 6,699,658.

The antibodies described herein can be further modified to generate CDR grafted and humanized parent antibodies. CDR-grafted parent antibodies comprise heavy and light chain variable region sequences from a human antibody wherein one or more of the CDR regions of V_H and/or V_L are replaced with CDR sequences of murine antibodies capable of binding antigen of interest. A framework sequence from any human antibody may serve as the template for CDR grafting. However, straight chain replacement onto such a framework often leads to some loss of binding affinity to the antigen. The more homologous a human antibody is to the original murine antibody, the less likely the possibility that combining the murine CDRs with the human framework will introduce distortions in the CDRs that could reduce affinity. Therefore, in an embodiment, the human variable framework that is chosen to replace the murine variable framework apart from the CDRs have at least a 65% sequence identity with the murine antibody variable region framework. In an embodiment, the human and murine variable regions apart from the CDRs have at least 70% sequence identify. In a particular embodiment, that the human and murine variable regions apart from the CDRs have at least 75% sequence identity. In another embodiment, the human and murine variable regions apart from the CDRs have at least 80% sequence identity. Methods for producing such antibodies are known in the art (see EP 239,400; PCT publication WO 91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Pat. No. 5,565,352); and anti-idiotypic antibodies.

Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human

immunoglobulin molecule. Known human Ig sequences are disclosed, e.g., www.ncbi.nlm.nih.gov/entrez- /query.fcgi; www.atcc.org/phage/hdb.html; www.sciquest.com/; www.abcam.com/; www.antibodyresource.com/onlinecomp.html; www.public.iastate.edu/.about.pedro/research_tools.html; www.mgen.uni-heidelberg.de/SD/IT/IT.html; www.whfreeman.com/immunology/CH- 05/kuby05.htm; www.library.thinkquest.org/12429/Immune/Antibody.html; www.hhmi.org/grants/lectures/1996/vlab/; www.path.cam.ac.uk/.about.mrc7/m-ikeimages.html; www.antibodyresource.com/; mcb.harvard.edu/BioLinks/Immunology.html.www.immunologylink.com/; pathbox.wustl.edu/.about.hcenter/index.- html; www.biotech.ufl.edu/.about.hcl/; www.pebio.com/pa/340913/340913.html- ; www.nal.usda.gov/awic/pubs/antibody/; www.m.ehime-u.acjp/.about.yasuhito-/Elisa.html; www.biodesign.com/table.asp; www.icnet.uk/axp/facs/davies/lin- ks.html; www.biotech.ufl.edu/.about.fccl/protocol.html; www.isac-net.org/sites_geo.html; aximtl.imt.uni-marburg.de/.about.rek/AEP- Start.html; baserv.uci.kun.nl/.about.jraats/linksl.html; www.recab.uni-hd.de/immuno.bme.nwu.edu/; www.mrc-cpe.cam.ac.uk/imt-doc/pu- blic/INTRO.html; www.ibt.unam.mx/vir/V_mice.html; imgt.cnusc.fr:8104/; www.biochem.ucl.ac.uk/.about.martin/abs/index.html; antibody.bath.ac.uk/; abgen.cvm.tamu.edu/lab/wwwabgen.html; www.unizh.ch/.about.honegger/AHOsem-inar/Slide01.html; www.cryst.bbk.ac.uk/.about.ubcg07s/; www.nimr.mrc.ac.uk/CC/ccaewg/ccaewg.htm; www.path.cam.ac.uk/.about.mrc7/h-umanisation/TAHHP.html; www.ibt.unam.mx/vir/structure/stat_aim.html; www.biosci.missouri.edu/smithgp/index.html; www.cryst.bioc.cam.ac.uk/.about.fmolina/Web-pages/Pept/spottech.html; www.jerini.de/fr roducts.htm; www.patents.ibm.com/ibm.html.Kabat et al., Sequences of Proteins of Immunological Interest, U.S. Dept. Health (1983). Such imported sequences can be used to reduce immunogenicity or reduce, enhance or modify binding, affinity, on-rate, off-rate, avidity, specificity, half-life, or any other suitable characteristic, as known in the art.

Framework residues in the human framework regions may be substituted with the corresponding residue from the CDR donor antibody to alter, e.g., improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify

framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Pat. No. 5,585,089; Riechmann et al., Nature 332:323 (1988). Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding. Antibodies can be humanized using a variety of techniques known in the art, such as but not limited to those described in Jones et al., Nature 321:522 (1986); Verhoeyen et al., Science 239:1534 (1988)), Sims et al., J. Immunol. 151: 2296 (1993); Chothia and Lesk, J. Mol. Biol. 196:901 (1987), Carter et al., Proc. Natl. Acad. Sci. U.S.A. 89:4285 (1992); Presta et al., J. Immunol. 151:2623 (1993), Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al. , PNAS 91:969-973 (1994); PCT publication WO 91/09967, PCT/: US98/16280, US96/18978, US91/09630, US91/05939, US94/01234, GB89/01334, GB91/01134, GB92/01755; WO90/14443, WO90/14424, WO90/14430, EP 229246, EP 592,106; EP 519,596, EP 239,400, U.S. Pat. Nos. 5,565,332, 5,723,323, 5,976,862, 5,824,514, 5,817,483, 5814476, 5763192, 5723323, 5,766886, 5,714,352, 6,204,023, 6,180,370, 5,693,762, 5,530,101, 5,585,089, 5,225,539; 4,816,567.

In another approach the parent antibodies can also be generated using standard molecule biology techniques based on the solved protein sequence for any antibodies. Protein sequence of any antibodies can be solved by a combination of Edman degradation, mass spectrum and BLAST analysis described in Edman, P. Acta Chem. Scand. 4: 283 (1950), Niall HD Methods Enzymol. 27: 942-1010 (1973), Vicki H.W. et al. Methods 35: 211-222 (2005) and NCBI Blast program: http://www.ncbi.nlm.nih.gov/blast/about/.

1.B: Criteria for selecting parent monoclonal antibodies

An embodiment of the invention pertains to selecting parent antibodies with at least one or more properties desired in the DVD-Ig molecule. In an embodiment, the desired property is selected from one or more antibody parameters. In another embodiment, the antibody parameters are selected from the group consisting of antigen specificity, affinity to antigen, potency, biological function, epitope recognition, stability, solubility, production efficiency, immunogenicity, pharmacokinetics, bioavailability, tissue cross reactivity, and orthologous antigen binding.

1.B.1: Affinity to Antigen

The desired affinity of a therapeutic mAb may depend upon the nature of the antigen, and the desired therapeutic end-point. In an embodiment, monoclonal antibodies have higher affinities (Kd = 0.01 – 0.50 pM) when blocking a cytokine-cytokine receptor interaction as such interaction are usually high affinity interactions (e.g.,<pM – <nM ranges). In such instances, the mAb affinity for its target should be equal to or better than the affinity of the cytokine (ligand) for its receptor. On the other hand, mAb with lesser affinity (> nM range) could be therapeutically effective e.g.,in clearing circulating potentially pathogenic proteins e.g.,monoclonal antibodies that bind to, sequester, and clear circulating species of A-β amyloid. In other instances, reducing the affinity of an existing high affinity mAb by site-directed mutagenesis or using a mAb with lower affinity for its target could be used to avoid potential side-effects e.g.,a high affinity mAb may sequester/neutralize all of its intended target, thereby completely depleting/eliminating the function(s) of the targeted protein. In this scenario, a low affinity mAb may sequester/neutralize a fraction of the target that may be responsible for the disease symptoms (the pathological or over-produced levels), thus allowing a fraction of the target to continue to perform its normal physiological function(s). Therefore, it may be possible to reduce the Kd to adjust dose and/or reduce side-effects. The affinity of the parental mAb might play a role in appropriately targeting cell surface molecules to achieve desired therapeutic out-come. For example, if a target is expressed on cancer cells with high density and on normal cells with low density, a lower affinity mAb will bind a greater number of targets on tumor cells than normal cells, resulting in tumor cell elimination via ADCC or CDC, and therefore might have therapeutically desirable effects. Thus selecting a mAb with desired affinity may be relevant for both soluble and surface targets.

Signaling through a receptor upon interaction with its ligand may depend upon the affinity of the receptor-ligand interaction. Similarly, it is conceivable that the affinity of a mAb for a surface receptor could determine the nature of intracellular signaling and whether the mAb may deliver an agonist or an antagonist signal. The affinity-based nature of mAb-mediated signaling may have an impact of its side-effect profile. Therefore, the desired affinity and desired functions of therapeutic monoclonal antibodies need to be determined carefully by in vitro and in vivo experimentation.

The desired Kd of a binding protein (e.g., an antibody) may be determined experimentally depending on the desired therapeutic outcome. In an embodiment parent antibodies with affinity (Kd) for a particular antigen equal to, or better than, the desired affinity of the DVD-Ig for the same antigen are selected. The antigen binding affinity and kinetics are assessed by BIAcore or another similar technique. In one embodiment, each parent antibody has a dissociation constant (Kd) to its antigen selected from the group consisting of: at most about 10^{-7} M; at most about 10^{-8} M; at most about 10^{-9} M; at most about 10^{-10} M; at most about 10^{-11} M; at most about 10^{-12} M; and at most 10^{-13} M. First parent antibody from which VD1 is obtained and second parent antibody from which VD2 is obtained may have similar or different affinity (K_D) for the respective antigen. Each parent antibody has an on rate constant (Kon) to the antigen selected from the group consisting of: at least about $10^{2}M^{-1}s^{-1}$; at least about $10^{3}M^{-1}s^{-1}$; at least about $10^{4}M^{-1}s^{-1}$; at least about $10^{5}M^{-1}s^{-1}$; and at least about $10^{6}M^{-1}s^{-1}$, as measured by surface plasmon resonance. The first parent antibody from which VD1 is obtained and the second parent antibody from which VD2 is obtained may have similar or different on rate constant (Kon) for the respective antigen. In one embodiment, each parent antibody has an off rate constant (Koff) to the antigen selected from the group consisting of: at most about $10^{-3}s^{-1}$; at most about $10^{-4}s^{-1}$; at most about $10^{-5}s^{-1}$; and at most about $10^{-6}s^{-1}$, as measured by surface plasmon resonance. The first parent antibody from which VD1 is obtained and the second parent antibody from which VD2 is obtained may have similar or different off rate constants (Koff) for the respective antigen.

1.B.2: Potency

The desired affinity/potency of parental monoclonal antibodies will depend on the desired therapeutic outcome. For example, for receptor-ligand (R-L) interactions the

affinity (kd) is equal to or better than the R-L kd (pM range). For simple clearance of a pathologic circulating protein, the kd could be in low nM range e.g.,clearance of various species of circulating A-β peptide. In addition, the kd will also depend on whether the target expresses multiple copies of the same epitope e.g a mAb targeting conformational epitope in Aβ oligomers.

Where VDI and VD2 bind the same antigen, but distint epitopes, the DVD-Ig will contain 4 binding sites for the same antigen, thus increasing avidity and thereby the apparent kd of the DVD-Ig. In an embodiment, parent antibodies with equal or lower kd than that desired in the DVD-Ig are chosen. The affinity considerations of a parental mAb may also depend upon whether the DVD-Ig contains four or more identical antigen binding sites (i.e; a DVD-Ig from a single mAb). In this case, the apparent kd would be greater than the mAb due to avidity. Such DVD-Igs can be employed for cross-linking surface receptor, increase neutralization potency, enhance clearance of pathological proteins etc.

In an embodiment parent antibodies with neutralization potency for specific antigen equal to or better than the desired neutralization potential of the DVD-Ig for the same antigen are selected. The neutralization potency can be assessed by a target-dependent bioassay where cells of appropriate type produce a measurable signal (i.e. proliferation or cytokine production) in response to target stimulation, and target neutralization by the mAb can reduce the signal in a dose-dependent manner.

1.B.: Biological Functions

Monoclonal antibodies can perform potentially several functions. Some of these functions are listed in Table 1. These functions can be assessed by both in vitro assays (e.g., cell-based and biochemical assays) and in vivo animal models.

Table 1: Some Potential Applications For Therapeutic Antibodies

Target (Class)	Mechanism of Action (target)
Soluble (cytokines,other)	Neutralization of activity (e.g., a cytokine) Enhance clearance (e.g., Aβ oligomers) Increase half-life (e.g., GLP 1)
Cell Surface	Agonist (e.g., GLP1 R; EPO R; etc.)

Target (Class)	Mechanism of Action (target)
(Receptors, other)	Antagonist (e.g., integrins; etc.) Cytotoxic (CD 20; etc.)
Protein deposits	Enhance clearance/degradation (e.g., Aβ plaques, amyloid deposits)

MAbs with distinct functions described in the examples herein in Table 1 can be selected to achieve desired therapeutic outcomes. Two or more selected parent monoclonal antibodies can then be used in DVD-Ig format to achieve two distinct functions in a single DVD-Ig molecule. For example, a DVD-Ig can be generated by selecting a parent mAb that neutralizes function of a specific cytokine, and selecting a parent mAb that enhances clearance of a pathological protein. Similarly, we can select two parent monoclonal antibodies that recognize two different cell surface receptors, one mAb with an agonist function on one receptor and the other mAb with an antagonist function on a different receptor. These two selected monoclonal antibodies each with a distinct function can be used to construct a single DVD-Ig molecule that will possess the two distinct functions (agonist and antagonist) of the selected monoclonal antibodies in a single molecule. Similarly, two antagonistic monoclonal antibodies to cell surface receptors each blocking binding of respective receptor ligands (e.g.,EGF and IGF) can be used in a DVD-Ig format. Conversely, an antagonistic anti-receptor mAb (e.g.,anti-EGFR) and a neutralizing anti-soluble mediator (e.g.,anti-IGF1/2) mAb can be selected to make a DVD-Ig.

1.B.4: Epitope Recognition:

Different regions of proteins may perform different functions. For example specific regions of a cytokine interact with the cytokine receptor to bring about receptor activation whereas other regions of the protein may be required for stabilizing the cytokine. In this instance one may select a mAb that binds specifically to the receptor interacting region(s) on the cytokine and thereby block cytokine-receptor interaction. In some cases, for example certain chemokine receptors that bind multiple ligands, a mAb that binds to the epitope (region on chemokine receptor) that interacts with only one ligand can be selected. In other instances, monoclonal antibodies can bind to epitopes on a target that are not directly responsible for physiological functions of the protein, but

binding of a mAb to these regions could either interfere with physiological functions (steric hindrance) or alter the conformation of the protein such that the protein cannot function (mAb to receptors with multiple ligand which alter the receptor conformation such that none of the ligand can bind). Anti-cytokine monoclonal antibodies that do not block binding of the cytokine to its receptor, but block signal transduction have also been identified (e.g., 125-2H, an anti-IL-18 mAb).

Examples of epitopes and mAb functions include, but are not limited to, blocking Receptor-Ligand (R-L) interaction (neutralizing mAb that binds R-interacting site); steric hindrance resulting in diminished or no R-binding. An Ab can bind the target at a site other than a receptor binding site, but still interferes with receptor binding and functions of the target by inducing conformational change and eliminate function (e.g., Xolair), binding to R but block signaling (125-2H).

In an embodiment, the parental mAb needs to target the appropriate epitope for maximum efficacy. Such epitope should be conserved in the DVD-Ig. The binding epitope of a mAb can be determined by several approaches, including co-crystallography, limited proteolysis of mAb-antigen complex plus mass spectrometric peptide mapping (Legros V. et al 2000 Protein Sci. 9:1002-10), phage displayed peptide libraries (O'Connor KH et al 2005 J Immunol Methods. 299:21-35), as well as mutagenesis (Wu C. et al . 2003 J Immunol 170:5571-7).

1.B.5: Physicochemical And Pharmaceutical Properties

Therapeutic treatment with antibodies often requires administration of high doses, often several mg/kg (due to a low potency on a mass basis as a consequence of a typically large molecular weight). In order to accommodate patient compliance and to adequately address chronic disease therapies and outpatient treatment, subcutaneous (s.c.) or intramuscular (i.m.) administration of therapeutic mAbs is desirable. For example, the maximum desirable volume for s.c. administration is ~1.0 mL, and therefore, concentrations of >100 mg/mL are desirable to limit the number of injections per dose. In an embodiment, the therapeutic antibody is administered in one dose. The development of such formulations is constrained, however, by protein-protein interactions (e.g., aggregation, which potentially increases immunogenicity risks) and by limitations during processing and delivery (e.g., viscosity). Consequently, the large quantities

required for clinical efficacy and the associated development constraints limit full exploitation of the potential of antibody formulation and s.c. administration in high-dose regimens. It is apparent that the physicochemical and pharmaceutical properties of a protein molecule and the protein solution are of utmost importance, e.g.,stability, solubility and viscosity features.

1.B.5.1: Stability

A "stable" antibody formulation is one in which the antibody therein essentially retains its physical stability and/or chemical stability and/or biological activity upon storage. Stability can be measured at a selected temperature for a selected time period. In an embodiment,, the antibody in the formulation is stable at room temperature (about 30°C) or at 40°C for at least 1 month and/or stable at about 2-8°C. for at least 1 year for at least 2 years. Furthermore, in an embodiment, the formulation is stable following freezing (to, e.g., -70°C) and thawing of the formulation, hereinafter referred to as a "freeze/thaw cycle." In another example, a "stable" formulation may be one wherein less than about 10% and less than about 5% of the protein is present as an aggregate in the formulation.

A DVD-Ig stable in vitro at various temperatures for an extended time period is desirable. One can achieve this by rapid screening of parental mAbs stable in vitro at elevated temperature, e.g.,at 40°C for 2-4 weeks, and then assess stability. During storage at 2-8°C, the protein reveals stability for at least 12 months, e.g., at least 24 months. Stability (% of monomeric, intact molecule) can be assessed using various techniques such as cation exchange chromatography, size exclusion chromatography, SDS-PAGE, as well as bioactivity testing. For a more comprehensive list of analytical techniques that may be employed to analyze covalent and conformational modifications see Jones, A. J. S. (1993) Analytical methods for the assessment of protein formulations and delivery systems. In: Cleland, J. L.; Langer, R., editors. Formulation and delivery of peptides and proteins, 1st edition, Washington, ACS, pg. 22-45; and Pearlman, R.; Nguyen, T. H.(1990) Analysis of protein drugs. In: Lee, V. H., editor. Peptide and protein drug delivery, 1st edition, New York, Marcel Dekker, Inc., pg. 247-301.

Heterogeneity and aggregate formation: stability of the antibody may be such that the formulation may reveal less than about 10%, and, in an embodiment, less than about

5%, in another embodiment, less than about 2%, or, in an embodiment, within the range of 0.5% to 1.5% or less in the GMP antibody material that is present as aggregate. Size exclusion chromatography is a method that is sensitive, reproducible, and very robust in the detection of protein aggregates.

In addition to low aggregate levels, the antibody must , in an embodiment, be chemically stable. Chemical stability may be determined by ion exchange chromatography (e.g.,cation or anion exchange chromatography), hydrophobic interaction chromatography, or other methods such as isoelectric focusing or capillary electrophoresis. For instance, chemical stability of the antibody may be such that after storage of at least 12 months at 2-8°C the peak representing unmodified antibody in a cation exchange chromatography may increase not more than 20%, in an embodiment, not more than 10%, or, in another embodiment, not more than 5% as compared to the antibody solution prior to storage testing.

In an embodiment, the parent antibodies display structural integrity; correct disulfide bond formation, and correct folding: Chemical instability due to changes in secondary or tertiary structure of an antibody may impact antibody activity. For instance, stability as indicated by activity of the antibody may be such that after storage of at least 12 months at 2-8°C the activity of the antibody may decrease not more than 50%, in an embodiment not more than 30%, or even not more than 10%, or in an embodiment not more than 5% or 1% as compared to the antibody solution prior to storage testing. Suitable antigen-binding assays can be employed to determine antibody activity.

1.B.5.2: Solubility

The "solubility" of a mAb correlates with the production of correctly folded, monomeric IgG. The solubility of the IgG may therefore be assessed by HPLC. For example, soluble (monomeric) IgG will give rise to a single peak on the HPLC chromatograph, whereas insoluble (e.g., multimeric and aggregated) will give rise to a plurality of peaks. A person skilled in the art will therefore be able to detect an increase or decrease in solubility of an IgG using routine HPLC techniques. For a more comprehensive list of analytical techniques that may be employed to analyze solubility (see Jones, A. G. Dep. Chem. Biochem. Eng., Univ. Coll. London, London, UK. Editor(s): Shamlou, P. Ayazi. Process. Solid-Liq. Suspensions (1993), 93-117.

Publisher: Butterworth-Heinemann, Oxford, UK and Pearlman, Rodney; Nguyen, Tue H, Advances in Parenteral Sciences (1990), 4 (Pept. Protein Drug Delivery), 247-301). Solubility of a therapeutic mAb is critical for formulating to high concentration often required for adequate dosing. As outlined herein, solubilities of >100 mg/mL may be required to accommodate efficient antibody dosing. For instance, antibody solubility may be not less than about 5 mg/mL in early research phase, in an embodiment not less than about 25 mg/mL in advanced process science stages, or in an embodiment not less than about 100 mg/mL, or in an embodiment not less than about 150 mg/mL. It is obvious to a person skilled in the art that the intrinsic properties of a protein molecule are important the physico-chemical properties of the protein solution, e.g.,stability, solubility, viscosity. However, a person skilled in the art will appreciate that a broad variety of excipients exist that may be used as additives to beneficially impact the characteristics of the final protein formulation. These excipients may include: (i) liquid solvents, cosolvents (e.g.,alcohols such as ethanol); (ii) buffering agents (e.g.,phosphate, acetate, citrate, amino acid buffers); (iii) sugars or sugar alcohols (e.g.,sucrose, trehalose, fructose, raffinose, mannitol, sorbitol, dextrans); (iv) surfactants (e.g.,polysorbate 20, 40, 60, 80, poloxamers); (v) isotonicity modifiers (e.g.,salts such as NaCl, sugars, sugar alcohols); and (vi) others (e.g.,preservatives, chelating agents, antioxidants, chelating substances (e.g.,EDTA), biodegradable polymers, carrier molecules (e.g.,HSA, PEGs).

Viscosity is a parameter of high importance with regard to antibody manufacture and antibody processing (e.g.,diafiltration/ultrafiltration), fill-finish processes (pumping aspects, filtration aspects) and delivery aspects (syringeability, sophisticated device delivery). Low viscosities enable the liquid solution of the antibody having a higher concentration. This enables the same dose may be administered in smaller volumes. Small injection volumes inhere the advantage of lower pain on injection sensations, and the solutions not necessarily have to be isotonic to reduce pain on injection in the patient. The viscosity of the antibody solution may be such that at shear rates of 100 (1/s) antibody solution viscosity is below 200 mPa s, in an embodiment below 125 mPa s, in another embodiment below 70 mPa s, and in yet another embodiment below 25 mPa s or even below 10 mPa s.

1.B.5.3: Production Efficiency

The generation of a DVD-Ig that is efficiently expressed in mammalian cells, such as Chinese hamster ovary cells (CHO), will in an embodiment require two parental monoclonal antibodies which are themselves expressed efficiently in mammalian cells. The production yield from a stable mammalian line (i.e. CHO) should be above about 0.5g/L, in an embodiment above about 1g/L, and in another embodiment in the range of about 2-5 g/L or more (Kipriyanov SM, Little M. 1999 Mol Biotechnol. 12:173-201; Carroll S, Al-Rubeai M. 2004 Expert Opin Biol Ther. 4:1821-9).

Production of antibodies and Ig fusion proteins in mammalian cells is influenced by several factors. Engineering of the expression vector via incorporation of strong promoters, enhancers and selection markers can maximize transcription of the gene of interest from an integrated vector copy. The identification of vector integration sites that are permissive for high levels of gene transcription can augment protein expression from a vector (Wurm et al, 2004, Nature Biotechnology , 2004, Vol/Iss/Pg. 22/11 (1393-1398)). Furthermore, levels of production are affected by the ratio of antibody heavy and light chains and various steps in the process of protein assembly and secretion (Jiang et al. 2006, Biotechnology Progress, Jan-Feb 2006, vol. 22, no. 1, p. 313-8).

1.B.6: Immunogenicity

Administration of a therapeutic mAb may results in certain incidence of an immune response (ie, the formation of endogenous antibodies directed against the therapeutic mAb). Potential elements that might induce immunogenicity should be analyzed during selection of the parental monoclonal antibodies, and steps to reduce such risk can be taken to optimize the parental monoclonal antibodies prior to DVD-Ig construction. Mouse-derived antibodies have been found to be highly immunogenic in patients. The generation of chimeric antibodies comprised of mouse variable and human constant regions presents a logical next step to reduce the immunogenicity of therapeutic antibodies (Morrison and Schlom, 1990). Alternatively, immunogenicity can be reduced by transferring murine CDR sequences into a human antibody framework (reshaping/CDR grafting/humanization), as described for a therapeutic antibody by Riechmann et al., 1988. Another method is referred to as "resurfacing" or "veneering", starting with the rodent variable light and heavy domains, only surface-accessible framework amino acids are altered to human ones, while the CDR and buried amino acids

remain from the parental rodent antibody (Roguska et al., 1996). In another type of humanization, instead of grafting the entire CDRs, one technique grafts only the "specificity-determining regions" (SDRs), defined as the subset of CDR residues that are involved in binding of the antibody to its target (Kashmiri et al., 2005). This necessitates identification of the SDRs either through analysis of available three-dimensional structures of antibody-target complexes or mutational analysis of the antibody CDR residues to determine which interact with the target. Alternatively, fully human antibodies may have reduced immunogenicity compared to murine, chimeric or humanized antibodies.

Another approach to reduce the immunogenicity of therapeutic antibodies is the elimination of certain specific sequences that are predicted to be immunogenic. In one approach, after a first generation biologic has been tested in humans and found to be unacceptably immunogenic, the B-cell epitopes can be mapped and then altered to avoid immune detection. Another approach uses methods to predict and remove potential T-cell epitopes. Computational methods have been developed to scan and to identify the peptide sequences of biologic therapeutics with the potential to bind to MHC proteins (Desmet et al., 2005). Alternatively a human dendritic cell-based method can be used to identify $CD4^+$ T-cell epitopes in potential protein allergens (Stickler et al., 2005; S.L. Morrison and J. Schlom, *Important Adv. Oncol.* (1990), pp. 3–18; Riechmann, L., Clark, M., Waldmann, H. and Winter, G. "*Reshaping human antibodies for therapy.*" Nature (1988) 332: 323-327; Roguska-M-A, Pedersen-J-T, Henry-A-H, Searle-S-M, Roja-C-M, Avery-B, Hoffee-M, Cook-S, Lambert-J-M, Blättler-W-A, Rees-A-R, Guild-B-C. A comparison of two murine mAbs humanized by CDR-grafting and variable domain resurfacing.Protein engineering, {Protein-Eng}, 1996, vol. 9, p. 895-904; Kashmiri-Syed-V-S, De-Pascalis-Roberto, Gonzales-Noreen-R, Schlom-Jeffrey. SDR grafting--a new approach to antibody humanization. Methods (San Diego Calif.), {Methods}, May 2005, vol. 36, no. 1, p. 25-34; Desmet-Johan, Meersseman-Geert, Boutonnet-Nathalie, Pletinckx-Jurgen, De-Clercq-Krista, Debulpaep-Maja, Braeckman-Tessa, Lasters-Ignace. Anchor profiles of HLA-specific peptides: analysis by a novel affinity scoring method and experimental validation. Proteins, 2005, vol. 58, p. 53-69; Stickler-M-M, Estell-D-A, Harding-F-A. CD4+ T-cell epitope determination using unexposed human

donor peripheral blood mononuclear cells. Journal of immunotherapy 2000, vol. 23, p. 654-60.)

1.B.7: In Vivo Efficacy

To generate a DVD-Ig molecule with desired in vivo efficacy, it is important to generate and select mAbs with similarly desired in vivo efficacy when given in combination. However, in some instances the DVD-Ig may exhibit in vivo efficacy that cannot be achieved with the combination of two separate mAbs. For instance, a DVD-Ig may bring two targets in close proximity leading to an activity that cannot be achieved with the combination of two separate mAbs. Additional desirable biological functions are described herein in section **B 3**. Parent antibodies with characteristics desirable in the DVD-Ig molecule may be selected based on factors such as pharmacokinetic t ½; tissue distribution; soluble versus cell surface targets; and target concentration- soluble/density –surface.

1.B.8: In Vivo Tissue Distribution

To generate a DVD-Ig molecule with desired in vivo tissue distribution, in an embodiment parent mAbs with similar desired in vivo tissue distribution profile must be selected. Alternatively, based on the mechanism of the dual-specific targeting strategy, it may at other times not be required to select parent mAbs with the similarly desired in vivo tissue distribution when given in combination. For instance, in the case of a DVD-Ig in which one binding component targets the DVD-Ig to a specific site thereby bringing the second binding component to the same target site. For example, one binding specificity of a DVD-Ig could target pancreas (islet cells) and the other specificity could bring GLP1 to the pancreas to induce insulin.

1.B.9: Isotype

To generate a DVD-Ig molecule with desired properties including, but not limited to, Isotype, Effector functions and the circulating half-life, in an embodiment parent mAbs with appropriate Fc-effector functions depending on the therapeutic utility and the desired therapeutic end-point are selected. There are five main heavy-chain classes or

isotypes some of which have several sub-types and these determine the effector functions of an antibody molecule. These effector functions reside in the hinge region, CH2 and CH3 domains of the antibody molecule. However, residues in other parts of an antibody molecule may have effects on effector functions as well. The hinge region Fc-effector functions include: (i) antibody-dependent cellular cytotoxicity, (ii) complement (C1q) binding, activation and complement-dependent cytotoxicity (CDC), (iii) phagocytosis/clearance of antigen-antibody complexes, and (iv) cytokine release in some instances. These Fc-effector functions of an antibody molecule are mediated through the interaction of the Fc-region with a set of class-specific cell surface receptors. Antibodies of the IgG1 isotype are most active while IgG2 and IgG4 having minimal or no effector functions. The effector functions of the IgG antibodies are mediated through interactions with three structurally homologous cellular Fc receptor types (and sub-types) (FcgR1, FcgRII and FcgRIII). These effector functions of an IgG1 can be eliminated by mutating specific amino acid residues in the lower hinge region (e.g., L234A, L235A) that are required for FcgR and C1q binding. Amino acid residues in the Fc region, in particular the CH2-CH3 domains, also determine the circulating half-life of the antibody molecule. This Fc function is mediated through the binding of the Fc-region to the neonatal Fc receptor (FcRn), which is responsible for recycling of antibody molecules from the acidic lysosomes back to the general circulation. In another embodiment, the activity of the Fc fragment can be greatly enhanced by sialylation of the N-linked glycan of the Fc portion (e.g., with 2,6-linkage to the penultimate galactose on the complex, biantennary glycan found at Asn 297 in immunoglobulin G (IgG)) (Anthony, RM (2008) Science 320:373-376).

Whether a mAb should have an active or an inactive isotype will depend on the desired therapeutic end-point for an antibody. Some examples of usage of isotypes and desired therapeutic outcome are listed below:

a) If the desired end-point is functional neutralization of a soluble cytokine then an inactive isotype may be used;

b) If the desired out-come is clearance of a pathological protein an active isotype may be used;

c) If the desired out-come is clearance of protein aggregates an active isotype may be used;

d) If the desired outcome is to antagonize a surface receptor an inactive isotype is used (Tysabri, IgG4; OKT3, mutated IgG1);

e) If the desired outcome is to eliminate target cells an active isotype is used (Herceptin, IgG1 (and with enhanced effector functions); and

f) If the desired outcome is to clear proteins from circulation without entering the CNS an IgM isotype may be used (e.g.,clearing circulating Ab peptide species).

The Fc effector functions of a parental mAb can be determined by various in vitro methods well known in the art.

As discussed, the selection of isotype, and thereby the effector functions will depend upon the desired therapeutic end-point. In cases where simple neutralization of a circulating target is desired, for example blocking receptor-ligand interactions, the effector functions may not be required. In such instances isotypes or mutations in the Fc-region of an antibody that eliminate effector functions are desirable. In other instances where elimination of target cells is the therapeutic end-point, for example elimination of tumor cells, isotypes or mutations or de-fucosylation in the Fc-region that enhance effector functions are desirable (Presta GL, Adv. Drug Delivery Rev. 58:640-656, 2006; Satoh M., Iida S., Shitara K. Expert Opinion Biol. Ther. 6:1161-1173, 2006). Similarly, depending up on the therapeutic utility, the circulating half-life of an antibody molecule can be reduced/prolonged by modulating antibody-FcRn interactions by introducing specific mutations in the Fc region (Dall'Acqua WF, Kiener PA, Wu H. J. Biol. Chem. 281:23514-23524, 2006; Petkova SB., Akilesh S., Sproule TJ. et al. Internat. Immunol. 18:1759-1769, 2006; Vaccaro C., Bawdon R., Wanjie S et al. PNAS 103:18709-18714, 2007).

The published information on the various residues that influence the different effector functions of a normal therapeutic mAb may need to be confirmed for DVD-Ig. It may be possible that in a DVD-Ig format additional (different) Fc-region residues, other than those identified for the modulation of monoclonal antibody effector functions, may be important.

Overall, the decision as to which Fc-effector functions (isotype) will be critical in the final DVD-Ig format will depend up on the disease indication, therapeutic target, desired therapeutic end-point and safety considerations. Listed below are exemplary appropriate heavy chain and light chain constant regions including, but not limited to:

- IgG1 – allotype: G1mz
- IgG1 mutant – A234, A235
- IgG2 – allotype: G2m(n-)
- Kappa – Km3
- Lambda

Fc Receptor and C1q Studies: The possibility of unwanted antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) by antibody complexing to any overexpressed target on cell membranes can be abrogated by the (for example, L234A, L235A) hinge-region mutations. These substituted amino acids, present in the IgG1 hinge region of mAb, are expected to result in diminished binding of mAb to human Fc receptors (but not FcRn), as FcgR binding is thought to occur within overlapping sites on the IgG1 hinge region. This feature of mAb may lead to an improved safety profile over antibodies containing a wild-type IgG. Binding of mAb to human Fc receptors can be determined by flow cytometry experiments using cell lines (e.g.,THP-1, K562) and an engineered CHO cell line that expresses FcgRIIb (or other FcgRs). Compared to IgG1 control monoclonal antibodies, mAb show reduced binding to FcgRI and FcgRIIa whereas binding to FcgRIIb is unaffected. The binding and activation of C1q by antigen/IgG immune complexes triggers the classical complement cascade with consequent inflammatory and/or immunoregulatory responses. The C1q binding site on IgGs has been localized to residues within the IgG hinge region. C1q binding to increasing concentrations of mAb was assessed by C1q ELISA. The results demonstrate that mAb is unable to bind to C1q, as expected when compared to the binding of a wildtype control IgG1. Overall, the L234A, L235A hinge region mutation abolishes binding of mAb to FcgRI, FcgRIIa and C1q but does not impact the interaction of mAb with FcgRIIb. This data suggests that in vivo, mAb with mutant Fc will interact

normally with the inhibitory FcgRIIb but will likely fail to interact with the activating FcgRI and FcgRIIa receptors or C1q.

Human FcRn binding: The neonatal receptor (FcRn) is responsible for transport of IgG across the placenta and to control the catabolic half-life of the IgG molecules. It might be desirable to increase the terminal half-life of an antibody to improve efficacy, to reduce the dose or frequency of administration, or to improve localization to the target. Alternatively, it might be advantageous to do the converse that is, to decrease the terminal half-life of an antibody to reduce whole body exposure or to improve the target-to-non-target binding ratios. Tailoring the interaction between IgG and its salvage receptor, FcRn, offers a way to increase or decrease the terminal half-life of IgG. Proteins in the circulation, including IgG, are taken up in the fluid phase through micropinocytosis by certain cells, such as those of the vascular endothelia. IgG can bind FcRn in endosomes under slightly acidic conditions (pH 6.0–6.5) and can recycle to the cell surface, where it is released under almost neutral conditions (pH 7.0–7.4). Mapping of the Fc-region-binding site on FcRn80, 16, 17 showed that two histidine residues that are conserved across species, His310 and His435, are responsible for the pH dependence of this interaction. Using phage-display technology, a mouse Fc-region mutation that increases binding to FcRn and extends the half-life of mouse IgG was identified (see Victor, G. et al.; Nature Biotechnology (1997), 15(7), 637-640). Fc-region mutations that increase the binding affinity of human IgG for FcRn at pH 6.0, but not at pH 7.4, have also been identified (see Dall'Acqua William F, et al., Journal of Immunology (2002), 169(9), 5171-80). Moreover, in one case, a similar pH-dependent increase in binding (up to 27-fold) was also observed for rhesus FcRn, and this resulted in a twofold increase in serum half-life in rhesus monkeys compared with the parent IgG (see Hinton, Paul R. et al., Journal of Biological Chemistry (2004), 279(8), 6213-6216). These findings indicate that it is feasible to extend the plasma half-life of antibody therapeutics by tailoring the interaction of the Fc region with FcRn. Conversely, Fc-region mutations that attenuate interaction with FcRn can reduce antibody half-life.

1.B.10: Pharmacokinetics (PK)

To generate a DVD-Ig molecule with desired pharmacokinetic profile, in an embodiment parent mAbs with the similarly desired pharmacokinetic profile are selected.

One consideration is that immunogenic response to monoclonal antibodies (ie, HAHA, human anti-human antibody response; HACA, human anti-chimeric antibody response) further complicates the pharmacokinetics of these therapeutic agents. In an embodiment, monoclonal antibodies with minimal or no immunogenicity are used for constructing DVD-Ig molecules such that the resulting DVD-Igs will also have minimal or no immunogenicity. Some of the factors that determine the PK of a mAb include, but are not limited to, Intrinsic properties of the mAb (VH amino acid sequence); immunogenicity; FcRn binding and Fc functions.

The PK profile of selected parental monoclonal antibodies can be easily determined in rodents as the PK profile in rodents correlates well with (or closely predicts) the PK profile of monoclonal antibodies in cynomolgus monkey and humans. The PK profile is determined as described in Example section 1.3.3.1.

After the parental monoclonal antibodies with desired PK characteristics (and other desired functional properties as discussed herein) are selected, the DVD-Ig is constructed. As the DVD-Ig molecules contain two antigen-binding domains from two parental monoclonal antibodies, the PK properties of the DVD-Ig are assessed as well. Therefore, while determining the PK properties of the DVD-Ig, PK assays may be employed that determine the PK profile based on functionality of both antigen-binding domains derived from the 2 parent monoclonal antibodies. The PK profile of a DVD-Ig can be determined as described in Example 1.3.3.1. Additional factors that may impact the PK profile of DVD-Ig include the antigen-binding domain (CDR) orientation; Linker size; and Fc / FcRn interactions. PK characteristics of parent antibodies can be evaluated by assessing the following parameters: absorption, distribution, metabolism and excretion.

Absorption: To date, administration of therapeutic monoclonal antibodies is via parenteral routes (e.g., intravenous [IV], subcutaneous [SC], or intramuscular [IM]). Absorption of a mAb into the systemic circulation following either SC or IM administration from the interstitial space is primarily through the lymphatic pathway. Saturable, presystemic, proteolytic degradation may result in variable absolute bioavailability following extravascular administration. Usually, increases in absolute bioavailability with increasing doses of monoclonal antibodies may be observed due to

saturated proteolytic capacity at higher doses. The absorption process for a mAb is usually quite slow as the lymph fluid drains slowly into the vascular system, and the duration of absorption may occur over hours to several days.The absolute bioavailability of monoclonal antibodies following SC administration generally ranges from 50% to 100%.

Distribution: Following IV administration, monoclonal antibodies usually follow a biphasic serum (or plasma) concentration-time profile, beginning with a rapid distribution phase, followed by a slow elimination phase. In general, a biexponential pharmacokinetic model best describes this kind of pharmacokinetic profile. The volume of distribution in the central compartment (Vc) for a mAb is usually equal to or slightly larger than the plasma volume (2-3 liters). A distinct biphasic pattern in serum (plasma) concentration versus time profile may not be apparent with other parenteral routes of administration, such as IM or SC, because the distribution phase of the serum (plasma) concentration-time curve is masked by the long absorption portion. Many factors, including physicochemical properties, site-specific and target-oriented receptor mediated uptake, binding capacity of tissue, and mAb dose can influence biodistribution of a mAb. Some of these factors can contribute to nonlinearity in biodistribution for a mAb.

Metabolism and Excretion: Due to the molecular size, intact monoclonal antibodies are not excreted into the urine via kidney. They are primarily inactivated by metabolism (e.g., catabolism). For IgG-based therapeutic monoclonal antibodies, half-lives typically ranges from hours or 1-2 days to over 20 days. The elimination of a mAb can be affected by many factors, including, but not limited to, affinity for the FcRn receptor, immunogenicity of the mAb, the degree of glycosylation of the mAb, the susceptibility for the mAb to proteolysis, and receptor-mediated elimination.

I.B.11: Tissue Cross-Reactivity Pattern On Human And Tox Species

Identical staining pattern suggests that potential human toxicity can be evaluated in tox species. Tox species are those animal in which unrelated toxicity is studied.

The individual antibodies are selected to meet two criteria. (1) Tissue staining appropriate for the known expression of the antibody target. (2) Similar staining pattern between human and tox species tissues from the same organ.

Criterion 1: Immunizations and/or antibody selections typically employ recombinant or synthesized antigens (proteins, carbohydrates or other molecules). Binding to the natural counterpart and counterscreen against unrelated antigens are often part of the screening funnel for therapeutic antibodies. However, screening against a multitude of antigens is often unpractical. Therefore tissue cross-reactivity studies with human tissues from all major organs serve to rule out unwanted binding of the antibody to any unrelated antigens.

Criterion 2: Comparative tissue cross reactivity studies with human and tox species tissues (cynomolgus monkey, dog, possibly rodents and others, the same 36 or 37 tissues are being tested as in the human study) help to validate the selection of a tox species. In the typical tissue cross-reactivity studies on frozen tissues sections therapeutic antibodies may demonstrate the expected binding to the known antigen and/or to a lesser degree binding to tissues based either on low level interactions (unspecific binding, low level binding to similar antigens, low level charge based interactions etc.). In any case the most relevant toxicology animal species is the one with the highest degree of coincidence of binding to human and animal tissue.

Tissue cross reactivity studies follow the appropriate regulatory guidelines including EC CPMP Guideline III/5271/94 "Production and quality control of mAbs" and the 1997 US FDA/CBER "Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use". . Cryosections (5 µm) of human tissues obtained at autopsy or biopsy were fixed and dried on object glass. The peroxidase staining of tissue sections was performed, using the avidin-biotin system. FDA's Guidance *"Points to Consider in the Manufacture and Testing of Monoclonal*

Antibody Products for Human Use". Relevant references include Clarke J 2004, Boon L. 2002a, Boon L 2002b, Ryan A 1999.

Tissue cross reactivity studies are often done in two stages, with the first stage including cryosections of 32 tissues (typically: Adrenal Gland, Gastrointestinal Tract, Prostate, Bladder, Heart, Skeletal Muscle, Blood Cells, Kidney, Skin, Bone Marrow, Liver, Spinal Cord, Breast, Lung, Spleen, Cerebellum, Lymph Node, Testes, Cerebral Cortex, Ovary, Thymus, Colon, Pancreas, Thyroid, Endothelium, Parathyroid, Ureter, Eye, Pituitary, Uterus, Fallopian Tube and Placenta) from one human donor. In the second phase a full cross reactivity study is performed with up to 38 tissues (including adrenal, blood, blood vessel, bone marrow, cerebellum, cerebrum, cervix, esophagus, eye, heart, kidney, large intestine, liver, lung, lymph node, breast mammary gland, ovary, oviduct, pancreas, parathyroid, peripheral nerve, pituitary, placenta, prostate, salivary gland, skin, small intestine, spinal cord, spleen, stomach, striated muscle, testis, thymus, thyroid, tonsil, ureter, urinary bladder, and uterus) from 3 unrelated adults. Studies are done typically at minimally two dose levels.

The therapeutic antibody (i.e. test article) and isotype matched control antibody may be biotinylated for avidin-biotin complex (ABC) detection; other detection methods may include tertiary antibody detection for a FITC (or otherwise) labeled test article, or precomplexing with a labeled anti-human IgG for an unlabeled test article.

Briefly, cryosections (about 5 µm) of human tissues obtained at autopsy or biopsy are fixed and dried on object glass. The peroxidase staining of tissue sections is performed, using the avidin-biotin system. First (in case of a precomplexing detection system), the test article is incubated with the secondary biotinylated anti-human IgG and developed into immune complex. The immune complex at the final concentrations of 2 and 10 µg/mL of test article is added onto tissue sections on object glass and then the tissue sections were reacted for 30 minutes with a avidin-biotin-peroxidase kit. Subsequently, DAB (3,3'-diaminobenzidine), a substrate for the peroxidase reaction, was applied for 4 minutes for tissue staining. Antigen-Sepharose beads are used as positive control tissue sections.

Any specific staining is judged to be either an expected (e.g.,consistent with antigen expression) or unexpected reactivity based upon known expression of the target

antigen in question. Any staining judged specific is scored for intensity and frequency. Antigen or serum competion or blocking studies can assist further in determining whether observed staining is specific or nonspecific.

If two selected antibodies are found to meet the selction criteria – appropriate tissue staining, matching staining between human and toxicology animal specific tissue – they can be selected for DVD-Ig generation.

The tissue cross reactivity study has to be repeated with the final DVD-Ig construct, but while these studies follow the same protocol as outline herein, they are more complex to evaluate because any binding can come from any of the two parent antibodies, and any unexplained binding needs to be confirmed with complex antigen competition studies.

It is readily apparent that the complex undertaking of tissue crossreactivity studies with a multispecific molecule like a DVD-Ig is greatly simplified if the two parental antibodies are selected for (1) lack of unexpected tissue cross reactivity findings and (2) for appropriate similarity of tissue cross reactivity findings between the corresponding human and toxicology animal species tissues.

1.B.12: Specificity And Selectivity

To generate a DVD-Ig molecule with desired specificity and selectivity, one needs to generate and select parent mAbs with the similarly desired specificity and selectivity profile.

Binding studies for specificity and selectivity with a DVD-Ig can be complex due to the four or more binding sites, two each for each antigen. Briefly, binding studies using ELISA, BIAcore. KinExA or other interaction studies with a DVD-Ig need to monitor the binding of one, two or more antigens to the DVD-Ig molecule. While BIAcore technology can resolve the sequential, independent binding of multiple antigens, more traditional methods including ELISA or more modern techniques like KinExA cannot. Therefore careful characterization of each parent antibody is critical. After each individual antibody has been characterized for specificity, confirmation of specificity retention of the individual binding sites in the DVD-Ig molecule is greatly simplified.

It is readily apparent that the complex undertaking of determining the specificity of a DVD-Ig is greatly simplified if the two parental antibodies are selected for specificity prior to being combined into a DVD-Ig.

Antigen–antibody interaction studies can take many forms, including many classical protein protein interaction studies, including ELISA (Enzyme linked immunosorbent assay), Mass spectrometry, chemical cross linking, SEC with light scattering, equilibrium dialysis, gel permeation, ultrafiltration, gel chromatography, large-zone analytical SEC, micropreparative ultracentrigugation (sedimentation equilibrium), spectroscopic methods, titration microcalorimetry, sedimentation equilibrium (in analytical ultracentrifuge), sedimentation velocity (in analytical centrifuge), surface plasmon resonance (including BIAcore). Relevant references include "Current Protocols in Protein Science", John E. Coligan, Ben M. Dunn, David W. Speicher, Paul T. Wingfield (eds.) Volume 3, chapters 19 and 20, published by John Wiley & Sons Inc., and references included therein and "Current Protocols in Immunology", John E. Coligan, Barbara E. Bierer, David H. Margulies, Ethan M. Shevach, Warren Strober (eds.) published by John Wiley & Sons Inc and relevant references included therein.

Cytokine Release in Whole Blood: The interaction of mAb with human blood cells can be investigated by a cytokine release assay (Wing, M. G. Therapeutic Immunology (1995), 2(4), 183-190; "Current Protocols in Pharmacology", S.J. Enna, Michael Williams, John W. Ferkany, Terry Kenakin, Paul Moser, (eds.) published by John Wiley & Sons Inc; Madhusudan, S. Clinical Cancer Research (2004), 10(19), 6528-6534; Cox, J. Methods (2006), 38(4), 274-282; Choi, I. European Journal of Immunology (2001), 31(1), 94-106). Briefly, various concentrations of mAb are incubated with human whole blood for 24 hours. The concentration tested should cover a wide range including final concentrations mimicking typical blood levels in patients (including but not limited to 100 ng/ml – 100μg/ml). Following the incubation, supernatants and cell lysates were analyzed for the presence of IL-1Rα, TNF-α, IL-1b, IL-6 and IL-8. Cytokine concentration profiles generated for mAb were compared to profiles produced by a negative human IgG control and a positive LPS or PHA control. The cytokine profile displayed by mAb from both cell supernatants and cell lysates was comparable to control human IgG. In an embodiment, the monoclonal antibody does not interact with human blood cells to spontaneously release inflammatory cytokines.

Cytokine release studies for a DVD-Ig are complex due to the four or more binding sites, two each for each antigen. Briefly, cytokine release studies as described herein measure the effect of the whole DVD-Ig molecule on whole blood or other cell systems, but can resolve which portion of the molecule causes cytokine release. Once cytokine release has been detected, the purity of the DVD-Ig preparation has to be ascertained, because some co-purifying cellular components can cause cytokine release on their own. If purity is not the issue, fragmentation of DVD-Ig (including but not limited to removal of Fc portion, separation of binding sites etc.), binding site mutagenesis or other methods may need to be employed to deconvolute any observations. It is readily apparent that this complex undertaking is greatly simplified if the two parental antibodies are selected for lack of cytokine release prior to being combined into a DVD-Ig.

1.B.13: Cross Reactivity To Other Species For Toxicological Studies

In an embodiment, the individual antibodies selected with sufficient cross-reactivity to appropriate tox species, for example, cynomolgus monkey. Parental antibodies need to bind to orthologous species target (i.e., cynomolgus monkey) and elicit appropriate response (modulation, neutralization, activation). In an embodiment, the cross-reactivity (affinity/potency) to orthologous species target should be within 10-fold of the human target. In practice, the parental antibodies are evaluated for multiple species, including mouse, rat, dog, monkey (and other non-human primates), as well as disease model species (i.e. sheep for asthma model). The acceptable cross-reactivity to tox species from the perantal monoclonal antibodies allows future toxicology studies of DVD-Ig-Ig in the same species. For that reason, the two parental monoclonal antibodies should have acceptable cross-reactivity for a common tox species therefore allowing toxicology studies of DVD-Ig in the same species.

Parent mAbs may be selected from various mAbs capable of binding specific targets and well known in the art. These include, but are not limited to anti-TNF antibody (US Patent No. 6,258,562), mouse or humanized anti-PGE2 antibody (US Application Serial Nos. 61/134,264 and 61/197,258 and Attorney Docket No. 9263.US.01, filed herewith, anti-IL-12 and/or anti-IL-12p40 antibody (US Patent No. 6,914,128); anti-IL-18 antibody (US 2005/0147610 A1), anti-C5, anti-CBL, anti-CD147, anti-gp120, anti-

VLA-4, anti-CD11a, anti-CD18, anti-VEGF, anti-CD40L, anti CD-40 (e.g., see WO2007124299) anti-Id, anti-ICAM-1, anti-CXCL13, anti-CD2, anti-EGFR, anti-TGF-beta 2, anti-HGF, anti-cMet, anti DLL-4, anti-NPR1, anti-PLGF, anti-E-selectin, anti-Fact VII, anti-Her2/neu, anti-F gp, anti-CD11/18, anti-CD14, anti-ICAM-3, anti-RON, anti CD-19, anti-CD80 (e.g., see WO2003039486, anti-CD4, anti-CD3, anti-CD23, anti-beta2-integrin, anti-alpha4beta7, anti-CD52, anti-HLA DR, anti-CD22 (e.g., see US Patent NO: 5,789,554), anti-CD20, anti-MIF, anti-CD64 (FcR), anti-TCR alpha beta, anti-CD2, anti-Hep B, anti-CA 125, anti-EpCAM, anti-gp120, anti-CMV, anti-gpIIbIIIa, anti-IgE, anti-CD25, anti-CD33, anti-HLA, anti-IGF1,2, anti IGFR, anti-VNRintegrin, anti-IL-1alpha, anti-IL-1beta, anti-IL-1 receptor, anti-IL-2 receptor, anti-IL-4, anti-IL-4 receptor, anti-IL5, anti-IL-5 receptor, anti-IL-6, anti- IL-6R, RANKL, NGF, DKK, alphaVbeta3, IL-17A, anti-IL-8, anti-IL-9, anti-IL-13, anti-IL-13 receptor, anti-IL-17, and anti-IL-23; IL-23p19; (see Presta LG. 2005 Selection, design, and engineering of therapeutic antibodies J Allergy Clin Immunol. 116:731-6 and http://www.path.cam.ac.uk/~mrc7/humanisation/antibodies.html).

Parent mAbs may also be selected from various therapeutic antibodies approved for use, in clinical trials, or in development for clinical use. Such therapeutic antibodies include, but are not limited to, rituximab (Rituxan®, IDEC/Genentech/Roche) (see for example U. S. Pat. No. 5,736,137), a chimeric anti-CD20 antibody approved to treat Non-Hodgkin's lymphoma; HuMax-CD20, an anti-CD20 currently being developed by Genmab, an anti-CD20 antibody described in U.S. Pat. No. 5, 500,362, AME-133 (Applied Molecular Evolution), hA20 (Immunomedics, Inc.), HumaLYM (Intracel), and PRO70769 (PCT/US2003/040426, entitled "Immunoglobulin Variants and Uses Thereof"), trastuzumab (Herceptin®, Genentech) (see for example U.S. Pat. No. 5,677,171), a humanized anti- Her2/neu antibody approved to treat breast cancer; pertuzumab (rhuMab-2C4, Omnitarg®), currently being developed by Genentech; an anti-Her2 antibody described in U.S. Pat. No. 4,753,894; cetuximab (Erbitux®, Imclone) (U.S. Pat. No. 4,943,533; PCT WO 96/40210), a chimeric anti-EGFR antibody in clinical trials for a variety of cancers; ABX-EGF (U.S. Pat. No. 6,235,883), currently being developed by Abgenix-Immunex-Amgen; HuMax- EGFr (U.S. Ser. No. 10/172,317), currently being developed by Genmab; 425, EMD55900, EMD62000, and EMD72000 (Merck KGaA) (U.S. Pat. No. 5,558,864; Murthy et al. 1987, Arch Biochem Biophys. 252(2):549-60; Rodeck et al., 1987, J Cell Biochem. 35(4):315-20; Kettleborough et al.,

1991, Protein Eng. 4(7):773-83); ICR62 (Institute of Cancer Research) (PCT WO 95/20045; Modjtahedi et al., 1993, J. Cell Biophys. 1993, 22(1-3):129-46; Modjtahedi et al., 1993, Br J Cancer. 1993, 67(2):247-53; Modjtahedi et al, 1996, Br J Cancer, 73(2):228-35; Modjtahedi et al, 2003, Int J Cancer, 105(2):273-80); TheraCIM hR3 (YM Biosciences, Canada and Centro de Immunologia Molecular, Cuba (U.S. Pat. No. 5,891,996; U.S. Pat. No. 6,506, 883; Mateo et al, 1997, Immunotechnology, 3(1):71-81); mAb-806 (Ludwig Institue for Cancer Research, Memorial Sloan-Kettering) (Jungbluth et al. 2003, Proc Natl Acad Sci USA. 100(2):639-44); KSB-102 (KS Biomedix); MR1-1 (IVAX, National Cancer Institute) (PCT WO 0162931A2); and SC100 (Scancell) (PCT WO 01/88138); alemtuzumab (Campath®, Millenium), a humanized mAb currently approved for treatment of B-cell chronic lymphocytic leukemia; muromonab-CD3 (Orthoclone OKT3®), an anti-CD3 antibody developed by Ortho Biotech/Johnson & Johnson, ibritumomab tiuxetan (Zevalin®), an anti-CD20 antibody developed by IDEC/Schering AG, gemtuzumab ozogamicin (Mylotarg®), an anti-CD33 (p67 protein) antibody developed by Celltech/Wyeth, alefacept (Amevive®), an anti-LFA-3 Fc fusion developed by Biogen), abciximab (ReoPro®), developed by Centocor/Lilly, basiliximab (Simulect®), developed by Novartis, palivizumab (Synagis®), developed by Medimmune, infliximab (Remicade®), an anti-TNFalpha antibody developed by Centocor, adalimumab (Humira®), an anti-TNFalpha antibody developed by Abbott, Humicade®, an anti-TNFalpha antibody developed by Celltech, golimumab (CNTO-148), a fully human TNF antibody developed by Centocor, etanercept (Enbrel®), an p75 TNF receptor Fc fusion developed by Immunex/Amgen, lenercept, an p55TNF receptor Fc fusion previously developed by Roche, ABX-CBL, an anti-CD147 antibody being developed by Abgenix, ABX-IL8, an anti-IL8 antibody being developed by Abgenix, ABX-MA1, an anti-MUC18 antibody being developed by Abgenix, Pemtumomab (R1549, 90Y-muHMFG1), an anti-MUC1 in development by Antisoma, Therex (R1550), an anti-MUC1 antibody being developed by Antisoma, AngioMab (AS1405), being developed by Antisoma, HuBC-1, being developed by Antisoma, Thioplatin (AS1407) being developed by Antisoma, Antegren® (natalizumab), an anti-alpha-4-beta-1 (VLA-4) and alpha-4-beta-7 antibody being developed by Biogen, VLA-1 mAb, an anti-VLA-1 integrin antibody being developed by Biogen, LTBR mAb, an anti-lymphotoxin beta receptor (LTBR) antibody being developed by Biogen, CAT-152, an anti-TGF-β2 antibody being developed by Cambridge Antibody Technology, ABT 874

(J695), an anti- IL-12 p40 antibody being developed by Abbott, CAT-192, an anti-TGFβ1 antibody being developed by Cambridge Antibody Technology and Genzyme, CAT-213, an anti-Eotaxin1 antibody being developed by Cambridge Antibody Technology, LymphoStat-B® an anti-Blys antibody being developed by Cambridge Antibody Technology and Human Genome Sciences Inc., TRAIL-R1mAb, an anti-TRAIL-R1 antibody being developed by Cambridge Antibody Technology and Human Genome Sciences, Inc. , Avastin® bevacizumab, rhuMAb-VEGF), an anti-VEGF antibody being developed by Genentech, an anti-HER receptor family antibody being developed by Genentech, Anti-Tissue Factor (ATF), an anti-Tissue Factor antibody being developed by Genentech, Xolair® (Omalizumab), an anti-IgE antibody being developed by Genentech, Raptiva® (Efalizumab), an anti- CD11a antibody being developed by Genentech and Xoma, MLN-02 Antibody (formerly LDP-02), being developed by Genentech and Millenium Pharmaceuticals, HuMax CD4, an anti-CD4 antibody being developed by Genmab, HuMax-IL15, an anti-IL15 antibody being developed by Genmab and Amgen, HuMax-Inflam, being developed by Genmab and Medarex, HuMax-Cancer, an anti-Heparanase I antibody being developed by Genmab and Medarex and Oxford GcoSciences, HuMax-Lymphoma, being developed by Genmab and Amgen, HuMax-TAC, being developed by Genmab, IDEC-131, and anti-CD40L antibody being developed by IDEC Pharmaceuticals, IDEC-151 (Clenoliximab), an anti- CD4 antibody being developed by IDEC Pharmaceuticals, IDEC-114, an anti-CD80 antibody being developed by IDEC Pharmaceuticals, IDEC-152, an anti- CD23 being developed by IDEC Pharmaceuticals, anti-macrophage migration factor (MIF) antibodies being developed by IDEC Pharmaceuticals, BEC2, an anti-idiotypic antibody being developed by Imclone, IMC-1C11, an anti-KDR antibody being developed by Imclone, DC101, an anti-flk-1 antibody being developed by Imclone, anti-VE cadherin antibodies being developed by Imclone, CEA-Cide® (labetuzumab), an anti-carcinoembryonic antigen (CEA) antibody being developed by Immunomedics, LymphoCide® (Epratuzumab), an anti-CD22 antibody being developed by Immunomedics, AFP-Cide, being developed by Immunomedics, MyelomaCide, being developed by Immunomedics, LkoCide, being developed by Immunomedics, ProstaCide, being developed by Immunomedics, MDX-010, an anti-CTLA4 antibody being developed by Medarex, MDX-060, an anti-CD30 antibody being developed by Medarex, MDX-070 being developed by Medarex, MDX-018 being developed by Medarex,

Osidem® (IDM-1), and anti-Her2 antibody being developed by Medarex and Immuno-Designed Molecules, HuMax®-CD4, an anti-CD4 antibody being developed by Medarex and Genmab, HuMax-IL15, an anti-IL15 antibody being developed by Medarex and Genmab, CNTO 148, an anti-TNFα antibody being developed by Medarex and Centocor/J&J, CNTO 1275, an anti-cytokine antibody being developed by Centocor/J&J, MOR101 and MOR102, anti-intercellular adhesion molecule-1 (ICAM-1) (CD54) antibodies being developed by MorphoSys, MOR201, an anti-fibroblast growth factor receptor 3 (FGFR-3) antibody being developed by MorphoSys, Nuvion® (visilizumab), an anti-CD3 antibody being developed by Protein Design Labs, HuZAF®, an anti-gamma interferon antibody being developed by Protein Design Labs, Anti-α 5β1 Integrin, being developed by Protein Design Labs, anti-IL-12, being developed by Protein Design Labs, ING-1, an anti-Ep-CAM antibody being developed by Xoma, Xolair® (Omalizumab) a humanized anti-IgE antibody developed by Genentech and Novartis, and MLN01, an anti-Beta2 integrin antibody being developed by Xoma. In another embodiment, the therapeutics include KRN330 (Kirin); huA33 antibody (A33, Ludwig Institute for Cancer Research); CNTO 95 (alpha V integrins, Centocor); MEDI-522 (alpha Vβ3 integrin, Medimmune); volociximab (alpha Vβ1 integrin, Biogen/PDL); Human mAb 216 (B cell glycosolated epitope, NCI); BiTE MT103 (bispecific CD19 x CD3, Medimmune); 4G7xH22 (Bispecific BcellxFcgammaR1, Medarex/Merck KGa); rM28 (Bispecific CD28 x MAPG, US Patent No. EP1444268); MDX447 (EMD 82633) (Bispecific CD64 x EGFR, Medarex); Catumaxomab (removab) (Bispecific EpCAM x anti-CD3, Trion/Fres); Ertumaxomab (bispecific HER2/CD3, Fresenius Biotech); oregovomab (OvaRex) (CA-125, ViRexx); Rencarex® (WX G250) (carbonic anhydrase IX, Wilex); CNTO 888 (CCL2, Centocor); TRC105 (CD105 (endoglin), Tracon); BMS-663513 (CD137 agonist, Brystol Myers Squibb); MDX-1342 (CD19, Medarex); Siplizumab (MEDI-507) (CD2, Medimmune); Ofatumumab (Humax-CD20) (CD20, Genmab); Rituximab (Rituxan) (CD20, Genentech); veltuzumab (hA20) (CD20, Immunomedics); Epratuzumab (CD22, Amgen); lumiliximab (IDEC 152) (CD23, Biogen); muromonab-CD3 (CD3, Ortho); HuM291 (CD3 fc receptor, PDL Biopharma); HeFi-1, CD30, NCI); MDX-060 (CD30, Medarex); MDX-1401 (CD30, Medarex); SGN-30 (CD30, Seattle Genentics); SGN-33 (Lintuzumab) (CD33, Seattle Genentics); Zanolimumab (HuMax-CD4) (CD4, Genmab); HCD122 (CD40, Novartis); SGN-40 (CD40, Seattle Genentics); Campath1h (Alemtuzumab) (CD52, Genzyme); MDX-1411

(CD70, Medarex); hLL1 (EPB-1) (CD74.38, Immunomedics); Galiximab (IDEC-144) (CD80, Biogen); MT293 (TRC093/D93) (cleaved collagen, Tracon); HuLuc63 (CS1, PDL Pharma); ipilimumab (MDX-010) (CTLA4, Brystol Myers Squibb); Tremelimumab (Ticilimumab, CP-675,2) (CTLA4, Pfizer); HGS-ETR1 (Mapatumumab) (DR4 TRAIL-R1 agonist, Human Genome Science /Glaxo Smith Kline); AMG-655 (DR5, Amgen); Apomab (DR5, Genentech); CS-1008 (DR5, Daiichi Sankyo); HGS-ETR2 (lexatumumab) (DR5 TRAIL-R2 agonist, HGS); Cetuximab (Erbitux) (EGFR, Imclone); IMC-11F8, (EGFR, Imclone); Nimotuzumab (EGFR, YM Bio); Panitumumab (Vectabix) (EGFR, Amgen); Zalutumumab (HuMaxEGFr) (EGFR, Genmab); CDX-110 (EGFRvIII, AVANT Immunotherapeutics); adecatumumab (MT201) (Epcam , Merck); edrecolomab (Panorex, 17-1A) (Epcam , Glaxo/Centocor); MORAb-003 (folate receptor a, Morphotech); KW-2871 (ganglioside GD3, Kyowa); MORAb-009 (GP-9, Morphotech); CDX-1307 (MDX-1307) (hCGb, Celldex); Trastuzumab (Herceptin) (HER2, Celldex); Pertuzumab (rhuMAb 2C4) (HER2 (DI), Genentech); apolizumab (HLA-DR beta chain, PDL Pharma); AMG-479 (IGF-1R, Amgen); anti-IGF-1R R1507 (IGF1-R, Roche); CP 751871 (IGF1-R, Pfizer); IMC-A12 (IGF1-R, Imclone); BIIB022 (IGF-1R , Biogen); Mik-beta-1 (IL-2Rb (CD122), Hoffman LaRoche); CNTO 328 (IL6, Centocor); Anti-KIR (1-7F9) (Killer cell Ig-like Receptor (KIR), Novo); Hu3S193 (Lewis (y), Wyeth, Ludwig Institute of Cancer Research); hCBE-11 (LTßR, Biogen); HuHMFG1 (MUC1, Antisoma/NCI); RAV12 (N-linked carbohydrate epitope, Raven); CAL (parathyroid hormone-related protein (PTH-rP), University of California); CT-011 (PD1, CureTech); MDX-1106 (ono-4538) (PD1, Medarex/Ono); MAb CT-011 (PD1, Curetech); IMC-3G3 (PDGFRa, Imclone); bavituximab (phosphatidylserine, Peregrine); huJ591 (PSMA, Cornell Research Foundation); muJ591 (PSMA, Cornell Research Foundation); GC1008 (TGFb (pan) inhibitor (IgG4), Genzyme); Infliximab (Remicade) (TNFa, Centocor); A27.15 (transferrin receptor, Salk Institute, INSERN WO 2005/111082); E2.3 (transferrin receptor, Salk Institute); Bevacizumab (Avastin) (VEGF, Genentech); HuMV833 (VEGF, Tsukuba Research Lab-WO/2000/034337, University of Texas); IMC-18F1 (VEGFR1, Imclone); IMC-1121 (VEGFR2, Imclone).

C: Construction of DVD Molecules

The dual variable domain immunoglobulin (DVD-Ig™) molecule is designed such that two different light chain variable domains (VL) from the two different parent

monoclonal antibodies are linked in tandem directly or via a short linker by recombinant DNA techniques, followed by the light chain constant domain. Similarly, the heavy chain comprises two different heavy chain variable domains (VH) linked in tandem, followed by the constant domain CH1 and Fc region (Figure 1A).

The variable domains can be obtained using recombinant DNA techniques from a parent antibody generated by any one of the methods described herein. In an embodiment, the variable domain is a murine heavy or light chain variable domain. In another embodiment, the variable domain is a CDR grafted or a humanized variable heavy or light chain domain. In an embodiment, the variable domain is a human heavy or light chain variable domain.

In one embodiment the first and second variable domains are linked directly to each other using recombinant DNA techniques. In another embodiment the variable domains are linked via a linker sequence. In an embodiment, two variable domains are linked. Three or more variable domains may also be linked directly or via a linker sequence. The variable domains may bind the same antigen or may bind different antigens. DVD molecules of the invention may include one immunoglobulin variable domain and one non- immunoglobulin variable domain such as ligand binding domain of a receptor, active domain of an enzyme. DVD molecules may also comprise 2 or more non-Ig domains.

The linker sequence may be a single amino acid or a polypeptide sequence. In an embodiment, the linker sequences are selected from the group consisting of AKTTPKLEEGEFSEAR (SEQ ID NO: 1); AKTTPKLEEGEFSEARV (SEQ ID NO: 2); AKTTPKLGG (SEQ ID NO: 3); SAKTTPKLGG (SEQ ID NO: 4); SAKTTP (SEQ ID NO: 5); RADAAP (SEQ ID NO: 6); RADAAPTVS (SEQ ID NO: 7); RADAAAAGGPGS (SEQ ID NO: 8); RADAAAA$(G_4S)_4$ (SEQ ID NO: 9); SAKTTPKLEEGEFSEARV (SEQ ID NO: 10); ADAAP (SEQ ID NO: 11); ADAAPTVSIFPP (SEQ ID NO: 12); TVAAP (SEQ ID NO: 13); TVAAPSVFIFPP (SEQ ID NO: 14); QPKAAP (SEQ ID NO: 15); QPKAAPSVTLFPP (SEQ ID NO: 16); AKTTPP (SEQ ID NO: 17); AKTTPPSVTPLAP (SEQ ID NO: 18); AKTTAP (SEQ ID NO: 19); AKTTAPSVYPLAP (SEQ ID NO: 20); ASTKGP (SEQ ID NO: 21); ASTKGPSVFPLAP (SEQ ID NO: 22), GGGGSGGGGSGGGGS (SEQ ID NO: 23);

GENKVEYAPALMALS (SEQ ID NO: 24); GPAKELTPLKEAKVS (SEQ ID NO: 25); and GHEAAAVMQVQYPAS (SEQ ID NO: 26). The choice of linker sequences is based on crystal structure analysis of several Fab molecules. There is a natural flexible linkage between the variable domain and the CH1/CL constant domain in Fab or antibody molecular structure. This natural linkage comprises approximately 10-12 amino acid residues, contributed by 4-6 residues from C-terminus of V domain and 4-6 residues from the N-terminus of CL/CH1 domain. DVD Igs of the invention were generated using N-terminal 5-6 amino acid residues, or 11-12 amino acid residues, of CL or CH1 as linker in light chain and heavy chain of DVD-Ig, respectively. The N-terminal residues of CL or CH1 domains, particularly the first 5-6 amino acid residues, adopt a loop conformation without strong secondary structures, therefore can act as flexible linkers between the two variable domains. The N-terminal residues of CL or CH1 domains are natural extension of the variable domains, as they are part of the Ig sequences, therefore minimize to a large extent any immunogenicity potentially arising from the linkers and junctions.

Other linker sequences may include any sequence of any length of CL/CH1 domain but not all residues of CL/CH1 domain; for example the first 5-12 amino acid residues of the CL/CH1 domains; the light chain linkers can be from Cκ or Cλ; and the heavy chain linkers can be derived from CH1 of any isotypes, including Cγ1, Cγ2, Cγ3, Cγ4, Cα1, Cα2, Cδ, Cε, and Cμ. Linker sequences may also be derived from other proteins such as Ig-like proteins, (e.g.TCR, FcR, KIR); G/S based sequences (e.g G4S repeats; SEQ ID NO: 27); hinge region-derived sequences; and other natural sequences from other proteins.

In an embodiment a constant domain is linked to the two linked variable domains using recombinant DNA techniques. In an embodiment, sequence comprising linked heavy chain variable domains is linked to a heavy chain constant domain and sequence comprising linked light chain variable domains is linked to a light chain constant domain. In an embodiment, the constant domains are human heavy chain constant domain and human light chain constant domain respectively. In an embodiment, the DVD heavy chain is further linked to an Fc region. The Fc region may be a native sequence Fc region, or a variant Fc region. In another embodiment, the Fc region is a human Fc region. In another embodiment the Fc region includes Fc region from IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgE, or IgD.

In another embodiment two heavy chain DVD polypeptides and two light chain DVD polypeptides are combined to form a DVD-Ig molecule. Table 2 lists amino acid sequences of VH and VL regions of exemplary antibodies for targets useful for treating disease, e.g., for treating cancer. In an embodiment, the invention provides a DVD comprising at least two of the VH and/or VL regions listed in Table 2, in any orientation. Example 3 provides DVD-Igs directed at the VH and VL regions listed in Table 2. Example 2 provides VH and VL regions for DVD-Igs directed to TNF and PGE2 (See Tables 5-8)

Table 2: List of Amino Acid Sequences of VH and VL regions of Antibodies for Generating DVD-Igs

SEQ ID No.	ABT Unique ID	Protein Region	Sequence 12345678901234567890123456789012345678
28	AB014VH	VH VEGF	EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNW VRQAPGKGLEWVGWINTYTGEPTYAADFKRRFTFSL DTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHW YFDVWGQGTLVTVSS
29	AB014VL	VL VEGF	DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWY QQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR
30	AB017VH	VH TNF	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHW VRQAPGKGLEWVSAITWNSGHIDYADSVEGRFTISR DNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSL DYWGQGTLVTVSS
31	AB017VL	Vl TNF	DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWY QQKPGKAPKLLIYAASTLQSGVPSRFSGSGSGTDFT LTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKR
32	AB020VH	VH NGF	QVQLQESGPGLVKPSETLSLTCTVSGFSLIGYDLNW IRQPPGKGLEWIGIIWGDGTTDYNSAVKSRVTISKD TSKNQFSLKLSSVTAADTAVYYCARGGYWYATSYYF DYWGQGTLVTVSS
33	AB020VL	VL NGF	DIQMTQSPSSLSASVGDRVTITCRASQSISNNLNWY QQKPGKAPKLLIYYTSRFHSGVPSRFSGSGSGTDFT FTISSLQPEDIATYYCQQEHTLPYTFGQGTKLEIKR
34	AB029VH	VH IL-17A	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMNW VRQAPGKGLEWVAAINQDGSEKYYVGSVKGRFTISR DNAKNSLYLQMNSLRVEDTAVYYCVRDYYDILTDYY IHYWYFDLWGRGTLVTVSS
35	AB029VL	VL IL-17A	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAW YQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDF TLTISRLEPEDFAVYYCQQYGSSPCTFGQGTRLEIK R
36	AB032VH	VH IL-1b	QVQLVESGGGVVQPGRSLRLSCAASGFTFSVYGMNW VRQAPGKGLEWVAIIWYDGDNQYYADSVKGRFTISR DNSKNTLYLQMNGLRAEDTAVYYCARDLRTGPFDYW GQGTLVTVSS
37	AB032VL	VL IL-1b	EIVLTQSPDFQSVTPKEKVTITCRASQSIGSSLHWY QQKPDQSPKLLIKYASQSFSGVPSRFSGSGSGTDFT LTINSLEAEDAAAYYCHQSSSLPFTFGPGTKVDIKR

SEQ ID No.	ABT Unique ID	Protein Region	Sequence 12345678901234567890123456789012345678901234567890123456
38	AB-040VH	VH IL-6	QVTLKESGPGILQPSQTLSLTCSFSGFSLSTNGMGV SWIRQPSGKGLEWLAHIYWDEDKRYNPSLKSRLTIS KDTSNNQVFLKITNVDTADTATYYCARRRIIYDVED YFDYWGQGTTLTVSS
39	AB040VL	VL IL-6	QIVLIQSPAIMSASPGEKVTMTCSASSSVSYMYWYQ QKPGSSPRLLIYDTSNLASGVPVRFSGSGSGTSYSL TISRMEAEDAATYYCQQWSGYPYTFGGGTKLEIKR
40	AB043VH	VH Abeta (seq. 1)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYGMSW VRQAPGKGLEWVASIRSGGGRTYYSDNVKGRFTISR DNSKNTLYLQMNSLRAEDTAVYYCVRYDHYSGSSDY WGQGTLVTVSS
41	AB043VL	VL Abeta (seq. 1)	DVVMTQSPLSLPVTPGEPASISCKSSQSLLDSDGKT YLNWLLQKPGQSPQRLIYLVSKLDSGVPDRFSGSGS GTDFTLKISRVEAEDVGVYYCWQGTHFPRTFGQGTK VEIKR
42	AB044VH	VH Abeta (seq. 2)	EVKLVESGGGLVKPGGSLRLSCAASGFTFSSYAMSW VRQAPGKGLEWVASIHNRGTIFYLDSVKGRFTISRD NVRNTLYLQMNSLRAEDTAVYYCTRGRSNSYAMDYW GQGTSVTVSS
43	AB044VL	VL Abeta (seq. 2)	DVLVTQSPLSLPVTPGEPASISCRSTQTLVHRNGDT YLEWYLQKPGQSPQSLIYKVSNRFSGVPDRFSGSGS GTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGQGTK LEIKR
44	AB045VH	VH Abeta (seq. 3)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMSW VRQAPGKGLELVASINSNGGSTYYPDSVKGRFTISR DNAKNTLYLQMNSLRAEDTAVYYCASGDYWGQGTLV TVSS
45	AB045VL	VL Abeta (seq. 3)	DIVMTQSPLSLPVTPGEPASISCRSSQSLVYSNGDT YLHWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGS GTDFTLKISRVEAEDVGVYYCSQSTHVPWTFGGGTK VEIKR
46	AB046VH	VH IL-18	EVQLVQSGTEVKKPGESLKISCKGSGYTVTSYWIGW VRQMPGKGLEWMGFIYPGDSETRYSPTFQGQVTISA DKSFNTAFLQWSSLKASDTAMYYCARVGSGWYPYTF DIWGQGTMVTVSS
47	AB046VL	VL IL-18	EIVMTQSPATLSVSPGERATLSCRASESISSNLAWY QQKPGQAPRLFIYTASTRATDIPARFSGSGSGTEFT LTISSLQSEDFAVYYCQQYNNWPSITFGQGTRLEIK R
48	AB048VH	VH PGE2	EVQLVQSGAEVKKPGASVKVSCKASGYTFTKYWLGW VRQAPGQGLEWMGDIYPGYDYTHYNEKFKDRVTLTT DTSTSTAYMELRSLRSDDTAVYYCARSDGSSTYWGQ GTLVTVSS
49	AB048VL	VL PGE2	DVLMTQTPLSLPVTPGEPASISCTSSQNIVHSNGNT YLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGS GTDFTLKISRVEAEDVGVYYCFQVSHVPYTFGGGTK VEIKR
50	AB049VH	VH IL-15	EVQLVQSGAEVKKPGESLKISCKVSGYFFTTYWIGW VRQMPGKGLEYMGIIYPGDSDTRYSPSFQGQVTISA DKSISTAYLQWSSLKASDTAMYYCARGGNWNCFDYW GQGTLVTVSS
51	AB049VL	VL IL-15	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAW YQQKPGQAPRLLIYGASRRATGIPDRFSGSGSGTDF TLTISRLEPEDFAVYYCQRYGSSHTFGQGTKLEISR
52	AB052VH	VH S1P	EVQLVQSGAEVKKPGESLKISCQSFGYIFIDHTIHW MRQMPGQGLEWMGAISPRHDITKYNEMFRGQVTISA DKSSSTAYLQWSSLKASDTAMYFCARGGFYGSTIWF DFWGQGTMVTVSS

SEQ ID No.	ABT Unique ID	Protein Region	Sequence 12345678901234567890123456789012345678
53	AB052VL	VL S1P	ETTVTQSPSFLSASVGDRVTITCITTTDIDDDMNWF QQEPGKAPKLLISEGNILRPGVPSRFSSSGYGTDFT LTISKLQPEDFATYYCLQSDNLPFTFGQGTKLEIKR
54	AB054VH	VH IL-6R	EVQLQESGPGLVRPSQTLSLTCTVSGYSITSDHAWS WVRQPPGRGLEWIGYISYSGITTYNPSLKSRVTMLR DTSKNQFSLRLSSVTAADTAVYYCARSLARTTAMDY WGQGSLVTVSS
55	AB054VL	VL IL-6R	DIQMTQSPSSLSASVGDRVTITCRASQDISSYLNWY QQKPGKAPKLLIYYTSRLHSGVPSRFSGSGSGTDFT FTISSLQPEDIATYYCQQGNTLPYTFGQGTKVEIKR
56	AB003VH	VH EGFR(1st seq)	QVQLQESGPGLVKPSETLSLTCTVSGGSVSSGDYYWTWIR QSPGKGLEWIGHIYYSGNTNYNPSLKSRLTISIDTSKTQF SLKLSSVTAADTAIYYCVRDRVTGAFDIWGQGTMVTVSS
57	AB003VL	VL EGFR(1st seq)	DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKP GKAPKLLIYDASNLETGVPSRFSGSGSGTDFTFTISSLQP EDIATYFCQHFDHLPLAFGGGTKVEIKR
58	AB033VH	VH EGFR (2nd seq)	QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQS PGKGLEWLGVIWSGGNTDYNTPFTSRLSINKDNSKSQVFF KMNSLQSNDTAIYYCARALTYYDYEFAYWGQGTLVTVSA
59	AB033VL	VL EGFR (2nd seq)	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRT NGSPRLLIKYASESISGIPSRFSGSGSGTDFTLSINSVES EDIADYYCQQNNNWPTTFGAGTKLELKR
60	AB011VH	VH IGF1R	EVQLLESGGGLVQPGGSLRLSCTASGFTFSSYAMNWVRQA PGKGLEWVSAISGSGGTTFYADSVKGRFTISRDNSRTTLY LQMNSLRAEDTAVYYCAKDLGWSDSYYYYYGMDVWGQGTT VTVSS
61	AB011VL	VL IGF1R	DIQMTQFPSSLSASVGDRVTITCRASQGIRNDLGWYQQKP GKAPKRLIYAASRLHRGVPSRFSGSGSGTEFTLTISSLQP EDFATYYCLQHNSYPCSFGQGTKLEIKR
62		VH Hu2B5.1	EVQLVQSGAEVKKPGASVKVSCKAS**GYTFTKYWLGW** VRQAPGQGLEWIG**DIYPGYDYTHYNEKFKD**RATLTV DTSTSTAYMELRSLRSDDTAVYYCAR**SDGSSTY**WGQ GTLVTVSS
63		VL HU2B5.1	DVVMTQTPLSLPVTPGEPASISC**TSSQNIVHSNGNT** **YLE**WYLQKPGQSPQLLIY**KVSNRFSG**VPDRFSGSGS GTDFTLKISRVEAEDVGVYYC**FQVSHVPYT**FGGGTK VEIKR
64		VH HU2B5.2	EVQLVQSGAEVKKPGASVKVSCKAS**GYTFTKYWLGW** VRQAPGQGLEWIG**DIYPGYDYTHYNEKFKD**RATLTV DTSTSTAYMELSSLRSDDTAVYYCAR**SDGSSTY**WGQ GTLVTVSS
65		VL HU2B5.2	DVVMTQTPLSLPVTPGEPASISC**TSSQNIVHSNGNT** **YLE**WYLQKPGQSPQLLIY**KVSNRFSG**VPDRFSGSGS GTDFTLKISRVEAEDVGVYYC**FQVSHVPYT**FGGGTK VEIKR
66		VH HU2B5.3	EVQLVQSGAEVKKPGASVKVSCKAS**GYTFTKYWLGW** VRQAPGQGLEWIG**DIYPGYDYTHYNEKFKD**RATLTV DTSTSTAYMELRSLRSDDTAVYYCAR**SDGSSTY**WGQ GTLVTVSS
67		VL HU2B5.3	DVLMTQTPLSLPVTPGEPASISC**TSSQNIVHSNGNT** **YLE**WYLQKPGQSPQLLIY**KVSNRF**SGVPDRFSGSGS GTDFTLKISRVEAEDVGVYYC**FQVSHVPYT**FGGGTK VEIKR
68		VH HU2B5.4	EVQLVQSGAEVKKPGASVKVSCKAS**GYTFTKYWLGW** VRQAPGQGLEWIG**DIYPGYDYTHYNEKFKD**RATLTV DTSTSTAYMELSSLRSDDTAVYYCAR**SDGSSTY**WGQ GTLVTVSS

SEQ ID No.	ABT Unique ID	Protein Region	Sequence 12345678901234567890123456789012345678
69		VL HU2B5.4	DVLMTQTPLSLPVTPGEPASISC**TSSQNIVHSNGNT** **YLE**WYLQKPGQSPQLLIY**KVSNRFSG**VPDRFSGSGS GTDFTLKISRVEAEDVGVYYC**FQVSHVPYT**FGGGTK VEIKR
70		VH HU2B5.5	EVQLVQSGAEVKKPGASVKVSCKAS**GYTFTKYWLG**W VRQAPGQGLEWMG**DIYPGYDYTHYNEKFKD**RVTLTT DTSTSTAYMELRSLRSDDTAVYYCAR**SDGSSTY**WGQ GTLVTVSS
71		VL HU2B5.5	DVVMTQTPLSLPVTPGEPASISC**TSSQNIVHSNGNT** **YLE**WYLQKPGQSPQLLIY**KVSNRF**SGVPDRFSGSGS GTDFTLKISRVEAEDVGVYYC**FQVSHVPYT**FGGGTK VEIKR
72		VH HU2B5.6	EVQLVQSGAEVKKPGASVKVSCKAS**GYTFTKYWLG**W VRQAPGQGLEWMG**DIYPGYDYTHYNEKFKD**RVTLTT DTSTSTAYMELSSLRSDDTAVYYCAR**SDGSSTY**WGQ GTLVTVSS
73		VL HU2B5.6	DVVMTQTPLSLPVTPGEPASISC**TSSQNIVHSNGNT** **YLE**WYLQKPGQSPQLLIY**KVSNRFSG**VPDRFSGSGS GTDFTLKISRVEAEDVGVYYC**FQVSHVPYT**FGGGTK VEIKR
74		VH HU2B5.7	EVQLVQSGAEVKKPGASVKVSCKAS**GYTFTKYWLG**W VRQAPGQGLEWMG**DIYPGYDYTHYNEKFKD**RVTLTT DTSTSTAYMELRSLRSDDTAVYYCAR**SDGSSTY**WGQ GTLVTVSS
75		VL HU2B5.7	DVLMTQTPLSLPVTPGEPASISC**TSSQNIVHSNGNT** **YLE**WYLQKPGQSPQLLIY**KVSNRFSG**VPDRFSGSGS GTDFTLKISRVEAEDVGVYYC**FQVSHVPYT**FGGGTK VEIKR
76		VH HU2B5.8	EVQLVQSGAEVKKPGASVKVSCKAS**GYTFTKYWLG**W VRQAPGQGLEWMG**DIYPGYDYTHYNEKFKD**RVTLTT DTSTSTAYMELSSLRSDDTAVYYCAR**SDGSSTY**WGQ GTLVTVSS
77		VL HU2B5.8	DVLMTQTPLSLPVTPGEPASISC**TSSQNIVHSNGNT** **YLE**WYLQKPGQSPQLLIY**KVSNRFSG**VPDRFSGSGS GTDFTLKISRVEAEDVGVYYC**FQVSHVPYT**FGGGTK VEIKR
78		VH HU2B5.9	EVQLVQSGAEVKKPGASVKVSCKAS**GYTFTKYWLG**W VRQAPGQGLEWMG**DIYPGYDYTHYNEKFKD**RVTMTT DTSTSTAYMELRSLRSDDTAVYYCAR**SDGSSTY**WGQ GTLVTVSS
79		VL HU2B5.9	DIVMTQTPLSLPVTPGEPASISC**TSSQNIVHSNGNT** **YLE**WYLQKPGQSPQLLIY**KVSNRFSG**VPDRFSGSGS GTDFTLKISRVEAEDVGVYYC**FQVSHVPYT**FGGGTK VEIKR

Detailed description of specific DVD-Ig molecules capable of binding specific targets, and methods of making the same, is provided in the Examples section below.

D: Production Of DVD Proteins

Binding proteins of the present invention may be produced by any of a number of techniques known in the art. For example, expression from host cells, wherein expression

vector(s) encoding the DVD heavy and DVD light chains is (are) transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, *e.g.*, electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is possible to express the DVD proteins of the invention in either prokaryotic or eukaryotic host cells, DVD proteins are expressed in eukaryotic cells, for example, mammalian host cells, because such eukaryotic cells (and in particular mammalian cells) are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active DVD protein.

Exemplary mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, *e.g.*, as described in R.J. Kaufman and P.A. Sharp (1982) *Mol. Biol.* 159:601-621), NS0 myeloma cells, COS cells, SP2 and PER.C6 cells. When recombinant expression vectors encoding DVD proteins are introduced into mammalian host cells, the DVD proteins are produced by culturing the host cells for a period of time sufficient to allow for expression of the DVD proteins in the host cells or secretion of the DVD proteins into the culture medium in which the host cells are grown. DVD proteins can be recovered from the culture medium using standard protein purification methods.

In an exemplary system for recombinant expression of DVD proteins of the invention, a recombinant expression vector encoding both the DVD heavy chain and the DVD light chain is introduced into dhfr- CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the DVD heavy and light chain genes are each operatively linked to CMV enhancer/AdMLP promoter regulatory elements to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the DVD heavy and light chains and intact DVD protein is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the

DVD protein from the culture medium. Still further the invention provides a method of synthesizing a DVD protein of the invention by culturing a host cell of the invention in a suitable culture medium until a DVD protein of the invention is synthesized. The method can further comprise isolating the DVD protein from the culture medium.

An important feature of DVD-Ig is that it can be produced and purified in a similar way as a conventional antibody. The production of DVD-Ig results in a homogeneous, single major product with desired dual-specific activity, without any sequence modification of the constant region or chemical modifications of any kind. Other previously described methods to generate "bi-specific", "multi-specific", and "multi-specific multivalent" full length binding proteins do not lead to a single primary product but instead lead to the intracellular or secreted production of a mixture of assembled inactive, mono-specific, multi-specific, multivalent, full length binding proteins, and multivalent full length binding proteins with combination of different binding sites. As an example, based on the design described by Miller and Presta (PCT publication WO2001/077342(A1), there are 16 possible combinations of heavy and light chains. Consequently only 6.25% of protein is likely to be in the desired active form, and not as a single major product or single primary product compared to the other 15 possible combinations. Separation of the desired, fully active forms of the protein from inactive and partially active forms of the protein using standard chromatography techniques, typically used in large scale manufacturing, is yet to be demonstrated.

Surprisingly the design of the "dual-specific multivalent full length binding proteins" of the present invention leads to a dual variable domain light chain and a dual variable domain heavy chain which assemble primarily to the desired "dual-specific multivalent full length binding proteins".

At least 50%, at least 75% and at least 90% of the assembled, and expressed dual variable domain immunoglobulin molecules are the desired dual-specific tetravalent protein. This aspect of the invention particularly enhances the commercial utility of the invention. Therefore, the present invention includes a method to express a dual variable domain light chain and a dual variable domain heavy chain in a single cell leading to a single primary product of a "dual-specific tetravalent full length binding protein".

The present invention provides a methods of expressing a dual variable domain light chain and a dual variable domain heavy chain in a single cell leading to a "primary product" of a "dual-specific tetravalent full length binding protein", where the "primary product" is more than 50% of all assembled protein, comprising a dual variable domain light chain and a dual variable domain heavy chain.

The present invention provides methods of expressing a dual variable domain light chain and a dual variable domain heavy chain in a single cell leading to a single "primary product" of a "dual-specific tetravalent full length binding protein", where the "primary product" is more than 75% of all assembled protein, comprising a dual variable domain light chain and a dual variable domain heavy chain.

The present invention provides methods of expressing a dual variable domain light chain and a dual variable domain heavy chain in a single cell leading to a single "primary product" of a "dual-specific tetravalent full length binding protein", where the "primary product" is more than 90% of all assembled protein, comprising a dual variable domain light chain and a dual variable domain heavy chain.

II. Derivatized DVD Binding Proteins

One embodiment provides a labeled binding protein wherein the binding protein of the invention is derivatized or linked to another functional molecule (e.g., another peptide or protein). For example, a labeled binding protein of the invention can be derived by functionally linking an binding protein of the invention (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (e.g., a bispecific antibody or a diabody), a detectable agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate association of the binding protein with another molecule (such as a streptavidin core region or a polyhistidine tag).

Useful detectable agents with which a binding protein of the invention may be derivatized include fluorescent compounds. Exemplary fluorescent detectable agents include fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-napthalenesulfonyl chloride, phycoerythrin and the like. A binding protein may also be derivatized with detectable enzymes, such as alkaline phosphatase, horseradish

peroxidase, glucose oxidase and the like. When a binding protein is derivatized with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a detectable reaction product. For example, when the detectable agent horseradish peroxidase is present, the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. a binding protein may also be derivatized with biotin, and detected through indirect measurement of avidin or streptavidin binding.

Another embodiment of the invention provides a crystallized binding protein and formulations and compositions comprising such crystals. In one embodiment the crystallized binding protein has a greater half-life in vivo than the soluble counterpart of the binding protein. In another embodiment the binding protein retains biological activity after crystallization.

Crystallized binding protein of the invention may be produced according to methods known in the art and as disclosed in WO 02072636.

Another embodiment of the invention provides a glycosylated binding protein wherein the antibody or antigen-binding portion thereof comprises one or more carbohydrate residues. Nascent *in vivo* protein production may undergo further processing, known as post-translational modification. In particular, sugar (glycosyl) residues may be added enzymatically, a process known as glycosylation. The resulting proteins bearing covalently linked oligosaccharide side chains are known as glycosylated proteins or glycoproteins. Antibodies are glycoproteins with one or more carbohydrate residues in the Fc domain, as well as the variable domain. Carbohydrate residues in the Fc domain have important effect on the effector function of the Fc domain, with minimal effect on antigen binding or half-life of the antibody (R. Jefferis, *Biotechnol. Prog.* **21** (2005), pp. 11–16). In contrast, glycosylation of the variable domain may have an effect on the antigen binding activity of the antibody. Glycosylation in the variable domain may have a negative effect on antibody binding affinity, likely due to steric hindrance (Co, M.S., et al., Mol. Immunol. (1993) 30:1361- 1367), or result in increased affinity for the antigen (Wallick, S.C., et al., Exp. Med. (1988) 168:1099-1109; Wright, A., et al., EMBO J. (1991) 10:2717 2723).

One aspect of the present invention is directed to generating glycosylation site mutants in which the O- or N-linked glycosylation site of the binding protein has been mutated. One skilled in the art can generate such mutants using standard well-known technologies. Glycosylation site mutants that retain the biological activity but have increased or decreased binding activity are another object of the present invention.

In still another embodiment, the glycosylation of the antibody or antigen-binding portion of the invention is modified. For example, an aglycoslated antibody can be made (i.e., the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for antigen. Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation may increase the affinity of the antibody for antigen. Such an approach is described in further detail in PCT Publication WO2003016466A2, and U.S. Pat. Nos. 5,714,350 and 6,350,861.

Additionally or alternatively, a modified binding protein of the invention can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues (see Kanda, Yutaka et al., Journal of Biotechnology (2007), 130(3), 300-310.) or an antibody having increased bisecting GlcNAc structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies of the invention to thereby produce an antibody with altered glycosylation. See, for example, Shields, R. L. et al. (2002) J. Biol. Chem. 277:26733-26740; Umana et al. (1999) Nat. Biotech. 17:176-1, as well as, European Patent No: EP 1,176,195; PCT Publications WO 03/035835; WO 99/54342 80.

Protein glycosylation depends on the amino acid sequence of the protein of interest, as well as the host cell in which the protein is expressed. Different organisms may produce different glycosylation enzymes (e.g., glycosyltransferases and glycosidases), and have different substrates (nucleotide sugars) available. Due to such

factors, protein glycosylation pattern, and composition of glycosyl residues, may differ depending on the host system in which the particular protein is expressed. Glycosyl residues useful in the invention may include, but are not limited to, glucose, galactose, mannose, fucose, n-acetylglucosamine and sialic acid. In an embodiment, the glycosylated binding protein comprises glycosyl residues such that the glycosylation pattern is human.

It is known to those skilled in the art that differing protein glycosylation may result in differing protein characteristics. For instance, the efficacy of a therapeutic protein produced in a microorganism host, such as yeast, and glycosylated utilizing the yeast endogenous pathway may be reduced compared to that of the same protein expressed in a mammalian cell, such as a CHO cell line. Such glycoproteins may also be immunogenic in humans and show reduced half-life *in vivo* after administration. Specific receptors in humans and other animals may recognize specific glycosyl residues and promote the rapid clearance of the protein from the bloodstream. Other adverse effects may include changes in protein folding, solubility, susceptibility to proteases, trafficking, transport, compartmentalization, secretion, recognition by other proteins or factors, antigenicity, or allergenicity. Accordingly, a practitioner may choose a therapeutic protein with a specific composition and pattern of glycosylation, for example glycosylation composition and pattern identical, or at least similar, to that produced in human cells or in the species-specific cells of the intended subject animal.

Expressing glycosylated proteins different from that of a host cell may be achieved by genetically modifying the host cell to express heterologous glycosylation enzymes. Using techniques known in the art a practitioner may generate antibodies or antigen-binding portions thereof exhibiting human protein glycosylation. For example, yeast strains have been genetically modified to express non-naturally occurring glycosylation enzymes such that glycosylated proteins (glycoproteins) produced in these yeast strains exhibit protein glycosylation identical to that of animal cells, especially human cells (U.S patent applications 20040018590 and 20020137134 and PCT publication WO2005100584 A2).

In addition to the binding proteins, the present invention is also directed to anti-idiotypic (anti-Id) antibodies specific for such binding proteins of the invention. An anti-

Id antibody is an antibody, which recognizes unique determinants generally associated with the antigen-binding region of another antibody. The anti-Id can be prepared by immunizing an animal with the binding protein or a CDR containing region thereof. The immunized animal will recognize, and respond to the idiotypic determinants of the immunizing antibody and produce an anti-Id antibody. It is readily apparent that it may be easier to generate anti-idiotypic antibodies to the two or more parent antibodies incorporated into a DVD-Ig molecule; and confirm binding studies by methods well recognized in the art (e.g., BIAcore, ELISA) to verify that anti-idiotypic antibodies specific for the idiotype of each parent antibody also recognize the idiotype (e.g., antigen binding site) in the context of the DVD-Ig. The anti-idiotypic antibodies specific for each of the two or more antigen binding sites of a DVD-Ig provide ideal reagents to measure DVD-Ig concentrations of a human DVD-Ig in patrient serum; DVD-Ig concentration assays can be established using a "sandwich assay ELISA format" with an antibody to a first antigen binding regions coated on the solid phase (e.g., BIAcore chip, ELISA plate etc.), rinsed with rinsing buffer, incubation with the serum sample, another rinsing step and ultimately incubation with another anti-idiotypic antibody to the another antigen binding site, itself labeled with an enzyme for quantitation of the binding reaction. In an embodiment, for a DVD-Ig with more than two different binding sites, anti-idiotypic antibodies to the two outermost binding sites (most distal and proximal from the constant region) will not only help in determining the DVD-Ig concentration in human serum but also document the integrity of the molecule *in vivo*. Each anti-Id antibody may also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody.

Further, it will be appreciated by one skilled in the art that a protein of interest may be expressed using a library of host cells genetically engineered to express various glycosylation enzymes, such that member host cells of the library produce the protein of interest with variant glycosylation patterns. A practitioner may then select and isolate the protein of interest with particular novel glycosylation patterns. In an embodiment, the protein having a particularly selected novel glycosylation pattern exhibits improved or altered biological properties.

III. Uses of DVD-Ig

Given their ability to bind to two or more antigens the binding proteins of the invention can be used to detect the antigens (*e.g.*, in a biological sample, such as serum or plasma), using a conventional immunoassay, such as an enzyme linked immunosorbent assays (ELISA), an radioimmunoassay (RIA) or tissue immunohistochemistry. The DVD-Ig is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, or ^{153}Sm.

In an embodiment, the binding proteins of the invention are capable of neutralizing the activity of the antigens both *in vitro* and *in vivo*. Accordingly, such DVD-Igs can be used to inhibit antigen activity, *e.g.*, in a cell culture containing the antigens, in human subjects or in other mammalian subjects having the antigens with which a binding protein of the invention cross-reacts. In another embodiment, the invention provides a method for reducing antigen activity in a subject suffering from a disease or disorder in which the antigen activity is detrimental. A binding protein of the invention can be administered to a human subject for therapeutic purposes.

As used herein, the term "a disorder in which antigen activity is detrimental" is intended to include diseases and other disorders in which the presence of the antigen in a subject suffering from the disorder has been shown to be or is suspected of being either responsible for the pathophysiology of the disorder or a factor that contributes to a worsening of the disorder. Accordingly, a disorder in which antigen activity is detrimental is a disorder in which reduction of antigen activity is expected to alleviate the symptoms and/or progression of the disorder. Such disorders may be evidenced, for example, by an increase in the concentration of the antigen in a biological fluid of a subject suffering from the disorder (*e.g.*, an increase in the concentration of antigen in serum, plasma, synovial fluid, *etc.* of the subject). Non-limiting examples of disorders

that can be treated with the binding proteins of the invention include those disorders discussed below and in the section pertaining to pharmaceutical compositions of the antibodies of the invention.

The DVD-Igs of the invention may bind one antigen or multiple antigens. Such antigens include, but are not limited to, the targets listed in the following databases. These target databases include those listings:

Therapeutic targets (http://xin.cz3.nus.edu.sg/group/cjttd/ttd.asp);

Cytokines and cytokine receptors (http://www.cytokinewebfacts.com/, http://www.copewithcytokines.de/cope.cgi, and

http://cmbi.bjmu.edu.cn/cmbidata/cgf/CGF_Database/cytokine.medic.kumamoto-u.ac.jp/CFC/indexR.html);

Chemokines (http://cytokine.medic.kumamoto-u.ac.jp/CFC/CK/Chemokine.html);

Chemokine receptors and GPCRs (http://csp.medic.kumamoto-u.ac.jp/CSP/Receptor.html, http://www.gpcr.org/7tm/);

Olfactory Receptors (http://senselab.med.yale.edu/senselab/ORDB/default.asp);

Receptors (http://www.iuphar-db.org/iuphar-rd/list/index.htm);

Cancer targets (http://cged.hgc.jp/cgi-bin/input.cgi);

Secreted proteins as potential antibody targets (http://spd.cbi.pku.edu.cn/);

Protein kinases (http://spd.cbi.pku.edu.cn/), and

Human CD markers (http://content.labvelocity.com/tools/6/1226/CD_table_final_locked.pdf) and (Zola H, 2005 CD molecules 2005: human cell differentiation molecules Blood, 106:3123-6).

DVD-Igs are useful as therapeutic agents to simultaneously block two different targets to enhance efficacy/safety and/or increase patient coverage. Such targets may include soluble targets (e.g., TNF and PGE2) and cell surface receptor targets (e.g., VEGFR and EGFR). It can also be used to induce redirected cytotoxicity between tumor

cells and T cells (e.g., Her2 and CD3) for cancer therapy, or between autoreactive cell and effector cells for autoimmune disease or transplantation, or between any target cell and effector cell to eliminate disease-causing cells in any given disease.

In addition, DVD-Ig can be used to trigger receptor clustering and activation when it is designed to target two different epitopes on the same receptor. This may have benefit in making agonistic and antagonistic anti-GPCR therapeutics. In this case, DVD-Ig can be used to target two different epitopes (including epitopes on both the loop regions and the extracellular domain) on one cell for clustering/signaling (two cell surface molecules) or signaling (on one molecule). Similarly, a DVD-Ig molecule can be designed to trigger CTLA-4 ligation, and a negative signal by targeting two different epitopes (or 2 copies of the same epitope) of CTLA-4 extracellular domain, leading to down regulation of the immune response. CTLA-4 is a clinically validated target for therapeutic treatment of a number of immunological disorders. CTLA-4/B7 interactions negatively regulate T cell activation by attenuating cell cycle progression, IL-2 production, and proliferation of T cells following activation, and CTLA-4 (CD152) engagement can down-regulate T cell activation and promote the induction of immune tolerance. However, the strategy of attenuating T cell activation by agonistic antibody engagement of CTLA-4 has been unsuccessful since CTLA-4 activation requires ligation. The molecular interaction of CTLA-4/B7 is in "skewed zipper" arrays, as demonstrated by crystal structural analysis (Stamper 2001 Nature 410:608). However none of the currently available CTLA-4 binding reagents have ligation properties, including anti-CTLA-4 mAbs. There have been several attempts to address this issue. In one case, a cell member-bound single chain antibody was generated, and significantly inhibited allogeneic rejection in mice (Hwang 2002 JI 169:633). In a separate case, artificial APC surface-linked single-chain antibody to CTLA-4 was generated and demonstrated to attenuate T cell responses (Griffin 2000 JI 164:4433). In both cases, CTLA-4 ligation was achieved by closely localized member-bound antibodies in artificial systems. While these experiments provide proof-of-concept for immune down-regulation by triggering CTLA-4 negative signaling, the reagents used in these reports are not suitable for therapeutic use. To this end, CTLA-4 ligation may be achieved by using a DVD-Ig molecule, which target two different epitopes (or 2 copies of the same epitope) of CTLA-4 extracellular domain. The rationale is that the distance spanning two binding sites of an

IgG, approximately 150-170Å, is too large for active ligation of CTLA-4 (30-50 Å between 2 CTLA-4 homodimer). However the distance between the two binding sites on DVD-Ig (one arm) is much shorter, also in the range of 30-50 Å, allowing proper ligation of CTLA-4.

Similarly, DVD-Ig can target two different members of a cell surface receptor complex (e.g.,IL-12R alpha and beta). Furthermore, DVD-Ig can target CR1 and a soluble protein/pathogen to drive rapid clearance of the target soluble protein/pathogen.

Additionally, DVD-Igs of the invention can be employed for tissue-specific delivery (target a tissue marker and a disease mediator for enhanced local PK thus higher efficacy and/or lower toxicity), including intracellular delivery (targeting an internalizing receptor and a intracellular molecule), delivering to inside brain (targeting transferrin receptor and a CNS disease mediator for crossing the blood-brain barrier). DVD-Ig can also serve as a carrier protein to deliver an antigen to a specific location via binding to a non-neutralizing epitope of that antigen and also to increase the half-life of the antigen. Furthermore, DVD-Ig can be designed to either be physically linked to medical devices implanted into patients or target these medical devices (see Burke, Sandra E.; Kuntz, Richard E.; Schwartz, Lewis B., Zotarolimus eluting stents. Advanced Drug Delivery Reviews (2006), 58(3), 437-446; Surface coatings for biological activation and functionalization of medical devices, Hildebrand, H. F.; Blanchemain, N.; Mayer, G.; Chai, F.; Lefebvre, M.; Boschin, F., Surface and Coatings Technology (2006), 200(22-23), 6318-6324; Drug/ device combinations for local drug therapies and infection prophylaxis, Wu, Peng; Grainger, David W., Biomaterials (2006), 27(11), 2450-2467; Mediation of the cytokine network in the implantation of orthopedic devices., Marques, A. P.; Hunt, J. A.; Reis, Rui L., Biodegradable Systems in Tissue Engineering and Regenerative Medicine (2005), 377-397). Briefly, directing appropriate types of cell to the site of medical implant may promote healing and restoring normal tissue function. Alternatively, inhibition of mediators (including but not limited to cytokines), released upon device implantation by a DVD coupled to or target to a device is also provided. For example, Stents have been used for years in interventional cardiology to clear blocked arteries and to improve the flow of blood to the heart muscle. However, traditional bare metal stents have been known to cause restenosis (re-narrowing of the artery in a treated area) in some patients and can lead to blood clots. Recently, an anti-CD34 antibody

coated stent has been described which reduced restenosis and prevents blood clots from occurring by capturing endothelial progenitor cells (EPC) circulating throughout the blood. Endothelial cells are cells that line blood vessels, allowing blood to flow smoothly. The EPCs adhere to the hard surface of the stent forming a smooth layer that not only promotes healing but prevents restenosis and blood clots, complications previously associated with the use of stents (Aoji et al. 2005 J Am Coll Cardiol. 45(10):1574-9). In addition to improving outcomes for patients requiring stents, there are also implications for patients requiring cardiovascular bypass surgery. For example, a prosthetic vascular conduit (artificial artery) coated with anti-EPC antibodies would eliminate the need to use arteries from patients legs or arms for bypass surgery grafts. This would reduce surgery and anesthesia times, which in turn will reduce coronary surgery deaths. DVD-Ig are designed in such a way that it binds to a cell surface marker (such as CD34) as well as a protein (or an epitope of any kind, including but not limited to proteins, lipids and polysaccharides) that has been coated on the implanted device to facilitate the cell recruitment. Such approaches can also be applied to other medical implants in general. Alternatively, DVD-Igs can be coated on medical devices and upon implantation and releasing all DVDs from the device (or any other need which may require additional fresh DVD-Ig, including aging and denaturation of the already loaded DVD-Ig) the device could be reloaded by systemic administration of fresh DVD-Ig to the patient, where the DVD-Ig is designed to binds to a target of interest (a cytokine, a cell surface marker (such as CD34) etc.) with one set of binding sites and to a target coated on the device (including a protein, an epitope of any kind, including but not limited to lipids, polysaccharides and polymers) with the other. This technology has the advantage of extending the usefulness of coated implants.

In an embodiment, the DVD-Ig is attached to a noncytotoxic carbon nano-tube (CNT) and targeted to a tissue, for example, a tumor tissue. CNTs emit heat when they absorb energy from near infrared (NIR) light. Once the DVD-Ig has bound to its tumor antigen(s), noninvasive exposure to NIR light ablates the tumor within the range of NIR. (Chakravarty, P. et al. (2008) Proc. Natl. Acad. Sci. 105:8697-8702).

A. Use of DVD-Igs in Various Diseases

DVD-Ig molecules of the invention are also useful as therapeutic molecules to treat various diseases. Such DVD molecules may bind one or more targets involved in a specific disease. Examples of such targets in various diseases are described below.

1. Human Autoimmune and Inflammatory Response

Many proteins have been implicated in general autoimmune and inflammatory responses, including C5, CCL1 (I-309), CCL11 (eotaxin), CCL13 (mcp-4), CCL15 (MIP-1d), CCL16 (HCC-4), CCL17 (TARC), CCL18 (PARC), CCL19, CCL2 (mcp-1), CCL20 (MIP-3a), CCL21 (MIP-2), CCL23 (MPIF-1), CCL24 (MPIF-2 / eotaxin-2), CCL25 (TECK), CCL26, CCL3 (MIP-1a), CCL4 (MIP-1b), CCL5 (RANTES), CCL7 (mcp-3), CCL8 (mcp-2), CXCL1, CXCL10 (IP-10), CXCL11 (I-TAC / IP-9), CXCL12 (SDF1), CXCL13, CXCL14, CXCL2, CXCL3, CXCL5 (ENA-78 / LIX), CXCL6 (GCP-2), CXCL9, IL13, IL8, CCL13 (mcp-4), CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CX3CR1, IL8RA, XCR1 (CCXCR1), IFNA2, IL10, IL13, IL17C, IL1A, IL1B, IL1F10, IL1F5, IL1F6, IL1F7, IL1F8, IL1F9, IL22, IL5, IL8, IL9, LTA, LTB, MIF, SCYE1 (endothelial Monocyte-activating cytokine), SPP1, TNF, TNFSF5, IFNA2, IL10RA, IL10RB, IL13, IL13RA1, IL5RA, IL9, IL9R, ABCF1, BCL6, C3, C4A, CEBPB, CRP, ICEBERG, IL1R1, IL1RN, IL8RB, LTB4R, TOLLIP, FADD, IRAK1, IRAK2, MYD88, NCK2, TNFAIP3, TRADD, TRAF1, TRAF2, TRAF3, TRAF4, TRAF5, TRAF6, ACVR1, ACVR1B, ACVR2, ACVR2B, ACVRL1, CD28, CD3E, CD3G, CD3Z, CD69, CD80, CD86, CNR1, CTLA4, CYSLTR1, FCER1A, FCER2, FCGR3A, GPR44, HAVCR2, OPRD1, P2RX7, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, BLR1, CCL1, CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL11, CCL13, CCL15, CCL16, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CX3CL1, CX3CR1, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL10, CXCL11, CXCL12, CXCL13, CXCR4, GPR2, SCYE1, SDF2, XCL1, XCL2, XCR1, AMH, AMHR2, BMPR1A, BMPR1B, BMPR2, C19orf10 (IL27w), CER1, CSF1, CSF2, CSF3, DKFZp451J0118, FGF2, GFI1, IFNA1, IFNB1, IFNG, IGF1, IL1A, IL1B, IL1R1, IL1R2, IL2, IL2RA, IL2RB, IL2RG, IL3, IL4, IL4R, IL5, IL5RA, IL6, IL6R, IL6ST, IL7, IL8, IL8RA, IL8RB, IL9, IL9R, IL10, IL10RA, IL10RB, IL11, IL11RA, IL12A, IL12B, IL12RB1, IL12RB2, IL13, IL13RA1, IL13RA2, IL15, IL15RA, IL16, IL17, IL17R, IL18, IL18R1, IL19, IL20, KITLG, LEP, LTA, LTB, LTB4R, LTB4R2, LTBR,

MIF, NPPB, PDGFB, TBX21, TDGF1, TGFA, TGFB1, TGFB1I1, TGFB2, TGFB3, TGFBI, TGFBR1, TGFBR2, TGFBR3, TH1L, TNF, TNFRSF1A, TNFRSF1B, TNFRSF7, TNFRSF8, TNFRSF9, TNFRSF11A, TNFRSF21, TNFSF4, TNFSF5, TNFSF6, TNFSF11, VEGF, ZFPM2, RNF110 (ZNF144), FGF family, PLGF, DLL4, and NPR-1. In one aspect, DVD-Igs capable of binding one or more of the targets listed herein are provided.

DVD Igs capable of binding the following pairs of targets to treat inflammatory disease are contemplated: mouse or human TNF and PGE2, NGF and PGE2, IL-17A and PGE2, IL-1b and PGE2, IL-6 and PGE2, IL-6R and PGE2, VEGF and PGE2, Abeta (seq. 1) and PGE2, Abeta (seq. 2) and PGE2, Abeta (seq. 3) and PGE2, IL-18 and PGE2, PGE2 and PGE2, IL-15 and PGE2, S1P and PGE2, EGFR (seq. 1) and PGE2, EGFR (seq. 2) and PGE2, and IGFR and PGE2 (see Examples).

2. Asthma

Allergic asthma is characterized by the presence of eosinophilia, goblet cell metaplasia, epithelial cell alterations, airway hyperreactivity (AHR), and Th2 and Th1 cytokine expression, as well as elevated serum IgE levels. It is now widely accepted that airway inflammation is the key factor underlying the pathogenesis of asthma, involving a complex interplay of inflammatory cells such as T cells, B cells, eosinophils, mast cells and macrophages, and of their secreted mediators including cytokines and chemokines. Corticosteroids are the most important anti-inflammatory treatment for asthma today, however their mechanism of action is non-specific and safety concerns exist, especially in the juvenile patient population. The development of more specific and targeted therapies is therefore warranted. There is increasing evidence that IL-13 in mice mimics many of the features of asthma, including AHR, mucus hypersecretion and airway fibrosis, independently of eosinophilic inflammation (Finotto et al., International Immunology (2005), 17(8), 993-1007; Padilla et al., Journal of Immunology (2005), 174(12), 8097-8105).

IL-13 has been implicated as having a pivotal role in causing pathological responses associated with asthma. The development of anti-IL-13 mAb therapy to reduce the effects of IL-13 in the lung is an exciting new approach that offers considerable promise as a novel treatment for asthma. However other mediators of differential

immunological pathways are also involved in asthma pathogenesis, and blocking these mediators, in addition to IL-13, may offer additional therapeutic benefit. Such target pairs include, but are not limited to, IL-13 and a pro-inflammatory cytokine, such as tumor necrosis factor-α (TNF-α). TNF-α may amplify the inflammatory response in asthma and may be linked to disease severity (McDonnell, et al., Progress in Respiratory Research (2001), 31(New Drugs for Asthma, Allergy and COPD), 247-250.). This suggests that blocking both IL-13 and TNF-α may have beneficial effects, particularly in severe airway disease. In another embodiment the DVD-Ig of the invention binds the targets IL-13 and TNFα or IL-13 and PGD2 and is used for treating asthma.

Animal models such as OVA-induced asthma mouse model, where both inflammation and AHR can be assessed, are known in the art and may be used to determine the ability of various DVD-Ig molecules to treat asthma. Animal models for studying asthma are disclosed in Coffman, et al., Journal of Experimental Medicine (2005), 201(12), 1875-1879; Lloyd, et al., Advances in Immunology (2001), 77, 263-295; Boyce et al., Journal of Experimental Medicine (2005), 201(12), 1869-1873; and Snibson, et al., Journal of the British Society for Allergy and Clinical Immunology (2005), 35(2), 146-52. In addition to routine safety assessments of these target pairs specific tests for the degree of immunosuppression may be warranted and helpful in selecting the best target pairs (see Luster et al., Toxicology (1994), 92(1-3), 229-43; Descotes, et al., Developments in biological standardization (1992), 77 99-102; Hart et al., Journal of Allergy and Clinical Immunology (2001), 108(2), 250-257).

Based on the rationale disclosed herein and using the same evaluation model for efficacy and safety other pairs of targets that DVD-Ig molecules can bind and be useful to treat asthma may be determined. In an embodiment, such targets include, but are not limited to, IL-13 and IL-1beta, since IL-1beta is also implicated in inflammatory response in asthma; IL-13 and cytokines and chemokines that are involved in inflammation, such as IL-13 and IL-9; IL-13 and IL-4; IL-13 and IL-5; IL-13 and IL-25; IL-13 and TARC; IL-13 and MDC; IL-13 and MIF; IL-13 and TGF-β; IL-13 and LHR agonist; IL-13 and CL25; IL-13 and SPRR2a; IL-13 and SPRR2b; and IL-13 and ADAM8. The present invention also provides DVD-Igs capable of binding one or more targets involved in asthma selected from the group consisting of PGD2, CSF1 (MCSF), CSF2 (GM-CSF), CSF3 (GCSF), FGF2, IFNA1, IFNB1, IFNG, histamine and histamine receptors, IL1A,

IL1B, IL2, IL3, IL4, IL5, IL6, IL7, IL8, IL9, IL10, IL11, IL12A, IL12B, IL13, IL14, IL15, IL16, IL17, IL18, IL19, KITLG, PDGFB, IL2RA, IL4R, IL5RA, IL8RA, IL8RB, IL12RB1, IL12RB2, IL13RA1, IL13RA2, IL18R1, TSLP, CCL1, CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL13, CCL17, CCL18, CCL19, CCL20, CCL22, CCL24,CX3CL1, CXCL1, CXCL2, CXCL3, XCL1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CX3CR1, GPR2, XCR1, FOS, GATA3, JAK1, JAK3, STAT6, TBX21, TGFB1, TNF, TNFSF6, YY1, CYSLTR1, FCER1A, FCER2, LTB4R, TB4R2, LTBR, PGD2 and Chitinase.

DVD Igs capable of binding the following pairs of targets to treat asthma are contemplated: mouse or human TNF and PGE2, NGF and PGE2, IL-17A and PGE2, IL-1b and PGE2, IL-6 and PGE2, IL-6R and PGE2, VEGF and PGE2, Abeta (seq. 1) and PGE2, Abeta (seq. 2) and PGE2, Abeta (seq. 3) and PGE2, IL-18 and PGE2, PGE2 and PGE2, IL-15 and PGE2, S1P and PGE2, EGFR (seq. 1) and PGE2, EGFR (seq. 2) and PGE2, and IGFR and PGE2 (see Examples).

3. Rheumatoid arthritis

Rheumatoid arthritis (RA), a systemic disease, is characterized by a chronic inflammatory reaction in the synovium of joints and is associated with degeneration of cartilage and erosion of juxta-articular bone. Many pro-inflammatory cytokines including TNF, chemokines, and growth factors are expressed in diseased joints. Systemic administration of anti-TNF antibody or sTNFR fusion protein to mouse models of RA was shown to be anti-inflammatory and joint protective. Clinical investigations in which the activity of TNF in RA patients was blocked with intravenously administered infliximab (Harriman G, Harper LK, Schaible TF. 1999 Summary of clinical trials in rheumatoid arthritis using infliximab, an anti-TNFalpha treatment. Ann Rheum Dis 58 Suppl 1:I61-4), a chimeric anti-TNF mAb, has provided evidence that TNF regulates IL-6, IL-8, MCP-1, and VEGF production, recruitment of immune and inflammatory cells into joints, angiogenesis, and reduction of blood levels of matrix metalloproteinases-1 and -3. A better understanding of the inflammatory pathway in rheumatoid arthritis has led to identification of other therapeutic targets involved in rheumatoid arthritis. Promising treatments such as interleukin-6 antagonists (IL-6 receptor antibody MRA, developed by Chugai, Roche (see Nishimoto, Norihiro et al., Arthritis & Rheumatism

(2004), 50(6), 1761-1769), CTLA4Ig (abatacept, Genovese Mc et al 2005 Abatacept for rheumatoid arthritis refractory to tumor necrosis factor alpha inhibition. N Engl J Med. 353:1114-23.), and anti-B cell therapy (rituximab, Okamoto H, Kamatani N. 2004 Rituximab for rheumatoid arthritis. N Engl J Med. 351:1909) have already been tested in randomized controlled trials over the past year. Other cytokines have been identified and have been shown to be of benefit in animal models, including interleukin-15 (therapeutic antibody HuMax-IL_15, AMG 714 see Baslund, Bo et al., Arthritis & Rheumatism (2005), 52(9), 2686-2692), interleukin-17, and interleukin-18, and clinical trials of these agents are currently under way. Dual-specific antibody therapy, combining anti-TNF and another mediator, has great potential in enhancing clinical efficacy and/or patient coverage. For example, blocking both TNF and PGE2 can potentially eradicate inflammation and angiogenesis, both of which are involved in pathophysiology of RA. Blocking other pairs of targets involved in RA including, but not limited to, TNF and PGE2; TNFα and IL-18; TNFα and IL-12; TNFα and IL-23; TNFα and IL-1beta; TNFα and MIF; TNFα and IL-17; TNFα and IL-17A; TNFα and IL-17F; TNFα and IL-17C; TNFα and IL-15; TNFα and VEGF; TNFα and OX40; TNFα and OX40L; TNFα and BAFF; TNFα and CD20; TNFα and HMGB1; TNFα and Histamine; TNFα and RAGE; TNFα and CXCL12; TNFα and CTLA4; TNFα and Cad-11; TNFα and Wnt5A; TNFα and ADMATS-4; TNFα and ADMATS-5; TNFα and ADMATS-4/5; TNFα and MMP13; TNFα and MMP1; TNFα and MMP3; TNFα and MMP4; TNFα and RANKL; TNFα and SOST; TNFα and DKK1; TNFα and LRP5/6; TNFα and Kremen; TNFα and SFRPS; TNFα and IL-6; TNFα and IL-32; TNFα and IL-33; TNFα and IL-6R; TNFα and Cathepsin K; TNFα and Bradykinin; TNFα and NGF; PGE2 and CTLA4; PGE2 and IL-1β; PGE2 and IL-12; PGE2 and IL-23; PGE2 and IL-15, PGE2 and IL-17; PGE2 and IL-17A; PGE2 and IL-17F; PGE2 and IL-17C; PGE2 and IL-18; PGE2 and IL-6; PGE2 and IL-6R; PGE2 and gp130; PGE2 and BAFF; S1P and PGE2, EGFR (seq. 1) and PGE2, EGFR (seq. 2) and PGE2, and IGFR and PGE2, PGE2 and Abeta (seq. 1, 2, or 3), PGE2 and ADMATS-4; PGE2 and ADMATS-5; PGE2 and ADMATS-4/5; PGE2 and MMP-1; PGE2 and MMP-3; PGE2 and MMP-4; PGE2 and MMP-13; PGE2 and Cathepsin K; PGE2 and Bradykinin; PGE2 and RANKL; PGE2 and DKK1; PGE2 and SOST; PGE2 and NGF; PGE2 and RAGE; PGE2 and S1P; PGE2 and IL-15; PGE2 and VEGF; PGE2 and Cadherin; with specific DVD Igs is also contemplated. In addition to

routine safety assessments of these target pairs, specific tests for the degree of immunosuppression may be warranted and helpful in selecting the best target pairs (see Luster et al., Toxicology (1994), 92(1-3), 229-43; Descotes, et al., Developments in biological standardization (1992), 77 99-102; Hart et al., Journal of Allergy and Clinical Immunology (2001), 108(2), 250-257). Whether a DVD Ig molecule will be useful for the treatment of rheumatoid arthritis can be assessed using pre-clinical animal RA models such as the collagen-induced arthritis mouse model. Other useful models are also well known in the art (see Brand DD., Comp Med. (2005) 55(2):114-22). Based on the cross-reactivity of the parental antibodies for human and mouse othologues (e.g.,reactivity for human and mouse TNF, human and mouse IL-15 etc.) validation studies in the mouse CIA model may be conducted with "matched surrogate antibody" derived DVD-Ig molecules; briefly, a DVD-Ig based on two (or more) mouse target specific antibodies may be matched to the extent possible to the characteristics of the parental human or humanized antibodies used for human DVD-Ig construction (similar affinity, similar neutralization potency, similar half-life etc.).

4. Systemic Lupus Erythematosis (SLE)

The immunopathogenic hallmark of SLE is the polyclonal B cell activation, which leads to hyperglobulinemia, autoantibody production and immune complex formation. The fundamental abnormality appears to be the failure of T cells to suppress the forbidden B cell clones due to generalized T cell dysregulation. In addition, B and T-cell interaction is facilitated by several cytokines such as IL-10 as well as co-stimulatory molecules such as CD40 and CD40L, B7 and CD28 and CTLA-4, which initiate the second signal. These interactions together with impaired phagocytic clearance of immune complexes and apoptotic material, perpetuate the immune response with resultant tissue injury. The following targets may be involved in SLE and can potentially be used for DVD-Ig approach for therapeutic intervention: B cell targeted therapies: CD-20, CD-22, CD-19, CD28, CD4, CD80, HLA-DRA, IL10, IL2, IL4, TNFRSF5, TNFRSF6, TNFSF5, TNFSF6, BLR1, HDAC4, HDAC5, HDAC7A, HDAC9, ICOSL, IGBP1, MS4A1, RGS1, SLA2, CD81, IFNB1, IL10, TNFRSF5, TNFRSF7, TNFSF5, AICDA, BLNK, GALNAC4S-6ST, HDAC4, HDAC5, HDAC7A, HDAC9, IL10, IL11, IL4, INHA, INHBA, KLF6, TNFRSF7, CD28, CD38, CD69, CD80, CD83, CD86, DPP4, FCER2, IL2RA, TNFRSF8, TNFSF7, CD24, CD37, CD40, CD72, CD74, CD79A, CD79B, CR2,

IL1R2, ITGA2, ITGA3, MS4A1, ST6GAL1, CD1C, CHST10, HLA-A, HLA-DRA, and NT5E.; co-stimulatory signals: CTLA4 or B7.1/B7.2; inhibition of B cell survival: BlyS, BAFF; Complement inactivation: C5; Cytokine modulation: the key principle is that the net biologic response in any tissue is the result of a balance between local levels of proinflammatory or anti-inflammatory cytokines (see Sfikakis PP et al 2005 Curr Opin Rheumatol 17:550-7). SLE is considered to be a Th-2 driven disease with documented elevations in serum IL-4, IL-6, IL-10. DVD Igs capable of binding one or more targets selected from the group consisting of IL-4, IL-6, IL-10, IFN-α, PGE2, and TNF-α are also contemplated. Combination of targets discussed herein will enhance therapeutic efficacy for SLE which can be tested in a number of lupus preclinical models (see Peng SL (2004) Methods Mol Med.;102:227-72). Based on the cross-reactivity of the parental antibodies for human and mouse othologues (e.g.,reactivity for human and mouse CD20, human and mouse Interferon alpha etc.) validation studies in a mouse lupus model may be conducted with "matched surrogate antibody" derived DVD-Ig molecules; briefly, a DVD-Ig based two (or more) mouse target specific antibodies may be matched to the extent possible to the characteristics of the parental human or humanized antibodies used for human DVD-Ig construction (similar affinity, similar neutralization potency, similar half-life etc.).

DVD Igs capable of binding the following pairs of targets to treat SLE are contemplated: mouse or human TNF and PGE2, NGF and PGE2, IL-17A and PGE2, IL-1b and PGE2, IL-6 and PGE2, IL-6R and PGE2, VEGF and PGE2, Abeta (seq. 1) and PGE2, Abeta (seq. 2) and PGE2, Abeta (seq. 3) and PGE2, IL-18 and PGE2, PGE2 and PGE2, IL-15 and PGE2, S1P and PGE2, EGFR (seq. 1) and PGE2, EGFR (seq. 2) and PGE2, and IGFR and PGE2 (see Examples).

5. Multiple Sclerosis

Multiple sclerosis (MS) is a complex human autoimmune-type disease with a predominantly unknown etiology. Immunologic destruction of myelin basic protein (MBP) throughout the nervous system is the major pathology of multiple sclerosis. MS is a disease of complex pathologies, which involves infiltration by CD4+ and CD8+ T cells and of response within the central nervous system. Expression in the CNS of cytokines, reactive nitrogen species and costimulator molecules have all been described in MS. Of major consideration are immunological mechanisms that contribute to the development of

autoimmunity. In particular, antigen expression, cytokine and leukocyte interactions, and regulatory T-cells, which help balance/modulate other T-cells such as Th1 and Th2 cells, are important areas for therapeutic target identification.

IL-12 is a proinflammatory cytokine that is produced by APC and promotes differentiation of Th1 effector cells. IL-12 is produced in the developing lesions of patients with MS as well as in EAE-affected animals. Previously it was shown that interference in IL-12 pathways effectively prevents EAE in rodents, and that in vivo neutralization of IL-12p40 using a anti-IL-12 mAb has beneficial effects in the myelin-induced EAE model in common marmosets.

TWEAK is a member of the TNF family, constitutively expressed in the central nervous system (CNS), with pro-inflammatory, proliferative or apoptotic effects depending upon cell types. Its receptor, Fn14, is expressed in CNS by endothelial cells, reactive astrocytes and neurons. TWEAK and Fn14 mRNA expression increased in spinal cord during experimental autoimmune encephalomyelitis (EAE). Anti-TWEAK antibody treatment in myelin oligodendrocyte glycoprotein (MOG) induced EAE in C57BL/6 mice resulted in a reduction of disease severity and leukocyte infiltration when mice were treated after the priming phase.

One aspect of the invention pertains to DVD Ig molecules capable of binding one or more, for example two, targets selected from the group consisting of PGE, PGE1, PGE2, S1P, S1P1, S1P2, S1P3, S1P4, S1P5, VLA-4, CD44, RAGE, phosphotidyl serine (PS), lysophosphatidic acid (LPA), IL-12, TWEAK, IL-23, CXCL13, CD40, CD40L, IL-18, VEGF, VLA-4, TNF, CD45RB, CD200, IFNgamma, GM-CSF, FGF, C5, CD52, and CCR2. An embodiment includes a dual-specific anti-IL-12/TWEAK DVD Ig as a therapeutic agent beneficial for the treatment of MS.

Several animal models for assessing the usefulness of the DVD molecules to treat MS are known in the art (see Steinman L, et al., (2005) Trends Immunol. 26(11):565-71; Lublin FD., et al., (1985) Springer Semin Immunopathol.8(3):197-208; Genain CP, et al., (1997) J Mol Med. 75(3):187-97; Tuohy VK, et al., (1999) J Exp Med. 189(7):1033-42; Owens T, et al., (1995) Neurol Clin.13(1):51-73; and 't Hart BA, et al., (2005) J Immunol 175(7):4761-8. Based on the cross-reactivity of the parental antibodies for human and animal species othologues (e.g.,reactivity for human and mouse IL-12, human and mouse

TWEAK etc.) validation studies in the mouse EAE model may be conducted with "matched surrogate antibody" derived DVD-Ig molecules; briefly, a DVD-Ig based on to (or more) mouse target specific antibodies may be matched to the extent possible to the characteristics of the parental human or humanized antibodies used for human DVD-Ig construction (similar affinity, similar neutralization potency, similar half-life etc.). The same concept applies to animal models in other non-rodent species, where a "matched surrogate antibody" derived DVD-Ig would be selected for the anticipated pharmacology and possibly safety studies. In addition to routine safety assessments of these target pairs specific tests for the degree of immunosuppression may be warranted and helpful in selecting the best target pairs (see Luster et al., Toxicology (1994), 92(1-3), 229-43; Descotes, et al., Developments in biological standardization (1992), 77 99-102; Jones R. 2000 Rovelizumab (ICOS Corp). IDrugs.3(4):442-6).

DVD Igs capable of binding the following pairs of targets to treat MS are contemplated: mouse or human TNF and PGE2, NGF and PGE2, IL-17A and PGE2, IL-1b and PGE2, IL-6 and PGE2, IL-6R and PGE2, VEGF and PGE2, Abeta (seq. 1) and PGE2, Abeta (seq. 2) and PGE2, Abeta (seq. 3) and PGE2, IL-18 and PGE2, PGE2 and PGE2, IL-15 and PGE2, S1P and PGE2, EGFR (seq. 1) and PGE2, EGFR (seq. 2) and PGE2, and IGFR and PGE2 (see Examples).

6. Sepsis

The pathophysiology of sepsis is initiated by the outer membrane components of both gram-negative organisms (lipopolysaccharide [LPS], lipid A, endotoxin) and gram-positive organisms (lipoteichoic acid, peptidoglycan). These outer membrane components are able to bind to the CD14 receptor on the surface of monocytes. By virtue of the recently described toll-like receptors, a signal is then transmitted to the cell, leading to the eventual production of the proinflammatory cytokines tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 (IL-1). Overwhelming inflammatory and immune responses are essential features of septic shock and play a central part in the pathogenesis of tissue damage, multiple organ failure, and death induced by sepsis. Cytokines, especially tumor necrosis factor (TNF) and interleukin (IL-1), have been shown to be critical mediators of septic shock. These cytokines have a direct toxic effect on tissues; they also activate phospholipase A2. These and other effects lead to increased concentrations of platelet-

activating factor, promotion of nitric oxide synthase activity, promotion of tissue infiltration by neutrophils, and promotion of neutrophil activity.

Tumor necrosis factor has an established role in the pathophysiology of sepsis, with biological effects that include hypotension, myocardial suppression, vascular leakage syndrome, organ necrosis, stimulation of the release of toxic secondary mediators and activation of the clotting cascade (Tracey, K. J. and Cerami, A. (1994) Annu. Rev. Med. 45:491-503; Russell, D and Thompson, R. C. (1993) Curr. Opin. Biotech. 4:714-721). Prostaglandin E2 synthesis and metabolism are increased in burn injury and trauma (Hahn, E. L. and Gamelli, R. L. (2000) J. Trauma. 49:1147-1154). Accordingly, the DVD-Ig™ molecules or DVD-Ig™ portions, of the invention can be used to treat sepsis in any of its clinical settings, including septic shock, burn injury, trauma, endotoxic shock, gram negative sepsis and toxic shock syndrome.

The treatment of sepsis and septic shock remains a clinical conundrum, and recent prospective trials with biological response modifiers (i.e. anti-TNF, anti-MIF) aimed at the inflammatory response have shown only modest clinical benefit. Recently, interest has shifted toward therapies aimed at reversing the accompanying periods of immune suppression. Studies in experimental animals and critically ill patients have demonstrated that increased apoptosis of lymphoid organs and some parenchymal tissues contribute to this immune suppression, anergy, and organ system dysfunction. During sepsis syndromes, lymphocyte apoptosis can be triggered by the absence of IL-2 or by the release of glucocorticoids, granzymes, or the so-called 'death' cytokines: tumor necrosis factor alpha or Fas ligand. Apoptosis proceeds via auto-activation of cytosolic and/or mitochondrial caspases, which can be influenced by the pro- and anti-apoptotic members of the Bcl-2 family. In experimental animals, not only can treatment with inhibitors of apoptosis prevent lymphoid cell apoptosis; it may also improve outcome. Although clinical trials with anti-apoptotic agents remain distant due in large part to technical difficulties associated with their administration and tissue targeting, inhibition of lymphocyte apoptosis represents an attractive therapeutic target for the septic patient. Likewise, a dual-specific agent targeting both inflammatory mediator and a apoptotic mediator, may have added benefit.

Furthermore, to treat sepsis, an DVD-Ig™ molecule or DVD-Ig™ portion, of the invention can be coadministered with one or more additional therapeutic agents that may further alleviate sepsis, such as an interleukin-1 inhibitor (such as those described in PCT Publication Nos. WO 92/16221 and WO 92/17583), the cytokine interleukin-6 (see e.g., PCT Publication No. WO 93/11793) or an antagonist of platelet activating factor (see e.g., European Patent Application Publication No. EP 374 510).

Additionally, in a preferred embodiment, an DVD-Ig™ molecule or DVD-Ig™ portion of the invention is administered to a human subject within a subgroup of sepsis patients having a serum or plasma concentration of IL-6 above 500 pg/ml, and more preferably 1000 pg/ml, at the time of treatment (see PCT Publication No. WO 95/20978 by Daum, L., et al.).

One aspect of the invention pertains to DVD Igs capable of binding one or more targets involved in sepsis, in an embodiment two targets, selected from the group consisting PGE, PGE1, PGE2, S1P, S1P1, S1P2, S1P3, S1P4, S1P5, RAGE, VLA-4, CD44, RAGE, HMGB1, S100, TNF, IL-1, MIF, IL-6, IL-8, IL-18, IL-12, IL-23, FasL, LPS, Toll-like receptors, TLR-4, tissue factor, MIP-2, ADORA2A, CASP1, CASP4, IL-10, IL-1B, NFKB1, PROC, TNFRSF1A, CSF3, CCR3, IL1RN, MIF, NFKB1, PTAFR, TLR2, TLR4, GPR44, HMOX1, midkine, IRAK1, NFKB2, SERPINA1, SERPINE1, and TREM1. The efficacy of such DVD Igs for sepsis can be assessed in preclinical animal models known in the art (see Buras JA, et al.,(2005) Nat Rev Drug Discov. 4(10):854-65 and Calandra T, et al., (2000) Nat Med. 6(2):164-70).

DVD Igs capable of binding the following pairs of targets to treat sepsis are contemplated: mouse or human TNF and PGE2, NGF and PGE2, IL-17A and PGE2, IL-1b and PGE2, IL-6 and PGE2, IL-6R and PGE2, VEGF and PGE2, Abeta (seq. 1) and PGE2, Abeta (seq. 2) and PGE2, Abeta (seq. 3) and PGE2, IL-18 and PGE2, PGE2 and PGE2, IL-15 and PGE2, S1P and PGE2, EGFR (seq. 1) and PGE2, EGFR (seq. 2) and PGE2, and IGFR and PGE2 (see Examples).

7. Neurological disorders

7.1. Neurodegenerative Diseases

Chronic neurodegenerative diseases are usually age-dependent diseases characterized by progressive loss of neuronal functions (neuronal cell death, demyelination), loss of mobility and loss of memory. Emerging knowledge of the mechanisms underlying chronic neurodegenerative diseases (e.g., Alzheimer's disease disease) show a complex etiology and a variety of factors have been recognized to contribute to their development and progression e.g.,age, glycemic status, amyloid production and multimerization, accumulation of advanced glycation-end products (AGE) which bind to their receptor RAGE (receptor for AGE), increased brain oxidative stress, decreased cerebral blood flow, neuroinflammation including release of inflammatory cytokines and chemokines, neuronal dysfunction and microglial activation. Thus these chronic neurodegenerative diseases represent a complex interaction between multiple cell types and mediators. Treatment strategies for such diseases are limited and mostly constitute either blocking inflammatory processes with non-specific anti-inflammatory agents (e.g., corticosteroids, COX inhibitors) or agents to prevent neuron loss and/or synaptic functions. These treatments fail to stop disease progression. Recent studies suggest that more targeted therapies such as antibodies to soluble A-b peptide (including the A-b oligomeric forms) can not only help stop disease progression but may help maintain memory as well. These preliminary observations suggest that specific therapies targeting more than one disease mediator (e.g.,A-b and a pro-inflammatory cytokine such as TNF) may provide even better therapeutic efficacy for chronic neurodegenerative diseases than observed with targeting a single disease mechanism (e.g.,soluble A-balone) (see C.E. Shepherd, et al, Neurobiol Aging. 2005 Oct 24; Nelson RB., Curr Pharm Des. 2005;11:3335; William L. Klein.; Neurochem Int. 2002 ;41:345; Michelle C Janelsins, et al., J Neuroinflammation. 2005 ;2:23; Soloman B., Curr Alzheimer Res. 2004;1:149; Igor Klyubin, et al., Nat Med. 2005;11:556-61; Arancio O, et al., EMBO Journal (2004) 1-10; Bornemann KD, et al., Am J Pathol. 2001;158:63; Deane R, et al., Nat Med. 2003;9:907-13; and Eliezer Masliah, et al., Neuron. 2005;46:857).

The DVD-Ig molecules of the invention can bind one or more targets involved in Chronic neurodegenerative diseases such as Alzheimers. Such targets include, but are not limited to, any mediator, soluble or cell surface, implicated in AD pathogenesis e.g AGE (S100 A, amphoterin), pro-inflammatory cytokines (e.g.,IL-1), chemokines (e.g.,MCP 1), molecules that inhibit nerve regeneration (e.g.,Nogo, RGM A), molecules that enhance

neurite growth (neurotrophins). The efficacy of DVD-Ig molecules can be validated in pre-clinical animal models such as the transgenic mice that over-express amyloid precursor protein or RAGE and develop Alzheimer's disease-like symptoms. In addition, DVD-Ig molecules can be constructed and tested for efficacy in the animal models and the best therapeutic DVD-Ig can be selected for testing in human patients. DVD-Ig molecules can also be employed for treatment of other neurodegenerative diseases such as Parkinson's disease. Alpha-Synuclein is involved in Parkinson's pathology. A DVD-Ig capable of targeting alpha-synuclein and inflammatory mediators such as PGE,PGE1, PGE2, RAGE, HMGB1, S100, TNF, IL-1, MCP-1 can prove effective therapy for Parkinson's disease and are contemplated in the invention.

DVD Igs capable of binding the following pairs of targets to treat neurological diseases are contemplated: mouse or human TNF and PGE2, NGF and PGE2, IL-17A and PGE2, IL-1b and PGE2, IL-6 and PGE2, IL-6R and PGE2, VEGF and PGE2, Abeta (seq. 1) and PGE2, Abeta (seq. 2) and PGE2, Abeta (seq. 3) and PGE2, IL-18 and PGE2, PGE2 and PGE2, IL-15 and PGE2, S1P and PGE2, EGFR (seq. 1) and PGE2, EGFR (seq. 2) and PGE2, and IGFR and PGE2 (see Examples).

7.2 Neuronal Regeneration and Spinal Cord Injury

Despite an increase in knowledge of the pathologic mechanisms, spinal cord injury (SCI) is still a devastating condition and represents a medical indication characterized by a high medical need. Most spinal cord injuries are contusion or compression injuries and the primary injury is usually followed by secondary injury mechanisms (inflammatory mediators e.g., cytokines and chemokines) that worsen the initial injury and result in significant enlargement of the lesion area, sometimes more than 10-fold. These primary and secondary mechanisms in SCI are very similar to those in brain injury caused by other means e.g., stroke. No satisfying treatment exists and high dose bolus injection of methylprednisolone (MP) is the only used therapy within a narrow time window of 8 h post injury. This treatment, however, is only intended to prevent secondary injury without causing any significant functional recovery. It is heavily critisized for the lack of unequivocal efficacy and severe adverse effects, like immunosuppression with subsequent infections and severe histopathological muscle alterations. No other drugs, biologics or small molecules, stimulating the endogenous

regenerative potential are approved, but promising treatment principles and drug candidates have shown efficacy in animal models of SCI in recent years. To a large extent the lack of functional recovery in human SCI is caused by factors inhibiting neurite growth, at lesion sites, in scar tissue, in myelin as well as on injury-associated cells. Such factors are the myelin-associated proteins NogoA, OMgp and MAG, RGM A, the scar-associated CSPG (Chondroitin Sulfate Proteoglycans) and inhibitory factors on reactive astrocytes (some semaphorins and ephrins). However, at the lesion site not only growth inhibitory molecules are found but also neurite growth stimulating factors like neurotrophins, laminin, L1 and others. This ensemble of neurite growth inhibitory and growth promoting molecules may explain that blocking single factors, like NogoA or RGM A, resulted in significant functional recovery in rodent SCI models, because a reduction of the inhibitory influences could shift the balance from growth inhibition to growth promotion. However, recoveries observed with blocking a single neurite outgrowth inhibitory molecule were not complete. To achieve faster and more pronounced recoveries either blocking two neurite outgrowth inhibitory molecules e.g Nogo and RGM A, or blocking an neurite outgrowth inhibitory molecule and enhancing functions of a neurite outgrowth enhancing molecule e.g Nogo and neurotrophins, or blocking a neurite outgrowth inhibitory moleclule e.g.,Nogo and a pro-inflammatory molecule e.g.,TNF, may be desirable (see McGee AW, et al., Trends Neurosci. 2003;26:193; Marco Domeniconi, et al., J Neurol Sci. 2005;233:43; Milan Makwana1, et al., FEBS J. 2005;272:2628; Barry J. Dickson, Science. 2002;298:1959; Felicia Yu Hsuan Teng, et al., J Neurosci Res. 2005;79:273; Tara Karnezis, et al., Nature Neuroscience 2004; 7, 736; Gang Xu, et al., J. Neurochem.2004; 91; 1018).

In one aspect, DVD-Igs capable of binding target pairs such as NgR and RGM A; NogoA and RGM A; MAG and RGM A; OMGp and RGM A; RGM A and RGM B; CSPGs and RGM A; aggrecan, midkine, neurocan, versican, phosphacan, Te38 and TNF-α; Aß globulomer-specific antibodies combined with antibodies promoting dendrite & axon sprouting are provided. Dendrite pathology is a very early sign of AD and it is known that NOGO A restricts dendrite growth. One can combine such type of ab with any of the SCI-candidate (myelin-proteins) Ab. Other DVD-Ig targets may include any combination of NgR-p75, NgR-Troy, NgR-Nogo66 (Nogo), NgR-Lingo, Lingo-Troy, Lingo-p75, MAG or Omgp. Additionally, targets may also include any mediator, soluble

or cell surface, implicated in inhibition of neurite e.g Nogo, Ompg, MAG, RGM A, semaphorins, ephrins, soluble A-b, pro-inflammatory cytokines (e.g.,IL-1), chemokines (e.g.,MIP 1a), molecules that inhibit nerve regeneration. The efficacy of anti-nogo / anti-RGM A or similar DVD-Ig molecules can be validated in pre-clinical animal models of spinal cord injury. In addition, these DVD-Ig molecules can be constructed and tested for efficacy in the animal models and the best therapeutic DVD-Ig can be selected for testing in human patients. In addition, DVD-Ig molecules can be constructed that target two distinct ligand binding sites on a single receptor e.g., Nogo receptor which binds three ligand Nogo, Ompg, and MAG and RAGE that binds A-b and S100 A. Furthermore, neurite outgrowth inihibitors e.g.,nogo and nogo receptor, also play a role in preventing nerve regeneration in immunological diseases like multiple sclerosis. Inhibition of nogo-nogo receptor interaction has been shown to enhance recovery in animal models of multiple sclerosis. Therefore, DVD-Ig molecules that can block the function of one immune mediator eg a cytokine like IL-12 and a neurite outgrowth inhibitor molecule eg nogo or RGM may offer faster and greater efficacy than blocking either an immune or an neurite outgrowth inhibitor molecule alone.

DVD Igs capable of binding the following pairs of targets to treat spinal cord injury or encourage neuronal regeneration are contemplated: mouse or human TNF and PGE2, NGF and PGE2, IL-17A and PGE2, IL-1b and PGE2, IL-6 and PGE2, IL-6R and PGE2, VEGF and PGE2, Abeta (seq. 1) and PGE2, Abeta (seq. 2) and PGE2, Abeta (seq. 3) and PGE2, IL-18 and PGE2, PGE2 and PGE2, IL-15 and PGE2, S1P and PGE2, EGFR (seq. 1) and PGE2, EGFR (seq. 2) and PGE2, and IGFR and PGE2 (see Examples).

8. Oncological Disorders

Monoclonal antibody therapy has emerged as an important therapeutic modality for cancer (von Mehren, M., et al. (2003) Annu. Rev. Med. 54:343-69). Antibodies may exert antitumor effects by inducing apoptosis, redirected cytotoxicity, interfering with ligand-receptor interactions, or preventing the expression of proteins that are critical to the neoplastic phenotype. In addition, antibodies can target components of the tumor microenvironment, perturbing vital structures such as the formation of tumor-associated vasculature. Antibodies can also target receptors whose ligands are growth factors, such

as the epidermal growth factor receptor. The antibody thus inhibits natural ligands that stimulate cell growth from binding to targeted tumor cells. Alternatively, antibodies may induce an anti-idiotype network, complement-mediated cytotoxicity, or antibody-dependent cellular cytotoxicity (ADCC). The use of dual-specific antibody that targets two separate tumor mediators will likely give additional benefit compared to a mono-specific therapy.

Tumor necrosis factor has been implicated in inducing cachexia, stimulating tumor growth, enhancing metastatic potential and mediating cytotoxicity in malignancies (see, e.g., Tracey and Cerami, supra). COX-2 is found to be overexpressed and lead to generation of aboundant prostaglandins including PGE_2 in cancers such as breast, gastric, lung and pancreatic, etc. NSAIDs and COX-1/2 inhibitors showed effective in tumor models. Anti-PGE_2 antibodies may also have broad implications for the prevention/treatment of a number of other malignancies (Chell, S. and Kaidi, A. Biochim Biophys Acta. (2006) 1766:104-119). Accordingly, the DVD-Ig™ molecules or DVD-Ig™ portions, of the invention, can be used in the treatment of malignancies, to inhibit tumor growth or metastasis for tumors including but not limited to headneck tumor, lung cancer, gastric cancer, prostate cancer, pancreatic cancer and/or to alleviate cachexia secondary to malignancy. The DVD-Ig™ molecules or DVD-Ig™ portions, may be administered systemically or locally to the tumor site.

In another embodiment, a DVD of the invention is capable of binding PGE2 and IGF1,2, PGE2 and Erb2B, PGE2 and VEGFR, PGE2 and IGFR, PGE2 and EGFR, PGE2 and CD20, PGE2 and CD138, PGE2 and CD40, PGE2 and CD38, VEGF and phosphatidylserine; VEGF and ErbB3; VEGF and PLGF; VEGF and ROBO4; VEGF and BSG2; VEGF and CDCP1; VEGF and ANPEP; VEGF and c-MET; HER-2 and ERB3; HER-2 and BSG2; HER-2 and CDCP1; HER-2 and ANPEP; EGFR and CD64; EGFR and BSG2; EGFR and CDCP1; EGFR and ANPEP; IGF1R and PDGFR; IGF1R and VEGF; IGF1R and CD20; CD20 and CD74; CD20 and CD30; CD20 and DR4; CD20 and VEGFR2; CD20 and CD52; CD20 and CD4; HGF and c-MET; HGF and NRP1; HGF and phosphatidylserine; ErbB3 and IGF1R; ErbB3 and IGF1,2; c-Met and Her-2; c-Met and NRP1; c-Met and IGF1R; IGF1,2 and PDGFR; IGF1,2 and CD20; IGF1,2 and IGF1R; IGF2 and EGFR; IGF2 and HER2; IGF2 and CD20; IGF2 and VEGF; IGF2 and IGF1R; IGF1 and IGF2; PDGFRa and VEGFR2; PDGFRa and PLGF; PDGFRa and

VEGF; PDGFRa and c-Met; PDGFRa and EGFR; PDGFRb and VEGFR2; PDGFRb and c-Met; PDGFRb and EGFR; RON and c-Met; RON and MTSP1; RON and MSP; RON and CDCP1; VGFR1 and PLGF; VGFR1 and RON; VGFR1 and EGFR; VEGFR2 and PLGF; VEGFR2 and NRP1; VEGFR2 and RON; VEGFR2 and DLL4; VEGFR2 and EGFR; VEGFR2 and ROBO4; VEGFR2 and CD55; LPA and S1P; EPHB2 and RON; CTLA4 and VEGF; CD3 and EPCAM; CD40 and IL6; CD40 and IGF; CD40 and CD56; CD40 and CD70; CD40 and VEGFR1; CD40 and DR5; CD40 and DR4; CD40 and APRIL; CD40 and BCMA; CD40 and RANKL; CD28 and MAPG; CD80 and CD40; CD80 and CD30; CD80 and CD33; CD80 and CD74; CD80 and CD2; CD80 and CD3; CD80 and CD19; CD80 and CD4; CD80 and CD52; CD80 and VEGF; CD80 and DR5; CD80 and VEGFR2; CD22 and CD20; CD22 and CD80; CD22 and CD40; CD22 and CD23; CD22 and CD33; CD22 and CD74; CD22 and CD19; CD22 and DR5; CD22 and DR4; CD22 and VEGF; CD22 and CD52; CD30 and CD20; CD30 and CD22; CD30 and CD23; CD30 and CD40; CD30 and VEGF; CD30 and CD74; CD30 and CD19; CD30 and DR5; CD30 and DR4; CD30 and VEGFR2; CD30 and CD52; CD30 and CD4; CD138 and RANKL; CD33 and FTL3; CD33 and VEGF; CD33 and VEGFR2; CD33 and CD44; CD33 and DR4; CD33 and DR5; DR4 and CD137; DR4 and IGF1,2; DR4 and IGF1R; DR4 and DR5; DR5 and CD40; DR5 and CD137; DR5 and CD20; DR5 and EGFR; DR5 and IGF1,2; DR5 and IGFR, DR5 and HER-2, and EGFR and DLL4. Other target combinations include one or more members of the EGF/erb-2/erb-3 family. Other targets (one or more) involved in oncological diseases that DVD Igs may bind include, but are not limited to those selected from the group consisting of: PGE2, Muc-1, TRAIL, CD52, CD20, CD19, CD3, CD4, CD8, BMP6, IL12A, IL1A, IL1B, IL2, IL24, INHA, TNF, TNFSF10, BMP6, EGF, FGF1, FGF10, FGF11, FGF12, FGF13, FGF14, FGF16, FGF17, FGF18, FGF19, FGF2, FGF20, FGF21, FGF22, FGF23, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, GRP, IGF1, IGF2, IL12A, IL1A, IL1B, IL2, INHA, TGFA, TGFB1, TGFB2, TGFB3, VEGF, CDK2, FGF10, FGF18, FGF2, FGF4, FGF7, IGF1R, IL2, BCL2, CD164, CDKN1A, CDKN1B, CDKN1C, CDKN2A, CDKN2B, CDKN2C, CDKN3, GNRH1, IGFBP6, IL1A, IL1B, ODZ1, PAWR, PLG, TGFB1I1, AR, BRCA1, CDK3, CDK4, CDK5, CDK6, CDK7, CDK9, E2F1, EGFR, ENO1, ERBB2, ESR1, ESR2, IGFBP3, IGFBP6, IL2, INSL4, MYC, NOX5, NR6A1, PAP, PCNA, PRKCQ, PRKD1, PRL, TP53, FGF22, FGF23, FGF9, IGFBP3, IL2, INHA, KLK6, TP53, CHGB, GNRH1, IGF1, IGF2, INHA, INSL3, INSL4, PRL, KLK6, SHBG, NR1D1, NR1H3,

NR1I3, NR2F6, NR4A3, ESR1, ESR2, NR0B1, NR0B2, NR1D2, NR1H2, NR1H4, NR1I2, NR2C1, NR2C2, NR2E1, NR2E3, NR2F1, NR2F2, NR3C1, NR3C2, NR4A1, NR4A2, NR5A1, NR5A2, NR6A1, PGR, RARB, FGF1, FGF2, FGF6, KLK3, KRT1, APOC1, BRCA1, CHGA, CHGB, CLU, COL1A1, COL6A1, EGF, ERBB2, ERK8, FGF1, FGF10, FGF11, FGF13, FGF14, FGF16, FGF17, FGF18, FGF2, FGF20, FGF21, FGF22, FGF23, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, GNRH1, IGF1, IGF2, IGFBP3, IGFBP6, IL12A, IL1A, IL1B, IL2, IL24, INHA, INSL3, INSL4, KLK10, KLK12, KLK13, KLK14, KLK15, KLK3, KLK4, KLK5, KLK6, KLK9, MMP2, MMP9, MSMB, NTN4, ODZ1, PAP, PLAU, PRL, PSAP, SERPINA3, SHBG, TGFA, TIMP3, CD44, CDH1, CDH10, CDH19, CDH20, CDH7, CDH9, CDH1, CDH10, CDH13, CDH18, CDH19, CDH20, CDH7, CDH8, CDH9, ROBO2, CD44, ILK, ITGA1, APC, CD164, COL6A1, MTSS1, PAP, TGFB1I1, AGR2, AIG1, AKAP1, AKAP2, CANT1, CAV1, CDH12, CLDN3, CLN3, CYB5, CYC1, DAB2IP, DES, DNCL1, ELAC2, ENO2, ENO3, FASN, FLJ12584, FLJ25530, GAGEB1, GAGEC1, GGT1, GSTP1, HIP1, HUMCYT2A, IL29, K6HF, KAI1, KRT2A, MIB1, PART1, PATE, PCA3, PIAS2, PIK3CG, PPID, PR1, PSCA, SLC2A2, SLC33A1, SLC43A1, STEAP, STEAP2, TPM1, TPM2, TRPC6, ANGPT1, ANGPT2, ANPEP, ECGF1, EREG, FGF1, FGF2, FIGF, FLT1, JAG1, KDR, LAMA5, NRP1, NRP2, PGF, PLXDC1, STAB1, VEGF, VEGFC, ANGPTL3, BAI1, COL4A3, IL8, LAMA5, NRP1, NRP2, STAB1, ANGPTL4, PECAM1, PF4, PROK2, SERPINF1, TNFAIP2, CCL11, CCL2, CXCL1, CXCL10, CXCL3, CXCL5, CXCL6, CXCL9, IFNA1, IFNB1, IFNG, IL1B, IL6, MDK, EDG1, EFNA1, EFNA3, EFNB2, EGF, EPHB4, FGFR3, HGF, IGF1, ITGB3, PDGFA, TEK, TGFA, TGFB1, TGFB2, TGFBR1, CCL2, CDH5, COL18A1, EDG1, ENG, ITGAV, ITGB3, THBS1, THBS2, BAD, BAG1, BCL2, CCNA1, CCNA2, CCND1, CCNE1, CCNE2, CDH1 (E-cadherin), CDKN1B (p27Kip1), CDKN2A (p16INK4a), COL6A1, CTNNB1 (b-catenin), CTSB (cathepsin B), ERBB2 (Her-2), ESR1, ESR2, F3 (TF), FOSL1 (FRA-1), GATA3, GSN (Gelsolin), IGFBP2, IL2RA, IL6, IL6R, IL6ST (glycoprotein 130), ITGA6 (a6 integrin), JUN, KLK5, KRT19, MAP2K7 (c-Jun), MKI67 (Ki-67), NGFB (NGF), NGFR, NME1 (NM23A), PGR, PLAU (uPA), PTEN, SERPINB5 (maspin), SERPINE1 (PAI-1), TGFA, THBS1 (thrombospondin-1), TIE (Tie-1), TNFRSF6 (Fas), TNFSF6 (FasL), TOP2A (topoisomerase Iia), TP53, AZGP1 (zinc-a-glycoprotein), BPAG1 (plectin), CDKN1A (p21Wap1/Cip1), CLDN7 (claudin-7), CLU (clusterin), ERBB2 (Her-2), FGF1, FLRT1 (fibronectin), GABRP (GABAa), GNAS1,

ID2, ITGA6 (a6 integrin), ITGB4 (b 4 integrin), KLF5 (GC Box BP), KRT19 (Keratin 19), KRTHB6 (hair-specific type II keratin), MACMARCKS, MT3 (metallothionectin-III), MUC1 (mucin), PTGS2 (COX-2), RAC2 (p21Rac2), S100A2, SCGB1D2 (lipophilin B), SCGB2A1 (mammaglobin 2), SCGB2A2 (mammaglobin 1), SPRR1B (Spr1), THBS1, THBS2, THBS4, and TNFAIP2 (B94), RON, c-Met, CD64, DLL4, PLGF, CTLA4, phophatidylserine, ROBO4, CD80, CD22, CD40, CD23, CD28, CD80, CD55, CD38, CD70, CD74, CD30, CD138, CD56, CD33, CD2, CD137, DR4, DR5, RANKL, VEGFR2, PDGFR, VEGFR1, MTSP1, MSP, EPHB2, EPHA1, EPHA2, EpCAM, PGE2, NKG2D, LPA, SIP, APRIL, BCMA, MAPG, FLT3, PDGFR alpha, PDGFR beta, ROR1, PSMA, PSCA, SCD1, and CD59. Preferable targets (one or more) involved in oncological diseases that the DVD Igs may bind in addition to PGE2 include, but are not limited to, those selected from the list above.

DVD Igs capable of binding the following pairs of targets to treat oncological disease are contemplated: mouse or human TNF and PGE2, NGF and PGE2, IL-17A and PGE2, IL-1b and PGE2, IL-6 and PGE2, IL-6R and PGE2, VEGF and PGE2, Abeta (seq. 1) and PGE2, Abeta (seq. 2) and PGE2, Abeta (seq. 3) and PGE2, IL-18 and PGE2, PGE2 and PGE2, IL-15 and PGE2, S1P and PGE2, EGFR (seq. 1) and PGE2, EGFR (seq. 2) and PGE2, and IGFR and PGE2 (see Examples).

9. Infectious Diseases

Tumor necrosis factor and prostaglandin E_2 have been implicated in mediating biological effects observed in a variety of infectious diseases. For example, TNFα has been implicated in mediating brain inflammation and capillary thrombosis and infarction in malaria (see, e.g., Tracey and Cerami, supra). TNFα also has been implicated in mediating brain inflammation, inducing breakdown of the blood-brain barrier, triggering septic shock syndrome and activating venous infarction in meningitis (see, e.g., Tracey and Cerami, supra). TNFα also has been implicated in inducing cachexia, stimulating viral proliferation and mediating central nervous system injury in acquired immune deficiency syndrome (AIDS) (see, e.g., Tracey and Cerami, supra). Accordingly, DVD-Ig™ molecules or DVD-Ig™ portions, of the invention, can be used in the treatment of infectious diseases, including bacterial meningitis (see e.g., European Pat. Application Publication No. EP 585 705), cerebral malaria, AIDS and AIDS-related complex (ARC)

(see, e.g., European Patent Application Publication No. EP 230 574), certain illnesses induced by viruses, such as Guillain Barre syndrome, infectious mononucleosis, other viral lymphadenopathies and infections with herpes virus; as well as cytomegalovirus infection secondary to transplantation (see e.g., Fietze, E., et al. (1994) Transplantation 58:675-680). The DVD-Ig™ molecules or DVD-Ig™ portions, of the invention, also can be used to alleviate symptoms associated with infectious diseases, including fever and myalgias due to infection (such as influenza) and cachexia secondary to infection (e.g., secondary to AIDS or ARC).

DVD Igs capable of binding the following pairs of targets to treat infectious disease are contemplated: mouse or human TNF and PGE2, NGF and PGE2, IL-17A and PGE2, IL-1b and PGE2, IL-6 and PGE2, IL-6R and PGE2, VEGF and PGE2, Abeta (seq. 1) and PGE2, Abeta (seq. 2) and PGE2, Abeta (seq. 3) and PGE2, IL-18 and PGE2, PGE2 and PGE2, IL-15 and PGE2, S1P and PGE2, EGFR (seq. 1) and PGE2, EGFR (seq. 2) and PGE2, and IGFR and PGE2 (see Examples).

10. Transplantation

Tumor necrosis factor and prostaglandin E_2 has been implicated as key mediators of allograft rejection and graft versus host disease (GVHD) and in mediating an adverse reaction that has been observed when the rat antibody OKT3, directed against the T cell receptor CD3 complex, is used to inhibit rejection of renal transplants (see, e.g., Tracey and Cerami, supra; Eason, J. D., et al. (1995) Transplantation 59:300-305; Suthanthiran, M. and Strom, T. B. (1994) New Engl. J. Med. 331:365-375). Accordingly, the antibodies, and antibody portions, of the invention, can be used to inhibit transplant rejection, including rejections of allografts and xenografts and to inhibit GVHD. Although the DVD-Ig™ molecule or DVD-Ig™ portion may be used alone, more preferably it is used in combination with one or more other agents that inhibit the immune response against the allograft or inhibit GVHD. For example, in one embodiment, a DVD-Ig™ molecule or DVD-Ig™ portion of the invention is used in combination with OKT3 to inhibit OKT3-induced reactions. In another embodiment, an antibody or antibody portion of the invention is used in combination with one or more antibodies directed at other targets involved in regulating immune responses, such as the cell surface molecules CD25 (interleukin-2 receptor-.alpha.), CD11a (LFA-1), CD54 (ICAM-1),

CD4, CD45, CD28/CTLA4, CD80 (B7-1) and/or CD86 (B7-2). In yet another embodiment, an antibody or antibody portion of the invention is used in combination with one or more general immunosuppressive agents, such as cyclosporin A or FK506.11.

Pulmonary Disorders

Tumor necrosis factor has been implicated in the pathophysiology of adult respiratory distress syndrome, including stimulating leukocyte-endothelial activation, directing cytotoxicity to pneumocytes and inducing vascular leakage syndrome (see, e.g., Tracey and Cerami, supra). Accordingly, the antibodies, and antibody portions, of the invention, can be used to treat various pulmonary disorders, including adult respiratory distress syndrome (see, e.g., PCT Publication No. WO 91/04054), shock lung, chronic pulmonary inflammatory disease, pulmonary sarcoidosis, pulmonary fibrosis and silicosis. The DVD-Ig™ molecules or DVD-Ig™ portions, may be administered systemically or locally to the lung surface, for example as an aerosol.

DVD Igs capable of binding the following pairs of targets to treat transplantation related disorders are contemplated: mouse or human TNF and PGE2, NGF and PGE2, IL-17A and PGE2, IL-1b and PGE2, IL-6 and PGE2, IL-6R and PGE2, VEGF and PGE2, Abeta (seq. 1) and PGE2, Abeta (seq. 2) and PGE2, Abeta (seq. 3) and PGE2, IL-18 and PGE2, PGE2 and PGE2, IL-15 and PGE2, S1P and PGE2, EGFR (seq. 1) and PGE2, EGFR (seq. 2) and PGE2, and IGFR and PGE2 (see Examples).

11. Intestinal Disorders

Tumor necrosis factor and prostaglandins has been implicated in the pathophysiology of inflammatory bowel disorders (see, e.g., Tracy, K. J., et al. (1986) Science 234:470-474; Sun, X-M., et al. (1988) J. Clin. Invest. 81:1328-133 1; MacDonald, T. T., et al. (1990) Clin. Exp. Immunol. 81:301-305). Chimeric murine anti-hTNFα antibodies have undergone clinical testing for treatment of Crohn's disease (van Dullemen, H. M., et al. (1995) Gastroenterology 109:129-135). The anti-TNFα antibody HUMIRA has been approved for the treatment of Crohn's disease. The DVD-Ig™ molecules or DVD-Ig™ portions, of the invention, also can be used to treat intestinal disorders, such as idiopathic inflammatory bowel disease, which includes two syndromes, Crohn's disease and ulcerative colitis.

DVD Igs capable of binding the following pairs of targets to treat intestinal disease are contemplated: mouse or human TNF and PGE2, NGF and PGE2, IL-17A and PGE2, IL-1b and PGE2, IL-6 and PGE2, IL-6R and PGE2, VEGF and PGE2, Abeta (seq. 1) and PGE2, Abeta (seq. 2) and PGE2, Abeta (seq. 3) and PGE2, IL-18 and PGE2, PGE2 and PGE2, IL-15 and PGE2, S1P and PGE2, EGFR (seq. 1) and PGE2, EGFR (seq. 2) and PGE2, and IGFR and PGE2 (see Examples).

12. Cardiac Disorders

The DVD-Ig™ molecules or DVD-Ig™ portions of the invention also can be used to treat various cardiac disorders, including ischemia of the heart (see, e.g., European Patent Application Publication No. EP 453 898) and heart insufficiency (weakness of the heart muscle)(see e.g., PCT Publication No. WO 94/20139).

DVD Igs capable of binding the following pairs of targets to treat cardiac disease are contemplated: mouse or human TNF and PGE2, NGF and PGE2, IL-17A and PGE2, IL-1b and PGE2, IL-6 and PGE2, IL-6R and PGE2, VEGF and PGE2, Abeta (seq. 1) and PGE2, Abeta (seq. 2) and PGE2, Abeta (seq. 3) and PGE2, IL-18 and PGE2, PGE2 and PGE2, IL-15 and PGE2, S1P and PGE2, EGFR (seq. 1) and PGE2, EGFR (seq. 2) and PGE2, and IGFR and PGE2 (see Examples).

13. Others

The DVD-Ig™ molecules or DVD-Ig™ portions of the invention also can be used to treat various other disorders in which TNFα and/or PGE_2 activities are detrimental. Examples of other diseases and disorders in which TNFα and/or PGE_2 activities have been implicated in the pathophysiology, and thus which can be treated using a DVD-Ig™ molecule or DVD-Ig™ portion of the invention include inflammatory bone disorders, bone growth disease and bone resorption disease (see, e.g., Bertolini, D. R., et al. (1986) Nature 319:516-518; Konig, A., et al. (1988) J. Bone Miner. Res. 3:621-627; Lerner, U. H. and Ohlin, A. (1993) J. Bone Miner. Res. 8:147-155; and Shankar, G. and Stem, P. H. (1993) Bone 14:871-876), hepatitis, including alcoholic hepatitis (see e.g., McClain, C. J. and Cohen, D. A. (1989) Hepatology 9:349-351; Felver, M. E., et al. (1990) Alcohol. Clin. Exp. Res. 14:255-259; and Hansen, J., et al. (1994) Hepatology 20:461-474) and viral hepatitis (Sheron, N., et al. (1991) J. Hepatol. 12:241-245; and Hussain, M. J., et al. (1994) J. Clin. Pathol. 47:1112-1115), coagulation disturbances (see e.g., van der Poll, T., et al. (1990) N. Engl. J. Med. 322:1622-1627; and van der Poll, T., et al. (1991) Prog. Clin. Biol. Res. 367:55-60), burns (see e.g., Giroir, B. P., et al. (1994) Am. J. Physiol. 267:H118-124; and Liu, X. S., et al. (1994) Burns 20:40-44), reperfusion injury (see e.g., Scales, W. E., et al. (1994) Am. J. Physiol. 267:G1122-1127; Serrick, C., et al. (1994) Transplantation 58:1158-1162; and Yao, Y. M., et al. (1995) Resuscitation 29:157-168), keloid formation (see e.g., McCauley, R. L., et al. (1992) J. Clin. Immunol. 12:300-308), scar tissue formation and pyrexia, allergic arthritis, hematological disorders, such as hemolytic anemias and thrombocytopenias, endocrinologic disorders, such as diabetes mellitus, Addison's disease, idiopathic hypoparathyroidism and chronic lymphocytic thyroiditis, disorders of reproduction such as amenorrhoea, infertility, recurrent abortions and eclampsia, and ocular disorders such as age-related macular degeneration (AMD).

DVD Igs capable of binding the following pairs of targets to treat the above diseases are contemplated: mouse or human TNF and PGE2, NGF and PGE2, IL-17A and PGE2, IL-1b and PGE2, IL-6 and PGE2, IL-6R and PGE2, VEGF and PGE2, Abeta (seq. 1) and PGE2, Abeta (seq. 2) and PGE2, Abeta (seq. 3) and PGE2, IL-18 and PGE2, PGE2 and PGE2, IL-15 and PGE2, S1P and PGE2, EGFR (seq. 1) and PGE2, EGFR (seq. 2) and PGE2, and IGFR and PGE2 (see Examples).

IV. Pharmaceutical Compositions

The invention also provides pharmaceutical compositions comprising a binding protein, of the invention and a pharmaceutically acceptable carrier. The pharmaceutical compositions comprising binding proteins of the invention are for use in, but not limited to, diagnosing, detecting, or monitoring a disorder, in preventing, treating, managing, or ameliorating of a disorder or one or more symptoms thereof, and/or in research. In a specific embodiment, a composition comprises one or more binding proteins of the invention. In another embodiment, the pharmaceutical composition comprises one or more binding proteins of the invention and one or more prophylactic or therapeutic agents other than binding proteins of the invention for treating a disorder. In an embodiment, the prophylactic or therapeutic agents known to be useful for or having been or currently being used in the prevention, treatment, management, or amelioration of a disorder or one or more symptoms thereof. In accordance with these embodiments, the composition may further comprise of a carrier, diluent or excipient.

The binding proteins of the invention can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises a binding protein of the invention and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In some embodiments, isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride, are included in the composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody or antibody portion.

Various delivery systems are known and can be used to administer one or more antibodies of the invention or the combination of one or more antibodies of the invention and a prophylactic agent or therapeutic agent useful for preventing, managing, treating, or ameliorating a disorder or one or more symptoms thereof, e.g., encapsulation in

liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or antibody fragment, receptor- mediated endocytosis (see, e. g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of administering a prophylactic or therapeutic agent of the invention include, but are not limited to, parenteral administration (e.g., intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous) , epidurala administration, intratumoral administration, and mucosal adminsitration (e.g., intranasal and oral routes). In addition, pulmonary administration can be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, e.g., U.S. Pat. Nos. 6,019,968; 5,985,320; 5,985,309; 5,934,272; 5,874,064; 5,855,913; 5,290,540; and 4,880,078; and PCT Publication Nos. WO 92/19244; WO 97/32572; WO 97/44013; WO 98/31346; and WO 99/66903. In one embodiment, a binding protein of the invention, combination therapy, or a composition of the invention is administered using Alkermes AIR® pulmonary drug delivery technology (Alkermes, Inc., Cambridge, Mass.). In a specific embodiment, prophylactic or therapeutic agents of the invention are administered intramuscularly, intravenously, intratumorally, orally, intranasally, pulmonary, or subcutaneously. The prophylactic or therapeutic agents may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

In an embodiment, specific binding of antibody-coupled carbon nanotubes (CNTs) to tumor cells in vitro, followed by their highly specific ablation with near-infrared (NIR) light can be used to target tumor cells. For example, biotinylated polar lipids can be used to prepare stable, biocompatible, noncytotoxic CNT dispersions that are then attached to one or two different neutralite avidin-derivatized DVD-Igs directed against one or more tumor antigens (e.g., CD22) (Chakravarty, P. et al. (2008) Proc. Natl. Acad. Sci. USA 105:8697-8702.

In a specific embodiment, it may be desirable to administer the prophylactic or therapeutic agents of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, the implant being of a porous or non-porous material, including

membranes and matrices, such as sialastic membranes, polymers, fibrous matrices (e.g., Tissuel®), or collagen matrices. In one embodiment, an effective amount of one or more antibodies of the invention antagonists is administered locally to the affected area to a subject to prevent, treat, manage, and/or ameliorate a disorder or a symptom thereof. In another embodiment, an effective amount of one or more antibodies of the invention is administered locally to the affected area in combination with an effective amount of one or more therapies (e. g., one or more prophylactic or therapeutic agents) other than a binding protein of the invention of a subject to prevent, treat, manage, and/or ameliorate a disorder or one or more symptoms thereof.

In another embodiment, the prophylactic or therapeutic agent can be delivered in a controlled release or sustained release system. In one embodiment, a pump may be used to achieve controlled or sustained release (see Langer, supra; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:20; Buchwald et al., 1980, Surgery 88:507; Saudek et al., 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric materials can be used to achieve controlled or sustained release of the therapies of the invention (see e.g., Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, J., Macromol. Sci. Rev. Macromol. Chem. 23:61; see also Levy et al., 1985, Science 228:190; During et al., 1989, Ann. Neurol. 25:351; Howard et al., 1989, J. Neurosurg. 7 1:105); U.S. Pat. No. 5,679,377; U.S. Pat. No. 5, 916,597; U. S. Pat. No. 5,912,015; U.S. Pat. No. 5,989,463; U.S. Pat. No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253. Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N- vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In an embodiment, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. In yet another embodiment, a controlled or sustained release system can be placed in proximity of the prophylactic or therapeutic target, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

Controlled release systems are discussed in the review by Langer (1990, Science 249:1527-1533). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more therapeutic agents of the invention. See, e.g., U. S. Pat. No. 4,526, 938, PCT publication WO 91/05548, PCT publication WO 96/20698, Ning et al. , 1996, "Intratumoral Radioimmunotheraphy of a Human Colon Cancer Xenograft Using a Sustained-Release Gel," Radiotherapy &Oncology 39:179-189, Song et al., 1995, "Antibody Mediated Lung Targeting of Long- Circulating Emulsions," PDA Journal of Pharmaceutical Science &Technology 50:372-397, Cleek et al., 1997, "Biodegradable Polymeric Carriers for a bFGF Antibody for Cardiovascular Application," Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854, and Lam et al., 1997, "Microencapsulation of Recombinant Humanized Monoclonal Antibody for Local Delivery," Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759- 760.

In a specific embodiment, where the composition of the invention is a nucleic acid encoding a prophylactic or therapeutic agent, the nucleic acid can be administered in vivo to promote expression of its encoded prophylactic or therapeutic agent, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U. S. Pat. No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see, e.g., Joliot et al., 1991, Proc. Natl. Acad. Sci. USA 88:1864-1868). Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include, but are not limited to, parenteral, e.g., intravenous, intradermal, subcutaneous, oral, intranasal (e.g., inhalation), transdermal (e.g., topical), transmucosal, and rectal administration. In a specific embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous, subcutaneous, intramuscular, oral, intranasal, or topical administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic

aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocamne to ease pain at the site of the injection.

If the compositions of the invention are to be administered topically, the compositions can be formulated in the form of an ointment, cream, transdermal patch, lotion, gel, shampoo, spray, aerosol, solution, emulsion, or other form well-known to one of skill in the art. See, e.g., Remington's Pharmaceutical Sciences and Introduction to Pharmaceutical Dosage Forms, 19th ed., Mack Pub. Co., Easton, Pa. (1995). In an embodiment, for non- sprayable topical dosage forms, viscous to semi-solid or solid forms comprising a carrier or one or more excipients compatible with topical application and having a dynamic viscosity greater than water are employed. Suitable formulations include, without limitation, solutions, suspensions, emulsions, creams, ointments, powders, liniments, salves, and the like, which are, if desired, sterilized or mixed with auxiliary agents (e.g., preservatives, stabilizers, wetting agents, buffers, or salts) for influencing various properties, such as, for example, osmotic pressure. Other suitable topical dosage forms include sprayable aerosol preparations wherein the active ingredient, in an embodiment, in combination with a solid or liquid inert carrier, is packaged in a mixture with a pressurized volatile (e.g., a gaseous propellant, such as freon) or in a squeeze bottle. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well-known in the art.

If the method of the invention comprises intranasal administration of a composition, the composition can be formulated in an aerosol form, spray, mist or in the form of drops. In particular, prophylactic or therapeutic agents for use according to the present invention can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant (e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas). In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges (composed of, e.g., gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

If the method of the invention comprises oral administration, compositions can be formulated orally in the form of tablets, capsules, cachets, gelcaps, solutions, suspensions, and the like. Tablets or capsules can be prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone, or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose, or calcium hydrogen phosphate) ; lubricants (e.g., magnesium stearate, talc, or silica); disintegrants (e.g., potato starch or sodium starch glycolate) ; or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well-known in the art. Liquid preparations for oral administration may take the form of, but not limited to, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may 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, ethyl alcohol, or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p- hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring, and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated for slow release, controlled release, or sustained release of a prophylactic or therapeutic agent(s).

The method of the invention may comprise pulmonary administration, e.g., by use of an inhaler or nebulizer, of a composition formulated with an aerosolizing agent. See, e.g., U.S. Pat. Nos. 6,019,968; 5,985,320; 5,985,309; 5,934,272; 5,874,064; 5,855,913; 5,290,540; and 4,880,078; and PCT Publication Nos. WO 92/19244; WO 97/32572; WO 97/44013; WO 98/31346; and WO 99/66903. In a specific embodiment, a binding protein of the invention, combination therapy, and/or composition of the invention is administered using Alkermes AIR® pulmonary drug delivery technology (Alkermes, Inc., Cambridge, Mass.).

The method of the invention may comprise administration of a composition formulated for parenteral administration by injection (e. g., by bolus injection or continuous infusion). Formulations for injection may be presented in unit dosage form (e.g., in ampoules or in multi-dose containers) with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or

aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle (e.g., sterile pyrogen-free water) before use.

The methods of the invention may additionally comprise of administration of compositions formulated as depot preparations. Such long acting formulations may be administered by implantation (e.g., subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compositions may be formulated with suitable polymeric or hydrophobic materials (e.g., as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives (e.g., as a sparingly soluble salt).

The methods of the invention encompasse administration of compositions formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

Generally, the ingredients of compositions are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the mode of administration is infusion, composition can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the mode of administration is by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

In particular, the invention also provides that one or more of the prophylactic or therapeutic agents, or pharmaceutical compositions of the invention is packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of the agent. In one embodiment, one or more of the prophylactic or therapeutic agents, or pharmaceutical compositions of the invention is supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted (e.g., with water or saline) to the appropriate concentration for administration to a subject. In an embodiment, one or more of the prophylactic or

therapeutic agents or pharmaceutical compositions of the invention is supplied as a dry sterile lyophilized powder in a hermetically sealed container at a unit dosage of at least 5 mg, at least 10 mg, at least 15 mg, at least 25 mg, at least 35 mg, at least 45 mg, at least 50 mg, at least 75 mg, or at least 100 mg. The lyophilized prophylactic or therapeutic agents or pharmaceutical compositions of the invention should be stored at between 2° C. and 8° C. in its original container and the prophylactic or therapeutic agents, or pharmaceutical compositions of the invention should be administered within 1 week, e.g., within 5 days, within 72 hours, within 48 hours, within 24 hours, within 12 hours, within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, one or more of the prophylactic or therapeutic agents or pharmaceutical compositions of the invention is supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the agent. In an embodiment, the liquid form of the administered composition is supplied in a hermetically sealed container at least 0.25 mg/ml, at least 0.5 mg/ml, at least 1 mg/ml, at least 2.5 mg/ml, at least 5 mg/ml, at least 8 mg/ml, at least 10 mg/ml, at least 15 mg/kg, at least 25 mg/ml, at least 50 mg/ml, at least 75 mg/ml, at least 100 mg/ml, at least 150mg/ml, or at least 200 mg/ml. The liquid form should be stored at between 2° C. and 8° C. in its original container.

The binding proteins of the invention can be incorporated into a pharmaceutical composition suitable for parenteral administration. In an embodiment, the antibody or antibody-portions will be prepared as an injectable solution containing 0.1-250 mg/ml binding protein. The injectable solution can be composed of either a liquid or lyophilized dosage form in a flint or amber vial, ampule or pre-filled syringe. The buffer can be L-histidine (1-50 mM), optimally 5-10mM, at pH 5.0 to 7.0 (optimally pH 6.0). Other suitable buffers include but are not limited to, sodium succinate, sodium citrate, sodium phosphate or potassium phosphate. Sodium chloride can be used to modify the toxicity of the solution at a concentration of 0-300 mM (optimally 150 mM for a liquid dosage form). Cryoprotectants can be included for a lyophilized dosage form, principally 0-10% sucrose (optimally 0.5-1.0%). Other suitable cryoprotectants include trehalose and lactose. Bulking agents can be included for a lyophilized dosage form, principally 1-10% mannitol (optimally 2-4%). Stabilizers can be used in both liquid and lyophilized dosage forms, principally 1-50 mM L-Methionine (optimally 5-10 mM). Other suitable bulking agents include glycine, arginine, can be included as 0-0.05% polysorbate-80 (optimally 0.005-0.01%). Additional surfactants include but are not limited to polysorbate 20 and

BRIJ surfactants. The pharmaceutical composition comprising the binding proteins of the invention prepared as an injectable solution for parenteral administration, can further comprise an agent useful as an adjuvant, such as those used to increase the absorption, or dispersion of a therapeutic protein (e.g., antibody). A particularly useful adjuvant is hyaluronidase, such as Hylenex® (recombinant human hyaluronidase). Addition of hyaluronidase in the injectable solution improves human bioavailability following parenteral administration, particularly subcutaneous administration. It also allows for greater injection site volumes (i.e. greater than 1 ml) with less pain and discomfort, and minimum incidence of injection site reactions. (see WO2004078140, and US2006104968).

The compositions of this invention may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (*e.g.*, injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The form chosen depends on the intended mode of administration and therapeutic application. Typical compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies. The chosen mode of administration is parenteral (*e.g.*, intravenous, subcutaneous, intraperitoneal, intramuscular). In an embodiment, the antibody is administered by intravenous infusion or injection. In another embodiment, the antibody is administered by intramuscular or subcutaneous injection.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active compound (*i.e.*, antibody or antibody portion) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated herein, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated herein. In the case of sterile, lyophilized powders for the preparation of sterile injectable solutions, the methods of preparation are vacuum drying and spray-drying that yields a powder of the active ingredient plus any additional

desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including, in the composition, an agent that delays absorption, for example, monostearate salts and gelatin.

The binding proteins of the present invention can be administered by a variety of methods known in the art, although for many therapeutic applications, in an embodiment, the route/mode of administration is subcutaneous injection, intravenous injection or infusion. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, *e.g.*, *Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

In certain embodiments, a binding protein of the invention may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound of the invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

Supplementary active compounds can also be incorporated into the compositions. In certain embodiments, a binding protein of the invention is coformulated with and/or coadministered with one or more additional therapeutic agents that are useful for treating

disorders with binding protein of the invention. For example, a binding protein of the invention may be coformulated and/or coadministered with one or more additional antibodies that bind other targets (*e.g.*, antibodies that bind other cytokines or that bind cell surface molecules). Furthermore, one or more antibodies of the invention may be used in combination with two or more of the foregoing therapeutic agents. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

In certain embodiments, a binding protein is linked to a half-life extending vehicle known in the art. Such vehicles include, but are not limited to, the Fc domain, polyethylene glycol, and dextran. Such vehicles are described, e.g., in U.S. Application Serial No. 09/428,082 and published PCT Application No. WO 99/25044.

In a specific embodiment, nucleic acid sequences encoding a binding protein of the invention or another prophylactic or therapeutic agent of the invention are administered to treat, prevent, manage, or ameliorate a disorder or one or more symptoms thereof by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded antibody or prophylactic or therapeutic agent of the invention that mediates a prophylactic or therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. For general reviews of the methods of gene therapy, see Goldspiel et al., 1993, Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, Science 260:926- 932 (1993); and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217; May, 1993, TIBTECH 11(5):155-215. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley &Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990). Detailed description of various methods of gene therapy are disclosed in US20050042664.

The binding proteins of the invention are useful in treating various diseases wherein the targets that are recognized by the binding proteins are detrimental. Such diseases include, but are not limited to, rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, septic arthritis, Lyme arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, inflammatory bowel disease, insulin dependent diabetes mellitus, thyroiditis, asthma, allergic diseases, psoriasis, dermatitis scleroderma, graft versus host disease, organ transplant rejection, acute or chronic immune disease associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, Wegener's granulomatosis, Henoch-Schoenlein purpurea, microscopic vasculitis of the kidneys, chronic active hepatitis, uveitis, septic shock, toxic shock syndrome, sepsis syndrome, cachexia, infectious diseases, parasitic diseases, acquired immunodeficiency syndrome, acute transverse myelitis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary cirrhosis, hemolytic anemia, malignancies, heart failure, myocardial infarction, Addison's disease, sporadic, polyglandular deficiency type I and polyglandular deficiency type II, Schmidt's syndrome, adult (acute) respiratory distress syndrome, alopecia, alopecia areata, seronegative arthopathy, arthropathy, Reiter's disease, psoriatic arthropathy, ulcerative colitic arthropathy, enteropathic synovitis, chlamydia, yersinia and salmonella associated arthropathy, spondyloarthopathy, atheromatous disease/arteriosclerosis, atopic allergy, autoimmune bullous disease, pemphigus vulgaris, pemphigus foliaceus, pemphigoid, linear IgA disease, autoimmune haemolytic anaemia, Coombs positive haemolytic anaemia, acquired pernicious anaemia, juvenile pernicious anaemia, myalgic encephalitis/Royal Free Disease, chronic mucocutaneous candidiasis, giant cell arteritis, primary sclerosing hepatitis, cryptogenic autoimmune hepatitis, Acquired Immunodeficiency Disease Syndrome, Acquired Immunodeficiency Related Diseases, Hepatitis B, Hepatitis C, common varied immunodeficiency (common variable hypogammaglobulinaemia), dilated cardiomyopathy, female infertility, ovarian failure, premature ovarian failure, fibrotic lung disease, cryptogenic fibrosing alveolitis, post-inflammatory interstitial lung disease, interstitial pneumonitis, connective tissue disease associated interstitial lung disease, mixed connective tissue disease associated lung disease, systemic sclerosis associated interstitial lung disease, rheumatoid arthritis associated interstitial lung disease, systemic

lupus erythematosus associated lung disease, dermatomyositis/polymyositis associated lung disease, Sjögren's disease associated lung disease, ankylosing spondylitis associated lung disease, vasculitic diffuse lung disease, haemosiderosis associated lung disease, drug-induced interstitial lung disease, fibrosis, radiation fibrosis, bronchiolitis obliterans, chronic eosinophilic pneumonia, lymphocytic infiltrative lung disease, postinfectious interstitial lung disease, gouty arthritis, autoimmune hepatitis, type-1 autoimmune hepatitis (classical autoimmune or lupoid hepatitis), type-2 autoimmune hepatitis (anti-LKM antibody hepatitis), autoimmune mediated hypoglycaemia, type B insulin resistance with acanthosis nigricans, hypoparathyroidism, acute immune disease associated with organ transplantation, chronic immune disease associated with organ transplantation, osteoarthrosis, primary sclerosing cholangitis, psoriasis type 1, psoriasis type 2, idiopathic leucopaenia, autoimmune neutropaenia, renal disease NOS, glomerulonephritides, microscopic vasulitis of the kidneys, lyme disease, discoid lupus erythematosus, male infertility idiopathic or NOS, sperm autoimmunity, multiple sclerosis (all subtypes), sympathetic ophthalmia, pulmonary hypertension secondary to connective tissue disease, Goodpasture's syndrome, pulmonary manifestation of polyarteritis nodosa, acute rheumatic fever, rheumatoid spondylitis, Still's disease, systemic sclerosis, Sjörgren's syndrome, Takayasu's disease/arteritis, autoimmune thrombocytopaenia, idiopathic thrombocytopaenia, autoimmune thyroid disease, hyperthyroidism, goitrous autoimmune hypothyroidism (Hashimoto's disease), atrophic autoimmune hypothyroidism, primary myxoedema, phacogenic uveitis, primary vasculitis, vitiligo acute liver disease, chronic liver diseases, alcoholic cirrhosis, alcohol-induced liver injury, choleosatatis, idiosyncratic liver disease, Drug-Induced hepatitis, Non-alcoholic Steatohepatitis, allergy and asthma, group B streptococci (GBS) infection, mental disorders (*e.g.*, depression and schizophrenia), Th2 Type and Th1 Type mediated diseases, acute and chronic pain (different forms of pain), and cancers such as lung, breast, stomach, bladder, colon, pancreas, ovarian, prostate and rectal cancer and hematopoietic malignancies (leukemia and lymphoma), Abetalipoprotemia, Acrocyanosis, acute and chronic parasitic or infectious processes, acute leukemia, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), acute or chronic bacterial infection, acute pancreatitis, acute renal failure, adenocarcinomas, aerial ectopic beats, AIDS dementia complex, alcohol-induced hepatitis, allergic conjunctivitis, allergic contact dermatitis, allergic rhinitis, allograft rejection, alpha-l- antitrypsin deficiency,

amyotrophic lateral sclerosis, anemia, angina pectoris, anterior horn cell degeneration, anti cd3 therapy, antiphospholipid syndrome, anti-receptor hypersensitivity reactions, aordic and peripheral aneuryisms, aortic dissection, arterial hypertension, arteriosclerosis, arteriovenous fistula, ataxia, atrial fibrillation (sustained or paroxysmal), atrial flutter, atrioventricular block, B cell lymphoma, bone graft rejection, bone marrow transplant (BMT) rejection, bundle branch block, Burkitt's lymphoma, Burns, cardiac arrhythmias, cardiac stun syndrome, cardiac tumors, cardiomyopathy, cardiopulmonary bypass inflammation response, cartilage transplant rejection, cerebellar cortical degenerations, cerebellar disorders, chaotic or multifocal atrial tachycardia, chemotherapy associated disorders, chromic myelocytic leukemia (CML), chronic alcoholism, chronic inflammatory pathologies, chronic lymphocytic leukemia (CLL), chronic obstructive pulmonary disease (COPD), chronic salicylate intoxication, colorectal carcinoma, congestive heart failure, conjunctivitis, contact dermatitis, cor pulmonale, coronary artery disease, Creutzfeldt-Jakob disease, culture negative sepsis, cystic fibrosis, cytokine therapy associated disorders, Dementia pugilistica, demyelinating diseases, dengue hemorrhagic fever, dermatitis, dermatologic conditions, diabetes, diabetes mellitus, diabetic ateriosclerotic disease, Diffuse Lewy body disease, dilated congestive cardiomyopathy, disorders of the basal ganglia, Down's Syndrome in middle age, drug-induced movement disorders induced by drugs which block CNS dopamine receptors, drug sensitivity, eczema, encephalomyelitis, endocarditis, endocrinopathy, epiglottitis, epstein-barr virus infection, erythromelalgia, extrapyramidal and cerebellar disorders, familial hematophagocytic lymphohistiocytosis, fetal thymus implant rejection, Friedreich's ataxia, functional peripheral arterial disorders, fungal sepsis, gas gangrene, gastric ulcer, glomerular nephritis, graft rejection of any organ or tissue, gram negative sepsis, gram positive sepsis, granulomas due to intracellular organisms, hairy cell leukemia, Hallerrorden-Spatz disease, hashimoto's thyroiditis, hay fever, heart transplant rejection, hemachromatosis, hemodialysis, hemolytic uremic syndrome/thrombolytic thrombocytopenic purpura, hemorrhage, hepatitis (A), His bundle arrythmias, HIV infection/HIV neuropathy, Hodgkin's disease, hyperkinetic movement disorders, hypersensitity reactions, hypersensitivity pneumonitis, hypertension, hypokinetic movement disorders, hypothalamic-pituitary-adrenal axis evaluation, idiopathic Addison's disease, idiopathic pulmonary fibrosis, antibody mediated cytotoxicity, Asthenia, infantile spinal muscular atrophy, inflammation of the aorta, influenza a, ionizing radiation

exposure, iridocyclitis/uveitis/optic neuritis, ischemia- reperfusion injury, ischemic stroke, juvenile rheumatoid arthritis, juvenile spinal muscular atrophy, Kaposi's sarcoma, kidney transplant rejection, legionella, leishmaniasis, leprosy, lesions of the corticospinal system, lipedema, liver transplant rejection, lymphederma, malaria, malignamt Lymphoma, malignant histiocytosis, malignant melanoma, meningitis, meningococcemia, metabolic/idiopathic, migraine headache, mitochondrial multi.system disorder, mixed connective tissue disease, monoclonal gammopathy, multiple myeloma, multiple systems degenerations (Mencel Dejerine- Thomas Shi-Drager and Machado-Joseph), myasthenia gravis, mycobacterium avium intracellulare, mycobacterium tuberculosis, myelodyplastic syndrome, myocardial infarction, myocardial ischemic disorders, nasopharyngeal carcinoma, neonatal chronic lung disease, nephritis, nephrosis, neurodegenerative diseases, neurogenic I muscular atrophies , neutropenic fever, non- hodgkins lymphoma, occlusion of the abdominal aorta and its branches, occulsive arterial disorders, okt3 therapy, orchitis/epidydimitis, orchitis/vasectomy reversal procedures, organomegaly, osteoporosis, pancreas transplant rejection, pancreatic carcinoma, paraneoplastic syndrome/hypercalcemia of malignancy, parathyroid transplant rejection, pelvic inflammatory disease, perennial rhinitis, pericardial disease, peripheral atherlosclerotic disease, peripheral vascular disorders, peritonitis, pernicious anemia, pneumocystis carinii pneumonia, pneumonia, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes syndrome), post perfusion syndrome, post pump syndrome, post-MI cardiotomy syndrome, preeclampsia, Progressive supranucleo Palsy, primary pulmonary hypertension, radiation therapy, Raynaud's phenomenon and disease, Raynoud's disease, Refsum's disease, regular narrow QRS tachycardia, renovascular hypertension, reperfusion injury, restrictive cardiomyopathy, sarcomas, scleroderma, senile chorea, Senile Dementia of Lewy body type, seronegative arthropathies, shock, sickle cell anemia, skin allograft rejection, skin changes syndrome, small bowel transplant rejection, solid tumors, specific arrythmias, spinal ataxia, spinocerebellar degenerations, streptococcal myositis, structural lesions of the cerebellum, Subacute sclerosing panencephalitis, Syncope, syphilis of the cardiovascular system, systemic anaphalaxis, systemic inflammatory response syndrome, systemic onset juvenile rheumatoid arthritis, T-cell or FAB ALL, Telangiectasia, thromboangitis obliterans, thrombocytopenia, toxicity, transplants, trauma/hemorrhage, type III hypersensitivity reactions, type IV hypersensitivity, unstable angina, uremia,

urosepsis, urticaria, valvular heart diseases, varicose veins, ,vasculitis, venous diseases, venous thrombosis, ventricular fibrillation, viral and fungal infections, vital encephalitis/aseptic meningitis, vital-associated hemaphagocytic syndrome, Wernicke-Korsakoff syndrome, Wilson's disease, xenograft rejection of any organ or tissue. (see Peritt et al. PCT publication No. WO2002097048A2, Leonard et al., PCT publication No. WO9524918 A1, and Salfeld et al., PCT publication No. WO00/56772A1).

The DVD-Igs of the invention may also treat one or more of the following diseases: Acute coronary syndromes, Acute Idiopathic Polyneuritis, Acute Inflammatory Demyelinating Polyradiculoneuropathy, Acute ischemia, Adult Still's Disease, Alopecia areata, Anaphylaxis, Anti-Phospholipid Antibody Syndrome, Aplastic anemia, Arteriosclerosis, Atopic eczema, Atopic dermatitis, Autoimmune dermatitis, Autoimmune disorder associated with Streptococcus infection, Autoimmune hearingloss, Autoimmune Lymphoproliferative Syndrome (ALPS), Autoimmune myocarditis, autoimmune thrombocytopenia (AITP), Blepharitis, Bronchiectasis, Bullous pemphigoid, Cardiovascular Disease, Catastrophic Antiphospholipid Syndrome, Celiac Disease, Cervical Spondylosis, Chronic ischemia, Cicatricial pemphigoid, Clinically isolated Syndrome (CIS) with Risk for Multiple Sclerosis, Conjunctivitis, Childhood Onset Psychiatric Disorder, Chronic obstructive pulmonary disease (COPD), Dacryocystitis, dermatomyositis, Diabetic retinopathy, Diabetes mellitus, Disk herniation, Disk prolaps, Drug induced immune hemolytic anemia, Endocarditis, Endometriosis, endophthalmitis, , Episcleritis, Erythema multiforme, erythema multiforme major, Gestational pemphigoid, Guillain-Barré Syndrome (GBS), Hay Fever, Hughes Syndrome , Idiopathic Parkinson's Disease, idiopathic interstitial pneumonia, IgE-mediated Allergy, Immune hemolytic anemia, Inclusion Body Myositis, Infectious ocular inflammatory disease, Inflammatory demyelinating disease, Inflammatory heart disease, Inflammatory kidney disease, IPF/UIP, Iritis, Keratitis, Keratojuntivitis sicca, Kussmaul disease or Kussmaul-Meier Disease, Landry's Paralysis, Langerhan's Cell Histiocytosis, Livedo reticularis, Macular Degeneration, malignancies, Microscopic Polyangiitis, Morbus Bechterev, Motor Neuron Disorders, Mucous membrane pemphigoid, Multiple Organ failure, Myasthenia Gravis, Myelodysplastic Syndrome, Myocarditis, Nerve Root Disorders, Neuropathy, Non-A Non-B Hepatitis, Optic Neuritis, Osteolysis, Ovarian cancer, Pauciarticular JRA, peripheral artery occlusive disease (PAOD), peripheral vascular disease (PVD), peripheral artery disease (PAD), Phlebitis, Polyarteritis nodosa (or periarteritis nodosa),

Polychondritis, Polymyalgia Rheumatica, Poliosis, Polyarticular JRA, Polyendocrine Deficiency Syndrome, Polymyositis, polymyalgia rheumatica (PMR), Post-Pump Syndrome, primary parkinsonism, prostate and rectal cancer and hematopoietic malignancies (leukemia and lymphoma), Prostatitis, Pure red cell aplasia, Primary Adrenal Insufficiency, Recurrent Neuromyelitis Optica, Restenosis, Rheumatic heart disease, SAPHO (synovitis, acne, pustulosis, hyperostosis, and osteitis), Scleroderma, Secondary Amyloidosis, Shock lung, Scleritis, Sciatica, Secondary Adrenal Insufficiency, Silicone associated connective tissue disease, Sneddon-Wilkinson Dermatosis, spondilitis ankylosans, Stevens-Johnson Syndrome (SJS), Systemic inflammatory response syndrome, Temporal arteritis, toxoplasmic retinitis, toxic epidermal necrolysis, Transverse myelitis, TRAPS (Tumor Necrosis Factor Receptor, Type 1 allergic reaction, Type II Diabetes, Urticaria, Usual interstitial pneumonia (UIP), Vasculitis, Vernal conjunctivitis, viral retinitis, Vogt-Koyanagi-Harada syndrome (VKH syndrome), Wet macular degeneration, and Wound healing.

The binding proteins of the invention can be used to treat humans suffering from autoimmune diseases, in particular those associated with inflammation, including, rheumatoid arthritis, spondylitis, allergy, autoimmune diabetes, autoimmune uveitis. In an embodiment, the binding proteins of the invention or antigen-binding portions thereof, are used to treat rheumatoid arthritis, Crohn's disease, multiple sclerosis, insulin dependent diabetes mellitus and psoriasis.

In an embodiment, diseases that can be treated or diagnosed with the compositions and methods of the invention include, but are not limited to, primary and metastatic cancers, including carcinomas of breast, colon, rectum, lung, oropharynx, hypopharynx, esophagus, stomach, pancreas, liver, gallbladder and bile ducts, small intestine, urinary tract (including kidney, bladder and urothelium), female genital tract (including cervix, uterus, and ovaries as well as choriocarcinoma and gestational trophoblastic disease), male genital tract (including prostate, seminal vesicles, testes and germ cell tumors), endocrine glands (including the thyroid, adrenal, and pituitary glands), and skin, as well as hemangiomas, melanomas, sarcomas (including those arising from bone and soft tissues as well as Kaposi's sarcoma), tumors of the brain, nerves, eyes, and meninges (including astrocytomas, gliomas, glioblastomas, retinoblastomas, neuromas, neuroblastomas, Schwannomas, and meningiomas), solid tumors arising from

hematopoietic malignancies such as leukemias, and lymphomas (both Hodgkin's and non-Hodgkin's lymphomas).

In an embodiment, the antibodies of the invention or antigen-binding portions thereof, are used to treat cancer or in the prevention of metastases from the tumors described herein either when used alone or in combination with radiotherapy and/or other chemotherapeutic agents.

The antibodies of the invention, or antigen binding portions thereof, may be combined with agents that include but are not limited to, antineoplastic agents, radiotherapy, chemotherapy such as DNA alkylating agents, cisplatin, carboplatin, anti-tubulin agents, paclitaxel, docetaxel, taxol, doxorubicin, gemcitabine, gemzar, anthracyclines, adriamycin, topoisomerase I inhibitors, topoisomerase II inhibitors, 5-fluorouracil (5-FU), leucovorin, irinotecan, receptor tyrosine kinase inhibitors (e.g., erlotinib, gefitinib), COX-2 inhibitors (e.g., celecoxib), kinase inhibitors, and siRNAs.

Preferably, the binding proteins of the invention or antigen-binding portions thereof, are used to treat rheumatoid arthritis, juvenile arthritis, rheumatoid spondylitis, ankylosing spondylitis, osteoarthritis, gouty arthritis, psoriatic arthritis, psoriasis, allergy, multiple sclerosis, demyelinating diseases, autoimmune diabetes, systemic lupus erythematosus, nephrotic syndrome, osteoarthritis pain, arthritis pain, neuronal pain, septic shock, burn injury, trauma, endotoxic shock, gram negative sepsis, toxic shock syndrome, transplantation, graft versus host disease (GVHD), host versus graft disease (HVGD), headneck tumor, lung cancer, gastric cancer, prostate cancer, pancreatic cancer, cachexia secondary to malignancy, bacterial meningitis, cerebral malaria, AIDS, AIDS-related complex (ARC), Guillain Barre syndrome, infectious mononucleosis, viral lymphadenopathies, herpes virus infections, cytomegalovirus infection secondary to transplantation, fever and myalgias due to infection (such as influenza), cachexia secondary to infection (e.g., secondary to AIDS or ARC), adult respiratory distress syndrome, shock lung, chronic pulmonary inflammatory disease, pulmonary sarcoidosis, pulmonary fibrosis and silicosis, idiopathic inflammatory bowel disease, Crohn's disease and ulcerative colitis, ischemia of the heart, heart insufficiency (weakness of the heart muscle), inflammatory bone disorders, bone growth disease, bone resorption disease, hepatitis, alcoholic hepatitis, viral hepatitis, coagulation disturbances, burns, reperfusion

injury, keloid formation, scar tissue formation, pyrexia, allergic arthritis, hematological disorders, such as hemolytic anemias and thrombocytopenias, endocrinologic disorders, such as diabetes mellitus, Addison's disease, idiopathic hypoparathyroidism, chronic lymphocytic thyroiditis, disorders of reproduction, amenorrhoea, infertility, recurrent abortions, eclampsia, ocular disorders, age-related macular degeneration (AMD).

A binding protein of the invention also can be administered with one or more additional therapeutic agents useful in the treatment of various diseases.

A binding protein of the invention can be used alone or in combination to treat such diseases. It should be understood that the binding proteins can be used alone or in combination with an additional agent, e.g., a therapeutic agent, the additional agent being selected by the skilled artisan for its intended purpose. For example, the additional agent can be a therapeutic agent art-recognized as being useful to treat the disease or condition being treated by the antibody of the present invention. The additional agent also can be an agent that imparts a beneficial attribute to the therapeutic composition e.g., an agent which effects the viscosity of the composition.

It should further be understood that the combinations which are to be included within this invention are those combinations useful for their intended purpose. The agents set forth below are illustrative for purposes and not intended to be limited. The combinations, which are part of this invention, can be the antibodies of the present invention and at least one additional agent selected from the lists below. The combination can also include more than one additional agent, e.g., two or three additional agents if the combination is such that the formed composition can perform its intended function.

Combinations to treat autoimmune and inflammatory diseases are non-steroidal anti-inflammatory drug(s) also referred to as NSAIDS which include drugs like ibuprofen. Other combinations are corticosteroids including prednisolone; the well known side-effects of steroid use can be reduced or even eliminated by tapering the steroid dose required when treating patients in combination with the DVD Igs of this invention. Non-limiting examples of therapeutic agents for rheumatoid arthritis with which an antibody, or antibody portion, of the invention can be combined include the following: cytokine suppressive anti-inflammatory drug(s) (CSAIDs); antibodies to or

antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-15, IL-16, IL-18, IL-21, IL-23, interferons, EMAP-II, GM-CSF, FGF, and PDGF. Binding proteins of the invention, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80 (B7.1), CD86 (B7.2), CD90, CTLA or their ligands including CD154 (gp39 or CD40L).

Combinations of therapeutic agents may interfere at different points in the autoimmune and subsequent inflammatory cascade; examples include TNF antagonists like chimeric, humanized or human TNF antibodies, Adalimumab, (PCT Publication No. WO 97/29131), CA2 (Remicade™), CDP 571, and soluble p55 or p75 TNF receptors, derivatives, thereof, (p75TNFR1gG (Enbrel™) or p55TNFR1gG (Lenercept), and also TNFα converting enzyme (TACE) inhibitors; similarly IL-1 inhibitors (Interleukin-1-converting enzyme inhibitors, IL-1RA etc.) may be effective for the same reason. Other combinations include Interleukin 11. Yet another combination includes key players of the autoimmune response which may act parallel to, dependent on or in concert with IL-12 function; especially are IL-18 antagonists including IL-18 antibodies or soluble IL-18 receptors, or IL-18 binding proteins. It has been shown that IL-12 and IL-18 have overlapping but distinct functions and a combination of antagonists to both may be most effective. Yet another combination are non-depleting anti-CD4 inhibitors. Yet other combinations include antagonists of the co-stimulatory pathway CD80 (B7.1) or CD86 (B7.2) including antibodies, soluble receptors or antagonistic ligands.

The binding proteins of the invention may also be combined with agents, such as methotrexate, 6-MP, azathioprine sulphasalazine, mesalazine, olsalazine chloroquinine/hydroxychloroquine, pencillamine, aurothiomalate (intramuscular and oral), azathioprine, cochicine, corticosteroids (oral, inhaled and local injection), beta-2 adrenoreceptor agonists (salbutamol, terbutaline, salmeteral), xanthines (theophylline, aminophylline), cromoglycate, nedocromil, ketotifen, ipratropium and oxitropium, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adensosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signalling by proinflammatory cytokines such as TNF-αor IL-1 (e.g.,IRAK, NIK, IKK , p38 or MAP kinase inhibitors), IL-1β converting enzyme

inhibitors, TNFαconverting enzyme (TACE) inhibitors, T-cell signalling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g.,soluble p55 or p75 TNF receptors and the derivatives p75TNFRIgG (Enbrel™ and p55TNFRIgG (Lenercept)), sIL-1RI, sIL-1RII, sIL-6R), antiinflammatory cytokines (e.g., IL-4, IL-10, IL-11, IL-13 and TGFβ), celecoxib, folic acid, hydroxychloroquine sulfate, rofecoxib, etanercept, infliximab, naproxen, valdecoxib, sulfasalazine, methylprednisolone, meloxicam, methylprednisolone acetate, gold sodium thiomalate, aspirin, triamcinolone acetonide, propoxyphene napsylate/apap, folate, nabumetone, diclofenac, piroxicam, etodolac, diclofenac sodium, oxaprozin, oxycodone hcl, hydrocodone bitartrate/apap, diclofenac sodium/misoprostol, fentanyl, anakinra, human recombinant, tramadol hcl, salsalate, sulindac, cyanocobalamin/fa/pyridoxine, acetaminophen, alendronate sodium, prednisolone, morphine sulfate, lidocaine hydrochloride, indomethacin, glucosamine sulf/chondroitin, amitriptyline hcl, sulfadiazine, oxycodone hcl/acetaminophen, olopatadine hcl, misoprostol, naproxen sodium, omeprazole, cyclophosphamide, rituximab, IL-1 TRAP, MRA, CTLA4-IG, IL-18 BP, anti-IL-18, Anti-IL15, BIRB-796, SCIO-469, VX-702, AMG-548, VX-740, Roflumilast, IC-485, CDC-801, and Mesopram. Combinations include methotrexate or leflunomide and in moderate or severe rheumatoid arthritis cases, cyclosporine.

Nonlimiting additional agents which can also be used in combination with a binding protein to treat rheumatoid arthritis include, but are not limited to, the following: non-steroidal anti-inflammatory drug(s) (NSAIDs); cytokine suppressive anti-inflammatory drug(s) (CSAIDs); CDP-571/BAY-10-3356 (humanized anti-TNFα antibody; Celltech/Bayer); cA2/infliximab (chimeric anti-TNFα antibody; Centocor); 75 kdTNFR-IgG/etanercept (75 kD TNF receptor-IgG fusion protein; Immunex; see *e.g.*, *Arthritis & Rheumatism* (1994) Vol. 37, S295; *J. Invest. Med.* (1996) Vol. 44, 235A); 55 kdTNF-IgG (55 kD TNF receptor-IgG fusion protein; Hoffmann-LaRoche); IDEC-CE9.1/SB 210396 (non-depleting primatized anti-CD4 antibody; IDEC/SmithKline; see *e.g.*, *Arthritis & Rheumatism* (1995) Vol. 38, S185); DAB 486-IL-2 and/or DAB 389-IL-2 (IL-2 fusion proteins; Seragen; see *e.g.*, *Arthritis & Rheumatism* (1993) Vol. 36, 1223); Anti-Tac (humanized anti-IL-2Rα; Protein Design Labs/Roche); IL-4 (anti-inflammatory

cytokine; DNAX/Schering); IL-10 (SCH 52000; recombinant IL-10, anti-inflammatory cytokine; DNAX/Schering); IL-4; IL-10 and/or IL-4 agonists (*e.g.*, agonist antibodies); IL-1RA (IL-1 receptor antagonist; Synergen/Amgen); anakinra (Kineret®/Amgen); TNF-bp/s-TNF (soluble TNF binding protein; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S284; *Amer. J. Physiol. - Heart and Circulatory Physiology* (1995) Vol. 268, pp. 37-42); R973401 (phosphodiesterase Type IV inhibitor; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282); MK-966 (COX-2 Inhibitor; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S81); Iloprost (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S82); methotrexate; thalidomide (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282) and thalidomide-related drugs (*e.g.*, Celgen); leflunomide (anti-inflammatory and cytokine inhibitor; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S131; *Inflammation Research* (1996) Vol. 45, pp. 103-107); tranexamic acid (inhibitor of plasminogen activation; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S284); T-614 (cytokine inhibitor; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282); prostaglandin E1 (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282); Tenidap (non-steroidal anti-inflammatory drug; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S280); Naproxen (non-steroidal anti-inflammatory drug; see *e.g.*, *Neuro Report* (1996) Vol. 7, pp. 1209-1213); Meloxicam (non-steroidal anti-inflammatory drug); Ibuprofen (non-steroidal anti-inflammatory drug); Piroxicam (non-steroidal anti-inflammatory drug); Diclofenac (non-steroidal anti-inflammatory drug); Indomethacin (non-steroidal anti-inflammatory drug); Sulfasalazine (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S281); Azathioprine (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S281); ICE inhibitor (inhibitor of the enzyme interleukin-1β converting enzyme); zap-70 and/or lck inhibitor (inhibitor of the tyrosine kinase zap-70 or lck); VEGF inhibitor and/or VEGF-R inhibitor (inhibitors of vascular endothelial cell growth factor or vascular endothelial cell growth factor receptor; inhibitors of angiogenesis); corticosteroid anti-inflammatory drugs (*e.g.*, SB203580); TNF-convertase inhibitors; anti-IL-12 antibodies; anti-IL-18 antibodies; interleukin-11 (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S296); interleukin-13 (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S308); interleukin -17 inhibitors (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S120);

gold; penicillamine; chloroquine; chlorambucil; hydroxychloroquine; cyclosporine; cyclophosphamide; total lymphoid irradiation; anti-thymocyte globulin; anti-CD4 antibodies; CD5-toxins; orally-administered peptides and collagen; lobenzarit disodium; Cytokine Regulating Agents (CRAs) HP228 and HP466 (Houghten Pharmaceuticals, Inc.); ICAM-1 antisense phosphorothioate oligo-deoxynucleotides (ISIS 2302; Isis Pharmaceuticals, Inc.); soluble complement receptor 1 (TP10; T Cell Sciences, Inc.); prednisone; orgotein; glycosaminoglycan polysulphate; minocycline; anti-IL2R antibodies; marine and botanical lipids (fish and plant seed fatty acids; see *e.g.*, DeLuca *et al.* (1995) *Rheum. Dis. Clin. North Am.* 21:759-777); auranofin; phenylbutazone; meclofenamic acid; flufenamic acid; intravenous immune globulin; zileuton; azaribine; mycophenolic acid (RS-61443); tacrolimus (FK-506); sirolimus (rapamycin); amiprilose (therafectin); cladribine (2-chlorodeoxyadenosine); methotrexate; bcl-2 inhibitors (see Bruncko, Milan et al., Journal of Medicinal Chemistry (2007), 50(4), 641-662); antivirals and immune modulating agents.

In one embodiment, the binding protein or antigen-binding portion thereof, is administered in combination with one of the following agents for the treatment of rheumatoid arthritis: small molecule inhibitor of KDR, small molecule inhibitor of Tie-2; methotrexate; prednisone; celecoxib; folic acid; hydroxychloroquine sulfate; rofecoxib; etanercept; infliximab; leflunomide; naproxen; valdecoxib; sulfasalazine; methylprednisolone; ibuprofen; meloxicam; methylprednisolone acetate; gold sodium thiomalate; aspirin; azathioprine; triamcinolone acetonide; propxyphene napsylate/apap; folate; nabumetone; diclofenac; piroxicam; etodolac; diclofenac sodium; oxaprozin; oxycodone hcl; hydrocodone bitartrate/apap; diclofenac sodium/misoprostol; fentanyl; anakinra, human recombinant; tramadol hcl; salsalate; sulindac; cyanocobalamin/fa/pyridoxine; acetaminophen; alendronate sodium; prednisolone; morphine sulfate; lidocaine hydrochloride; indomethacin; glucosamine sulfate/chondroitin; cyclosporine; amitriptyline hcl; sulfadiazine; oxycodone hcl/acetaminophen; olopatadine hcl; misoprostol; naproxen sodium; omeprazole; mycophenolate mofetil; cyclophosphamide; rituximab; IL-1 TRAP; MRA; CTLA4-IG; IL-18 BP; IL-12/23; anti-IL 18; anti-IL 15; BIRB-796; SCIO-469; VX-702; AMG-548; VX-740; Roflumilast; IC-485; CDC-801; and mesopram.

Non-limiting examples of therapeutic agents for inflammatory bowel disease with which a binding protein of the invention can be combined include the following: budenoside; epidermal growth factor; corticosteroids; cyclosporin, sulfasalazine; aminosalicylates; 6-mercaptopurine; azathioprine; metronidazole; lipoxygenase inhibitors; mesalamine; olsalazine; balsalazide; antioxidants; thromboxane inhibitors; IL-1 receptor antagonists; anti-IL-1β mAbs; anti-IL-6 mAbs; growth factors; elastase inhibitors; pyridinyl-imidazole compounds; antibodies to or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-15, IL-16, IL-17, IL-18, EMAP-II, GM-CSF, FGF, and PDGF. Antibodies of the invention, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD90 or their ligands. The antibodies of the invention, or antigen binding portions thereof, may also be combined with agents, such as methotrexate, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signalling by proinflammatory cytokines such as TNFα or IL-1 (e.g.,IRAK, NIK, IKK, p38 or MAP kinase inhibitors), IL-1β converting enzyme inhibitors, TNFα converting enzyme inhibitors, T-cell signalling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g.,soluble p55 or p75 TNF receptors, sIL-1RI, sIL-1RII, sIL-6R) and antiinflammatory cytokines (e.g.,IL-4, IL-10, IL-11, IL-13 and TGFβ) and bcl-2 inhibitors.

Examples of therapeutic agents for Crohn's disease in which a binding protein can be combined include the following: TNF antagonists, for example, anti-TNF antibodies, Adalimumab (PCT Publication No. WO 97/29131; HUMIRA), CA2 (REMICADE), CDP 571, TNFR-Ig constructs, (p75TNFRIgG (ENBREL) and p55TNFRIgG (LENERCEPT)) inhibitors and PDE4 inhibitors. Antibodies of the invention, or antigen binding portions thereof, can be combined with corticosteroids, for example, budenoside and dexamethasone. Binding proteins of the invention or antigen binding portions thereof, may also be combined with agents such as sulfasalazine, 5-aminosalicylic acid and olsalazine, and agents which interfere with synthesis or action of proinflammatory

cytokines such as IL-1, for example, IL-1β converting enzyme inhibitors and IL-1ra. Antibodies of the invention or antigen binding portion thereof may also be used with T cell signaling inhibitors, for example, tyrosine kinase inhibitors 6-mercaptopurines. Binding proteins of the invention, or antigen binding portions thereof, can be combined with IL-11. Binding proteins of the invention, or antigen binding portions thereof, can be combined with mesalamine, prednisone, azathioprine, mercaptopurine, infliximab, methylprednisolone sodium succinate, diphenoxylate/atrop sulfate, loperamide hydrochloride, methotrexate, omeprazole, folate, ciprofloxacin/dextrose-water, hydrocodone bitartrate/apap, tetracycline hydrochloride, fluocinonide, metronidazole, thimerosal/boric acid, cholestyramine/sucrose, ciprofloxacin hydrochloride, hyoscyamine sulfate, meperidine hydrochloride, midazolam hydrochloride, oxycodone hcl/acetaminophen, promethazine hydrochloride, sodium phosphate, sulfamethoxazole/trimethoprim, celecoxib, polycarbophil, propoxyphene napsylate, hydrocortisone, multivitamins, balsalazide disodium, codeine phosphate/apap, colesevelam hcl, cyanocobalamin, folic acid, levofloxacin, methylprednisolone, natalizumab and interferon-gamma

Non-limiting examples of therapeutic agents for multiple sclerosis with which binding proteins of the invention can be combined include the following: corticosteroids; prednisolone; methylprednisolone; azathioprine; cyclophosphamide; cyclosporine; methotrexate; 4-aminopyridine; tizanidine; interferon-β1a (AVONEX; Biogen); interferon-β1b (BETASERON; Chiron/Berlex); interferon α-n3) (Interferon Sciences/Fujimoto), interferon-α (Alfa Wassermann/J&J), interferon β1A-IF (Serono/Inhale Therapeutics), Peginterferon α 2b (Enzon/Schering-Plough), Copolymer 1 (Cop-1; COPAXONE; Teva Pharmaceutical Industries, Inc.); hyperbaric oxygen; intravenous immunoglobulin; clabribine; antibodies to or antagonists of other human cytokines or growth factors and their receptors, for example, TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-23, IL-15, IL-16, IL-18, EMAP-II, GM-CSF, FGF, and PDGF. Binding proteins of the invention can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD19, CD20, CD25, CD28, CD30, CD40, CD45, CD69, CD80, CD86, CD90 or their ligands. Binding proteins of the invention, may also be combined with agents, such as methotrexate, cyclosporine, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids

such as prednisolone, phosphodiesterase inhibitors, adensosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signalling by proinflammatory cytokines such as TNFα or IL-1 (e.g.,IRAK, NIK, IKK, p38 or MAP kinase inhibitors), IL-1β converting enzyme inhibitors, TACE inhibitors, T-cell signaling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g.,soluble p55 or p75 TNF receptors, sIL-1RI, sIL-1RII, sIL-6R), antiinflammatory cytokines (e.g.,IL-4, IL-10, IL-13 and TGFβ) and bcl-2 inhibitors.

Examples of therapeutic agents for multiple sclerosis in which binding proteins of the invention can be combined tinclude interferon-β, for example, IFNβ1a and IFNβ1b; copaxone, corticosteroids, caspase inhibitors, for example inhibitors of caspase-1, IL-1 inhibitors, TNF inhibitors, and antibodies to CD40 ligand and CD80.

The binding proteins of the invention, may also be combined with agents, such as alemtuzumab, dronabinol, Unimed, daclizumab, mitoxantrone, xaliproden hydrochloride, fampridine, glatiramer acetate, natalizumab, sinnabidol, a-immunokine NNSO3, ABR-215062, AnergiX.MS, chemokine receptor antagonists, BBR-2778, calagualine, CPI-1189, LEM (liposome encapsulated mitoxantrone), THC.CBD (cannabinoid agonist) MBP-8298, mesopram (PDE4 inhibitor), MNA-715, anti-IL-6 receptor antibody, neurovax, pirfenidone allotrap 1258 (RDP-1258), sTNF-R1, talampanel, teriflunomide,TGF-beta2, tiplimotide, VLA-4 antagonists (for example, TR-14035, VLA4 Ultrahaler, Antegran-ELAN/Biogen), interferon gamma antagonists, IL-4 agonists.

Non-limiting examples of therapeutic agents for Angina with which binding proteins of the invention can be combined include the following: aspirin, nitroglycerin, isosorbide mononitrate, metoprolol succinate, atenolol, metoprolol tartrate, amlodipine besylate, diltiazem hydrochloride, isosorbide dinitrate, clopidogrel bisulfate, nifedipine, atorvastatin calcium, potassium chloride, furosemide, simvastatin, verapamil hcl, digoxin, propranolol hydrochloride, carvedilol, lisinopril, spironolactone, hydrochlorothiazide, enalapril maleate, nadolol, ramipril, enoxaparin sodium, heparin sodium, valsartan, sotalol hydrochloride, fenofibrate, ezetimibe, bumetanide, losartan potassium, lisinopril/hydrochlorothiazide, felodipine, captopril, bisoprolol fumarate.

Non-limiting examples of therapeutic agents for Ankylosing Spondylitis with which binding proteins of the invention can be combined include the following: ibuprofen, diclofenac and misoprostol, naproxen, meloxicam, indomethacin, diclofenac, celecoxib, rofecoxib, Sulfasalazine, Methotrexate, azathioprine, minocyclin, prednisone, etanercept, infliximab.

Non-limiting examples of therapeutic agents for Asthma with which binding proteins of the invention can be combined include the following: albuterol, salmeterol/fluticasone, montelukast sodium, fluticasone propionate, budesonide, prednisone, salmeterol xinafoate, levalbuterol hcl, albuterol sulfate/ipratropium, prednisolone sodium phosphate, triamcinolone acetonide, beclomethasone dipropionate, ipratropium bromide, azithromycin, pirbuterol acetate, prednisolone, theophylline anhydrous, methylprednisolone sodium succinate, clarithromycin, zafirlukast, formoterol fumarate, influenza virus vaccine, methylprednisolone, amoxicillin trihydrate, flunisolide, allergy injection, cromolyn sodium, fexofenadine hydrochloride, flunisolide/menthol, amoxicillin/clavulanate, levofloxacin, inhaler assist device, guaifenesin, dexamethasone sodium phosphate, moxifloxacin hcl, doxycycline hyclate, guaifenesin/d-methorphan, p-ephedrine/cod/chlorphenir, gatifloxacin, cetirizine hydrochloride, mometasone furoate, salmeterol xinafoate, benzonatate, cephalexin, pe/hydrocodone/chlorphenir, cetirizine hcl/pseudoephed, phenylephrine/cod/promethazine, codeine/promethazine, cefprozil, dexamethasone, guaifenesin/pseudoephedrine, chlorpheniramine/hydrocodone, nedocromil sodium, terbutaline sulfate, epinephrine, methylprednisolone, metaproterenol sulfate.

Non-limiting examples of therapeutic agents for COPD with which binding proteins of the invention can be combined include the following: albuterol sulfate/ipratropium, ipratropium bromide, salmeterol/fluticasone, albuterol, salmeterol xinafoate, fluticasone propionate, prednisone, theophylline anhydrous, methylprednisolone sodium succinate, montelukast sodium, budesonide, formoterol fumarate, triamcinolone acetonide, levofloxacin, guaifenesin, azithromycin, beclomethasone dipropionate, levalbuterol hcl, flunisolide, ceftriaxone sodium, amoxicillin trihydrate, gatifloxacin, zafirlukast, amoxicillin/clavulanate, flunisolide/menthol, chlorpheniramine/hydrocodone, metaproterenol sulfate, methylprednisolone, mometasone furoate, p-ephedrine/cod/chlorphenir, pirbuterol

acetate, p-ephedrine/loratadine, terbutaline sulfate, tiotropium bromide, (R,R)-formoterol, TgAAT, Cilomilast, Roflumilast.

Non-limiting examples of therapeutic agents for HCV with which binding proteins of the invention can be combined include the following: Interferon-alpha-2a, Interferon-alpha-2b, Interferon-alpha con1, Interferon-alpha-n1, Pegylated interferon-alpha-2a, Pegylated interferon-alpha-2b, ribavirin, Peginterferon alfa-2b + ribavirin, Ursodeoxycholic Acid, Glycyrrhizic Acid, Thymalfasin, Maxamine, VX-497 and any compounds that are used to treat HCV through intervention with the following targets: HCV polymerase, HCV protease, HCV helicase, HCV IRES (internal ribosome entry site).

Non-limiting examples of therapeutic agents for Idiopathic Pulmonary Fibrosis with which binding proteins of the invention can be combined include the following: prednisone, azathioprine, albuterol, colchicine, albuterol sulfate, digoxin, gamma interferon, methylprednisolone sod succ, lorazepam, furosemide, lisinopril, nitroglycerin, spironolactone, cyclophosphamide, ipratropium bromide, actinomycin d, alteplase, fluticasone propionate, levofloxacin, metaproterenol sulfate, morphine sulfate, oxycodone hcl, potassium chloride, triamcinolone acetonide, tacrolimus anhydrous, calcium, interferon-alpha, methotrexate, mycophenolate mofetil, Interferon-gamma-1β.

Non-limiting examples of therapeutic agents for Myocardial Infarction with which binding proteins of the invention can be combined include the following: aspirin, nitroglycerin, metoprolol tartrate, enoxaparin sodium, heparin sodium, clopidogrel bisulfate, carvedilol, atenolol, morphine sulfate, metoprolol succinate, warfarin sodium, lisinopril, isosorbide mononitrate, digoxin, furosemide, simvastatin, ramipril, tenecteplase, enalapril maleate, torsemide, retavase, losartan potassium, quinapril hcl/mag carb, bumetanide, alteplase, enalaprilat, amiodarone hydrochloride, tirofiban hcl m-hydrate, diltiazem hydrochloride, captopril, irbesartan, valsartan, propranolol hydrochloride, fosinopril sodium, lidocaine hydrochloride, eptifibatide, cefazolin sodium, atropine sulfate, aminocaproic acid, spironolactone, interferon, sotalol hydrochloride, potassium chloride, docusate sodium, dobutamine hcl, alprazolam, pravastatin sodium, atorvastatin calcium, midazolam hydrochloride, meperidine hydrochloride, isosorbide

dinitrate, epinephrine, dopamine hydrochloride, bivalirudin, rosuvastatin, ezetimibe/simvastatin, avasimibe, cariporide.

Non-limiting examples of therapeutic agents for Psoriasis with which binding proteins of the invention can be combined include the following: small molecule inhibitor of KDR, small molecule inhibitor of Tie-2, calcipotriene, clobetasol propionate, triamcinolone acetonide, halobetasol propionate, tazarotene, methotrexate, fluocinonide, betamethasone diprop augmented, fluocinolone acetonide, acitretin, tar shampoo, betamethasone valerate, mometasone furoate, ketoconazole, pramoxine/fluocinolone, hydrocortisone valerate, flurandrenolide, urea, betamethasone, clobetasol propionate/emoll, fluticasone propionate, azithromycin, hydrocortisone, moisturizing formula, folic acid, desonide, pimecrolimus, coal tar, diflorasone diacetate, etanercept folate, lactic acid, methoxsalen, hc/bismuth subgal/znox/resor, methylprednisolone acetate, prednisone, sunscreen, halcinonide, salicylic acid, anthralin, clocortolone pivalate, coal extract, coal tar/salicylic acid, coal tar/salicylic acid/sulfur, desoximetasone, diazepam, emollient, fluocinonide/emollient, mineral oil/castor oil/na lact, mineral oil/peanut oil, petroleum/isopropyl myristate, psoralen, salicylic acid, soap/tribromsalan, thimerosal/boric acid, celecoxib, infliximab, cyclosporine, alefacept, efalizumab, tacrolimus, pimecrolimus, PUVA, UVB, sulfasalazine.

Non-limiting examples of therapeutic agents for Psoriatic Arthritis with which binding proteins of the invention can be combined include the following: methotrexate, etanercept, rofecoxib, celecoxib, folic acid, sulfasalazine, naproxen, leflunomide, methylprednisolone acetate, indomethacin, hydroxychloroquine sulfate, prednisone, sulindac, betamethasone diprop augmented, infliximab, methotrexate, folate, triamcinolone acetonide, diclofenac, dimethylsulfoxide, piroxicam, diclofenac sodium, ketoprofen, meloxicam, methylprednisolone, nabumetone, tolmetin sodium, calcipotriene, cyclosporine, diclofenac sodium/misoprostol, fluocinonide, glucosamine sulfate, gold sodium thiomalate, hydrocodone bitartrate/apap, ibuprofen, risedronate sodium, sulfadiazine, thioguanine, valdecoxib, alefacept, efalizumab and bcl-2 inhibitors.

Non-limiting examples of therapeutic agents for Restenosis with which binding proteins of the invention can be combined include the following: sirolimus, paclitaxel, everolimus, tacrolimus, Zotarolimus, acetaminophen.

Non-limiting examples of therapeutic agents for Sciatica with which binding proteins of the invention can be combined include the following: hydrocodone bitartrate/apap, rofecoxib, cyclobenzaprine hcl, methylprednisolone, naproxen, ibuprofen, oxycodone hcl/acetaminophen, celecoxib, valdecoxib, methylprednisolone acetate, prednisone, codeine phosphate/apap, tramadol hcl/acetaminophen, metaxalone, meloxicam, methocarbamol, lidocaine hydrochloride, diclofenac sodium, gabapentin, dexamethasone, carisoprodol, ketorolac tromethamine, indomethacin, acetaminophen, diazepam, nabumetone, oxycodone hcl, tizanidine hcl, diclofenac sodium/misoprostol, propoxyphene napsylate/apap, asa/oxycod/oxycodone ter, ibuprofen/hydrocodone bit, tramadol hcl, etodolac, propoxyphene hcl, amitriptyline hcl, carisoprodol/codeine phos/asa, morphine sulfate, multivitamins, naproxen sodium, orphenadrine citrate, temazepam.

Examples of therapeutic agents for SLE (Lupus) in which binding proteins of the invention can be combined include the following: NSAIDS, for example, diclofenac, naproxen, ibuprofen, piroxicam, indomethacin; COX2 inhibitors, for example, Celecoxib, rofecoxib, valdecoxib; anti-malarials, for example, hydroxychloroquine; Steroids, for example, prednisone, prednisolone, budenoside, dexamethasone; Cytotoxics, for example, azathioprine, cyclophosphamide, mycophenolate mofetil, methotrexate; inhibitors of PDE4 or purine synthesis inhibitor, for example Cellcept. Binding proteins of the invention, may also be combined with agents such as sulfasalazine, 5-aminosalicylic acid, olsalazine, Imuran and agents which interfere with synthesis, production or action of proinflammatory cytokines such as IL-1, for example, caspase inhibitors like IL-1β converting enzyme inhibitors and IL-1ra. Binding proteins of the invention may also be used with T cell signaling inhibitors, for example, tyrosine kinase inhibitors; or molecules that target T cell activation molecules, for example, CTLA-4-IgG or anti-B7 family antibodies, anti-PD-1 family antibodies. Binding proteins of the invention, can be combined with IL-11 or anti-cytokine antibodies, for example, fonotolizumab (anti-IFNg antibody), or anti-receptor receptor antibodies, for example, anti-IL-6 receptor antibody and antibodies to B-cell surface molecules. Antibodies of the invention or antigen binding portion thereof may also be used with LJP 394 (abetimus), agents that deplete or inactivate B-cells, for example, Rituximab (anti-CD20 antibody), lymphostat-B (anti-BlyS antibody), TNF antagonists, for example, anti-TNF antibodies, Adalimumab (PCT

Publication No. WO 97/29131; HUMIRA), CA2 (REMICADE), CDP 571, TNFR-Ig constructs, (p75TNFRIgG (ENBREL) and p55TNFRIgG (LENERCEPT)) and bcl-2 inhibitors, because bcl-2 overexpression in transgenic mice has been demonstrated to cause a lupus like phenotype (see Marquina, Regina et al., Journal of Immunology (2004), 172(11), 7177-7185), therefore inhibition is expected to have therapeutic effects.

The pharmaceutical compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of a binding protein of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the binding protein may be determined by a person skilled in the art and may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the binding protein to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody, or antibody portion, are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Dosage regimens may be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the

limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of a binding protein of the invention is 0.1-20 mg/kg, for example, 1-10 mg/kg. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. 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 dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the methods of the invention described herein are obvious and may be made using suitable equivalents without departing from the scope of the invention or the embodiments disclosed herein. Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included for purposes of illustration only and are not intended to be limiting of the invention.

V. Diagnostics

The disclosure herein also provides diagnostic applications. This is further elucidated below.

I.Method of Assay

The present disclosure also provides a method for determining the presence, amount or concentration of an analyte (or a fragment thereof) in a test sample using at least one DVD-Ig as described herein. Any suitable assay as is known in the art can be used in the method. Examples include, but are not limited to, immunoassay, such as sandwich immunoassay (e.g., monoclonal, polyclonal and/or DVD-Ig sandwich immunoassays or any variation thereof (e.g., monoclonal/DVD-Ig, DVD-Ig/polyclonal, etc.), including radioisotope detection (radioimmunoassay (RIA)) and enzyme detection (enzyme immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA) (e.g., Quantikine ELISA assays, R&D Systems, Minneapolis, MN))), competitive inhibition

immunoassay (e.g., forward and reverse), fluorescence polarization immunoassay (FPIA), enzyme multiplied immunoassay technique (EMIT), bioluminescence resonance energy transfer (BRET), and homogeneous chemiluminescent assay, etc. In a SELDI-based immunoassay, a capture reagent that specifically binds an analyte (or a fragment thereof) of interest is attached to the surface of a mass spectrometry probe, such as a pre-activated protein chip array. The analyte (or a fragment thereof) is then specifically captured on the biochip, and the captured analyte (or a fragment thereof) is detected by mass spectrometry. Alternatively, the analyte (or a fragment thereof) can be eluted from the capture reagent and detected by traditional MALDI (matrix-assisted laser desorption/ionization) or by SELDI. A chemiluminescent microparticle immunoassay, in particular one employing the ARCHITECT® automated analyzer (Abbott Laboratories, Abbott Park, IL), is an example of a preferred immunoassay.

Methods well-known in the art for collecting, handling and processing urine, blood, serum and plasma, and other body fluids, are used in the practice of the present disclosure, for instance, when a DVD-Ig as described herein is employed as an immunodiagnostic reagent and/or in an analyte immunoassay kit. The test sample can comprise further moieties in addition to the analyte of interest, such as antibodies, antigens, haptens, hormones, drugs, enzymes, receptors, proteins, peptides, polypeptides, oligonucleotides and/or polynucleotides. For example, the sample can be a whole blood sample obtained from a subject. It can be necessary or desired that a test sample, particularly whole blood, be treated prior to immunoassay as described herein, e.g., with a pretreatment reagent. Even in cases where pretreatment is not necessary (e.g., most urine samples), pretreatment optionally can be done (e.g., as part of a regimen on a commercial platform).

The pretreatment reagent can be any reagent appropriate for use with the immunoassay and kits of the invention. The pretreatment optionally comprises: (a) one or more solvents (e.g., methanol and ethylene glycol) and optionally, salt, (b) one or more solvents and salt, and optionally, detergent, (c) detergent, or (d) detergent and salt. Pretreatment reagents are known in the art, and such pretreatment can be employed, e.g., as used for assays on Abbott TDx, AxSYM®, and ARCHITECT® analyzers (Abbott Laboratories, Abbott Park, IL), as described in the literature (see, e.g., Yatscoff et al., Abbott TDx Monoclonal Antibody Assay Evaluated for Measuring Cyclosporine in

Whole Blood, Clin. Chem. 36: 1969-1973 (1990), and Wallemacq et al., Evaluation of the New AxSYM Cyclosporine Assay: Comparison with TDx Monoclonal Whole Blood and EMIT Cyclosporine Assays, Clin. Chem. 45: 432-435 (1999)), and/or as commercially available. Additionally, pretreatment can be done as described in Abbott's U.S. Pat. No. 5,135,875, European Pat. Pub. No. 0 471 293, U.S Provisional Pat. App. 60/878,017, filed December 29, 2006, and U.S. Pat. App. Pub. No. 2008/0020401 (incorporated by reference in its entirety for its teachings regarding pretreatment). The pretreatment reagent can be a heterogeneous agent or a homogeneous agent.

With use of a heterogeneous pretreatment reagent, the pretreatment reagent precipitates analyte binding protein (e.g., protein that can bind to an analyte or a fragment thereof) present in the sample. Such a pretreatment step comprises removing any analyte binding protein by separating from the precipitated analyte binding protein the supernatant of the mixture formed by addition of the pretreatment agent to sample. In such an assay, the supernatant of the mixture absent any binding protein is used in the assay, proceeding directly to the antibody capture step.

With use of a homogeneous pretreatment reagent there is no such separation step. The entire mixture of test sample and pretreatment reagent are contacted with a labeled specific binding partner for analyte (or a fragment thereof), such as a labeled anti-analyte antibody (or an antigenically reactive fragment thereof). The pretreatment reagent employed for such an assay typically is diluted in the pretreated test sample mixture, either before or during capture by the first specific binding partner. Despite such dilution, a certain amount of the pretreatment reagent is still present (or remains) in the test sample mixture during capture. According to the invention, the labeled specific binding partner can be a DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof).

In a heterogeneous format, after the test sample is obtained from a subject, a first mixture is prepared. The mixture contains the test sample being assessed for an analyte (or a fragment thereof) and a first specific binding partner, wherein the first specific binding partner and any analyte contained in the test sample form a first specific binding partner-analyte complex. Preferably, the first specific binding partner is an anti-analyte antibody or a fragment thereof. The first specific binding partner can be a DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof) as described herein. The order in

which the test sample and the first specific binding partner are added to form the mixture is not critical. Preferably, the first specific binding partner is immobilized on a solid phase. The solid phase used in the immunoassay (for the first specific binding partner and, optionally, the second specific binding partner) can be any solid phase known in the art, such as, but not limited to, a magnetic particle, a bead, a test tube, a microtiter plate, a cuvette, a membrane, a scaffolding molecule, a film, a filter paper, a disc and a chip.

After the mixture containing the first specific binding partner-analyte complex is formed, any unbound analyte is removed from the complex using any technique known in the art. For example, the unbound analyte can be removed by washing. Desirably, however, the first specific binding partner is present in excess of any analyte present in the test sample, such that all analyte that is present in the test sample is bound by the first specific binding partner.

After any unbound analyte is removed, a second specific binding partner is added to the mixture to form a first specific binding partner-analyte-second specific binding partner complex. The second specific binding partner is preferably an anti-analyte antibody that binds to an epitope on analyte that differs from the epitope on analyte bound by the first specific binding partner. Moreover, also preferably, the second specific binding partner is labeled with or contains a detectable label as described above. The second specific binding partner can be a DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof) as described herein.

Any suitable detectable label as is known in the art can be used. For example, the detectable label can be a radioactive label (such as 3H, 125I, 35S, 14C, 32P, and 33P), an enzymatic label (such as horseradish peroxidase, alkaline peroxidase, glucose 6-phosphate dehydrogenase, and the like), a chemiluminescent label (such as acridinium esters, thioesters, or sulfonamides; luminol, isoluminol, phenanthridinium esters, and the like), a fluorescent label (such as fluorescein (e.g., 5-fluorescein, 6-carboxyfluorescein, 3'6-carboxyfluorescein, 5(6)-carboxyfluorescein, 6-hexachloro-fluorescein, 6-tetrachlorofluorescein, fluorescein isothiocyanate, and the like)), rhodamine, phycobiliproteins, R-phycoerythrin, quantum dots (e.g., zinc sulfide-capped cadmium selenide), a thermometric label, or an immuno-polymerase chain reaction label. An introduction to labels, labeling procedures and detection of labels is found in Polak and

Van Noorden, Introduction to Immunocytochemistry, 2nd ed., Springer Verlag, N.Y. (1997), and in Haugland, Handbook of Fluorescent Probes and Research Chemicals (1996), which is a combined handbook and catalogue published by Molecular Probes, Inc., Eugene, Oregon. A fluorescent label can be used in FPIA (see, e.g., U.S. Patent Nos. 5,593,896, 5,573,904, 5,496,925, 5,359,093, and 5,352,803, which are hereby incorporated by reference in their entireties). An acridinium compound can be used as a detectable label in a homogeneous or heterogeneous chemiluminescent assay (see, e.g., Adamczyk et al., Bioorg. Med. Chem. Lett. 16: 1324-1328 (2006); Adamczyk et al., Bioorg. Med. Chem. Lett. 4: 2313-2317 (2004); Adamczyk et al., Biorg. Med. Chem. Lett. 14: 3917-3921 (2004); and Adamczyk et al., Org. Lett. 5: 3779-3782 (2003)).

A preferred acridinium compound is an acridinium-9-carboxamide. Methods for preparing acridinium 9-carboxamides are described in Mattingly, J. Biolumin. Chemilumin. 6: 107-114 (1991); Adamczyk et al., J. Org. Chem. 63: 5636-5639 (1998); Adamczyk et al., Tetrahedron 55: 10899-10914 (1999); Adamczyk et al., Org. Lett. 1: 779-781 (1999); Adamczyk et al., Bioconjugate Chem. 11: 714-724 (2000); Mattingly et al., In Luminescence Biotechnology: Instruments and Applications; Dyke, K. V. Ed.; CRC Press: Boca Raton, pp. 77–105 (2002); Adamczyk et al., Org. Lett. 5: 3779-3782 (2003); and U.S. Pat. Nos. 5,468,646, 5,543,524 and 5,783,699 (each of which is incorporated herein by reference in its entirety for its teachings regarding same). Another preferred acridinium compound is an acridinium-9-carboxylate aryl ester. An example of an acridinium-9-carboxylate aryl ester is 10-methyl-9-(phenoxycarbonyl)acridinium fluorosulfonate (available from Cayman Chemical, Ann Arbor, MI). Methods for preparing acridinium 9-carboxylate aryl esters are described in McCapra et al., Photochem. Photobiol. 4: 1111-21 (1965); Razavi et al., Luminescence 15: 245-249 (2000); Razavi et al., Luminescence 15: 239-244 (2000); and U.S. Patent No. 5,241,070 (each of which is incorporated herein by reference in its entirety for its teachings regarding same). Further details regarding acridinium-9-carboxylate aryl ester and its use are set forth in US 2008-0248493.

Chemiluminescent assays (e.g., using acridinium as described above or other chemiluminescent agents) can be performed in accordance with the methods described in Adamczyk et al., Anal. Chim. Acta 579(1): 61-67 (2006). While any suitable assay format can be used, a microplate chemiluminometer (Mithras LB-940, Berthold

Technologies U.S.A., LLC, Oak Ridge, TN) enables the assay of multiple samples of small volumes rapidly.

The order in which the test sample and the specific binding partner(s) are added to form the mixture for chemiluminescent assay is not critical. If the first specific binding partner is detectably labeled with a chemiluminescent agent such as an acridinium compound, detectably labeled first specific binding partner-analyte complexes form. Alternatively, if a second specific binding partner is used and the second specific binding partner is detectably labeled with a chemiluminescent agent such as an acridinium compound, detectably labeled first specific binding partner-analyte-second specific binding partner complexes form. Any unbound specific binding partner, whether labeled or unlabeled, can be removed from the mixture using any technique known in the art, such as washing.

Hydrogen peroxide can be generated in situ in the mixture or provided or supplied to the mixture (e.g., the source of the hydrogen peroxide being one or more buffers or other solutions that are known to contain hydrogen peroxide) before, simultaneously with, or after the addition of an above-described acridinium compound. Hydrogen peroxide can be generated in situ in a number of ways such as would be apparent to one skilled in the art.

Upon the simultaneous or subsequent addition of at least one basic solution to the sample, a detectable signal, namely, a chemiluminescent signal, indicative of the presence of analyte is generated. The basic solution contains at least one base and has a pH greater than or equal to 10, preferably, greater than or equal to 12. Examples of basic solutions include, but are not limited to, sodium hydroxide, potassium hydroxide, calcium hydroxide, ammonium hydroxide, magnesium hydroxide, sodium carbonate, sodium bicarbonate, calcium hydroxide, calcium carbonate, and calcium bicarbonate. The amount of basic solution added to the sample depends on the concentration of the basic solution. Based on the concentration of the basic solution used, one skilled in the art can easily determine the amount of basic solution to add to the sample.

The chemiluminescent signal that is generated can be detected using routine techniques known to those skilled in the art. Based on the intensity of the signal generated, the amount of analyte in the sample can be quantified. Specifically, the

amount of analyte in the sample is proportional to the intensity of the signal generated. The amount of analyte present can be quantified by comparing the amount of light generated to a standard curve for analyte or by comparison to a reference standard. The standard curve can be generated using serial dilutions or solutions of known concentrations of analyte by mass spectroscopy, gravimetric methods, and other techniques known in the art. While the above is described with emphasis on use of an acridinium compound as the chemiluminescent agent, one of ordinary skill in the art can readily adapt this description for use of other chemiluminescent agents.

Analyte immunoassays generally can be conducted using any format known in the art, such as, but not limited to, a sandwich format. Specifically, in one immunoassay format, at least two antibodies are employed to separate and quantify analyte, such as human analyte, or a fragment thereof in a sample. More specifically, the at least two antibodies bind to different epitopes on an analyte (or a fragment thereof) forming an immune complex, which is referred to as a "sandwich." Generally, in the immunoassays one or more antibodies can be used to capture the analyte (or a fragment thereof) in the test sample (these antibodies are frequently referred to as a "capture" antibody or "capture" antibodies) and one or more antibodies can be used to bind a detectable (namely, quantifiable) label to the sandwich (these antibodies are frequently referred to as the "detection antibody," the "detection antibodies," the "conjugate," or the "conjugates"). Thus, in the context of a sandwich immunoassay format, a DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof) as described herein can be used as a capture antibody, a detection antibody, or both. For example, one DVD-Ig having a domain that can bind a first epitope on an analyte (or a fragment thereof) can be used as a capture antibody and/or another DVD-Ig having a domain that can bind a second epitope on an analyte (or a fragment thereof) can be used as a detection antibody. In this regard, a DVD-Ig having a first domain that can bind a first epitope on an analyte (or a fragment thereof) and a second domain that can bind a second epitope on an analyte (or a fragment thereof) can be used as a capture antibody and/or a detection antibody. Alternatively, one DVD-Ig having a first domain that can bind an epitope on a first analyte (or a fragment thereof) and a second domain that can bind an epitope on a second analyte (or a fragment thereof) can be used as a capture antibody and/or a detection antibody to detect, and optionally quantify, two or more analytes. In the event that an analyte can be present in a

sample in more than one form, such as a monomeric form and a dimeric/multimeric form, which can be homomeric or heteromeric, one DVD-Ig having a domain that can bind an epitope that is only exposed on the monomeric form and another DVD-Ig having a domain that can bind an epitope on a different part of a dimeric/multimeric form can be used as capture antibodies and/or detection antibodies, thereby enabling the detection, and optional quantification, of different forms of a given analyte. Furthermore, employing DVD-Igs with differential affinities within a single DVD-Ig and/or between DVD-Igs can provide an avidity advantage. In the context of immunoassays as described herein, it generally may be helpful or desired to incorporate one or more linkers within the structure of a DVD-Ig. When present, optimally the linker should be of sufficient length and structural flexibility to enable binding of an epitope by the inner domains as well as binding of another epitope by the outer domains. In this regard, if a DVD-Ig can bind two different analytes and one analyte is larger than the other, desirably the larger analyte is bound by the outer domains.

Generally speaking, a sample being tested for (for example, suspected of containing) analyte (or a fragment thereof) can be contacted with at least one capture antibody (or antibodies) and at least one detection antibody (which can be a second detection antibody or a third detection antibody or even a successively numbered antibody, e.g., as where the capture and/or detection antibody comprise multiple antibodies) either simultaneously or sequentially and in any order. For example, the test sample can be first contacted with at least one capture antibody and then (sequentially) with at least one detection antibody. Alternatively, the test sample can be first contacted with at least one detection antibody and then (sequentially) with at least one capture antibody. In yet another alternative, the test sample can be contacted simultaneously with a capture antibody and a detection antibody.

In the sandwich assay format, a sample suspected of containing analyte (or a fragment thereof) is first brought into contact with at least one first capture antibody under conditions that allow the formation of a first antibody/analyte complex. If more than one capture antibody is used, a first capture antibody/analyte complex comprising two or more capture antibodies is formed. In a sandwich assay, the antibodies, i.e., preferably, the at least one capture antibody, are used in molar excess amounts of the maximum amount of analyte (or a fragment thereof) expected in the test sample. For

example, from about 5 µg to about 1 mg of antibody per mL of buffer (e.g., microparticle coating buffer) can be used.

Competitive inhibition immunoassays, which are often used to measure small analytes because binding by only one antibody is required, comprise sequential and classic formats. In a sequential competitive inhibition immunoassay a capture antibody to an analyte of interest is coated onto a well of a microtiter plate or other solid support. When the sample containing the analyte of interest is added to the well, the analyte of interest binds to the capture antibody. After washing, a known amount of labeled (e.g., biotin or horseradish peroxidase (HRP)) analyte is added to the well. A substrate for an enzymatic label is necessary to generate a signal. An example of a suitable substrate for HRP is 3,3',5,5'-tetramethylbenzidine (TMB). After washing, the signal generated by the labeled analyte is measured and is inversely proportional to the amount of analyte in the sample. In a classic competitive inhibition immunoassay an antibody to an analyte of interest is coated onto a solid support (e.g., a well of a microtiter plate). However, unlike the sequential competitive inhibition immunoassay, the sample and the labeled analyte are added to the well at the same time. Any analyte in the sample competes with labeled analyte for binding to the capture antibody. After washing, the signal generated by the labeled analyte is measured and is inversely proportional to the amount of analyte in the sample.

Optionally, prior to contacting the test sample with the at least one capture antibody (for example, the first capture antibody), the at least one capture antibody can be bound to a solid support, which facilitates the separation of the first antibody/analyte (or a fragment thereof) complex from the test sample. The substrate to which the capture antibody is bound can be any suitable solid support or solid phase that facilitates separation of the capture antibody-analyte complex from the sample.

Examples include a well of a plate, such as a microtiter plate, a test tube, a porous gel (e.g., silica gel, agarose, dextran, or gelatin), a polymeric film (e.g., polyacrylamide), beads (e.g., polystyrene beads or magnetic beads), a strip of a filter/membrane (e.g., nitrocellulose or nylon), microparticles (e.g., latex particles, magnetizable microparticles (e.g., microparticles having ferric oxide or chromium oxide cores and homo- or hetero-polymeric coats and radii of about 1-10 microns). The substrate can comprise a suitable

porous material with a suitable surface affinity to bind antigens and sufficient porosity to allow access by detection antibodies. A microporous material is generally preferred, although a gelatinous material in a hydrated state can be used. Such porous substrates are preferably in the form of sheets having a thickness of about 0.01 to about 0.5 mm, preferably about 0.1 mm. While the pore size may vary quite a bit, preferably the pore size is from about 0.025 to about 15 microns, more preferably from about 0.15 to about 15 microns. The surface of such substrates can be activated by chemical processes that cause covalent linkage of an antibody to the substrate. Irreversible binding, generally by adsorption through hydrophobic forces, of the antigen or the antibody to the substrate results; alternatively, a chemical coupling agent or other means can be used to bind covalently the antibody to the substrate, provided that such binding does not interfere with the ability of the antibody to bind to analyte. Alternatively, the antibody can be bound with microparticles, which have been previously coated with streptavidin (e.g., DYNAL® Magnetic Beads, Invitrogen, Carlsbad, CA) or biotin (e.g., using Power-BindTM-SA-MP streptavidin-coated microparticles (Seradyn, Indianapolis, IN)) or anti-species-specific monoclonal antibodies. If necessary, the substrate can be derivatized to allow reactivity with various functional groups on the antibody. Such derivatization requires the use of certain coupling agents, examples of which include, but are not limited to, maleic anhydride, N-hydroxysuccinimide, and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide. If desired, one or more capture reagents, such as antibodies (or fragments thereof), each of which is specific for analyte(s) can be attached to solid phases in different physical or addressable locations (e.g., such as in a biochip configuration (see, e.g., U.S. Pat. No. 6,225,047; Int'l Pat. App. Pub. No. WO 99/51773; U.S. Pat. No. 6,329,209; Int'l Pat. App. Pub. No. WO 00/56934, and U.S. Pat. No. 5,242,828). If the capture reagent is attached to a mass spectrometry probe as the solid support, the amount of analyte bound to the probe can be detected by laser desorption ionization mass spectrometry. Alternatively, a single column can be packed with different beads, which are derivatized with the one or more capture reagents, thereby capturing the analyte in a single place (see, antibody-derivatized, bead-based technologies, e.g., the xMAP technology of Luminex (Austin, TX)).

After the test sample being assayed for analyte (or a fragment thereof) is brought into contact with the at least one capture antibody (for example, the first capture

antibody), the mixture is incubated in order to allow for the formation of a first antibody (or multiple antibody)-analyte (or a fragment thereof) complex. The incubation can be carried out at a pH of from about 4.5 to about 10.0, at a temperature of from about 2°C to about 45°C, and for a period from at least about one (1) minute to about eighteen (18) hours, preferably from about 1 to about 24 minutes, most preferably for about 4 to about 18 minutes. The immunoassay described herein can be conducted in one step (meaning the test sample, at least one capture antibody and at

least one detection antibody are all added sequentially or simultaneously to a reaction vessel) or in more than one step, such as two steps, three steps, etc.

After formation of the (first or multiple) capture antibody/analyte (or a fragment thereof) complex, the complex is then contacted with at least one detection antibody under conditions which allow for the formation of a (first or multiple) capture antibody/analyte (or a fragment thereof)/second detection antibody complex). While captioned for clarity as the "second" antibody (e.g., second detection antibody), in fact, where multiple antibodies are used for capture and/or detection, the at least one detection antibody can be the second, third, fourth, etc. antibodies used in the immunoassay. If the capture antibody/analyte (or a fragment thereof) complex is contacted with more than one detection antibody, then a (first or multiple) capture antibody/analyte (or a fragment thereof)/(multiple) detection antibody complex is formed. As with the capture antibody (e.g., the first capture antibody), when the at least one (e.g., second and any subsequent) detection antibody is brought into contact with the capture antibody/analyte (or a fragment thereof) complex, a period of incubation under conditions similar to those described above is required for the formation of the (first or multiple) capture antibody/analyte (or a fragment thereof)/(second or multiple) detection antibody complex. Preferably, at least one detection antibody contains a detectable label. The detectable label can be bound to the at least one detection antibody (e.g., the second detection antibody) prior to, simultaneously with, or after the formation of the (first or multiple) capture antibody/analyte (or a fragment thereof)/(second or multiple) detection antibody complex. Any detectable label known in the art can be used (see discussion above, including of the Polak and Van Noorden (1997) and Haugland (1996) references).

The detectable label can be bound to the antibodies either directly or through a coupling agent. An example of a coupling agent that can be used is EDAC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, hydrochloride), which is commercially available from Sigma-Aldrich, St. Louis, MO. Other coupling agents that can be used are known in the art. Methods for binding a detectable label to an antibody are known in the art. Additionally, many detectable labels can be purchased or synthesized that already contain end groups that facilitate the coupling of the detectable label to the antibody, such as CPSP-Acridinium Ester (i.e., 9-[N-tosyl-N-(3-carboxypropyl)]-10-(3-sulfopropyl)acridinium carboxamide) or SPSP-Acridinium Ester (i.e., N10-(3-sulfopropyl)-N-(3-sulfopropyl)-acridinium-9-carboxamide).

The (first or multiple) capture antibody/analyte/(second or multiple) detection antibody complex can be, but does not have to be, separated from the remainder of the test sample prior to quantification of the label. For example, if the at least one capture antibody (e.g., the first capture antibody) is bound to a solid support, such as a well or a bead, separation can be accomplished by removing the fluid (of the test sample) from contact with the solid support. Alternatively, if the at least first capture antibody is bound to a solid support, it can be simultaneously contacted with the analyte-containing sample and the at least one second detection antibody to form a first (multiple) antibody/analyte/second (multiple) antibody complex, followed by removal of the fluid (test sample) from contact with the solid support. If the at least one first capture antibody is not bound to a solid support, then the (first or multiple) capture antibody/analyte/(second or multiple) detection antibody complex does not have to be removed from the test sample for quantification of the amount of the label.

After formation of the labeled capture antibody/analyte/detection antibody complex (e.g., the first capture antibody/analyte/second detection antibody complex), the amount of label in the complex is quantified using techniques known in the art. For example, if an enzymatic label is used, the labeled complex is reacted with a substrate for the label that gives a quantifiable reaction such as the development of color. If the label is a radioactive label, the label is quantified using appropriate means, such as a scintillation counter. If the label is a fluorescent label, the label is quantified by stimulating the label with a light of one color (which is known as the "excitation wavelength") and detecting another color (which is known as the "emission wavelength")

that is emitted by the label in response to the stimulation. If the label is a chemiluminescent label, the label is quantified by detecting the light emitted either visually or by using luminometers, x-ray film, high speed photographic film, a CCD camera, etc. Once the amount of the label in the complex has been quantified, the concentration of analyte or a fragment thereof in the test sample is determined by appropriate means, such as by use of a standard curve that has been generated using serial dilutions of analyte or a fragment thereof of known concentration. Other than using serial dilutions of analyte or a fragment thereof, the standard curve can be generated gravimetrically, by mass spectroscopy and by other techniques known in the art.

In a chemiluminescent microparticle assay employing the ARCHITECT® analyzer, the conjugate diluent pH should be about 6.0 +/- 0.2, the microparticle coating buffer should be maintained at about room temperature (i.e., at from about 17 to about 27 ðC), the microparticle coating buffer pH should be about 6.5 +/- 0.2, and the microparticle diluent pH should be about 7.8 +/- 0.2. Solids preferably are less than about 0.2%, such as less than about 0.15%, less than about 0.14%, less than about 0.13%, less than about 0.12%, or less than about 0.11%, such as about 0.10%.

FPIAs are based on competitive binding immunoassay principles. A fluorescently labeled compound, when excited by a linearly polarized light, will emit fluorescence having a degree of polarization inversely proportional to its rate of rotation. When a fluorescently labeled tracer-antibody complex is excited by a linearly polarized light, the emitted light remains highly polarized because the fluorophore is constrained from rotating between the time light is absorbed and the time light is emitted. When a "free" tracer compound (i.e., a compound that is not bound to an antibody) is excited by linearly polarized light, its rotation is much faster than the corresponding tracer-antibody conjugate produced in a competitive binding immunoassay. FPIAs are advantageous over RIAs inasmuch as there are no radioactive substances requiring special handling and disposal. In addition, FPIAs are homogeneous assays that can be easily and rapidly performed.

In view of the above, a method of determining the presence, amount, or concentration of analyte (or a fragment thereof) in a test sample is provided. The method comprises assaying the test sample for an analyte (or a fragment thereof) by an assay (i)

employing (i') at least one of an antibody, a fragment of an antibody that can bind to an analyte, a variant of an antibody that can bind to an analyte, a fragment of a variant of an antibody that can bind to an analyte, and a DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof) that can bind to an analyte, and (ii') at least one detectable label and (ii) comprising comparing a signal generated by the detectable label as a direct or indirect indication of the presence, amount or concentration of analyte (or a fragment thereof) in the test sample to a signal generated as a direct or indirect indication of the presence, amount or concentration of analyte (or a fragment thereof) in a control or calibrator. The calibrator is optionally part of a series of calibrators, in which each of the calibrators differs from the other calibrators by the concentration of analyte.

The method can comprise (i) contacting the test sample with at least one first specific binding partner for analyte (or a fragment thereof) selected from the group consisting of an antibody, a fragment of an antibody that can bind to an analyte, a variant of an antibody that can bind to an analyte, a fragment of a variant of an antibody that can bind to an analyte, and a DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof) that can bind to an analyte so as to form a first specific binding partner/analyte (or fragment thereof) complex, (ii) contacting the first specific binding partner/analyte (or fragment thereof) complex with at least one second specific binding partner for analyte (or fragment thereof) selected from the group consisting of a detectably labeled anti-analyte antibody, a detectably labeled fragment of an anti-analyte antibody that can bind to analyte, a detectably labeled variant of an anti-analyte antibody that can bind to analyte, a detectably labeled fragment of a variant of an anti-analyte antibody that can bind to analyte, and a detectably labeled DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof) so as to form a first specific binding partner/analyte (or fragment thereof)/second specific binding partner complex, and (iii) determining the presence, amount or concentration of analyte in the test sample by detecting or measuring the signal generated by the detectable label in the first specific binding partner/analyte (or fragment thereof)/second specific binding partner complex formed in (ii). A method in which at least one first specific binding partner for analyte (or a fragment thereof) and/or at least one second specific binding partner for analyte (or a fragment thereof) is a DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof) as described herein can be preferred.

Alternatively, the method can comprise contacting the test sample with at least one first specific binding partner for analyte (or a fragment thereof) selected from the group consisting of an antibody, a fragment of an antibody that can bind to an analyte, a variant of an antibody that can bind to an analyte, a fragment of a variant of an antibody that can bind to an analyte, and a DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof) and simultaneously or sequentially, in either order, contacting the test sample with at least one second specific binding partner, which can compete with analyte (or a fragment thereof) for binding to the at least one first specific binding partner and which is selected from the group consisting of a detectably labeled analyte, a detectably labeled fragment of analyte that can bind to the first specific binding partner, a detectably labeled variant of analyte that can bind to the first specific binding partner, and a detectably labeled fragment of a variant of analyte that can bind to the first specific binding partner. Any analyte (or a fragment thereof) present in the test sample and the at least one second specific binding partner compete with each other to form a first specific binding partner/analyte (or fragment thereof) complex and a first specific binding partner/second specific binding partner complex, respectively. The method further comprises determining the presence, amount or concentration of analyte in the test sample by detecting or measuring the signal generated by the detectable label in the first specific binding partner/second specific binding partner complex formed in (ii), wherein the signal generated by the detectable label in the first specific binding partner/second specific binding partner complex is inversely proportional to the amount or concentration of analyte in the test sample.

The above methods can further comprise diagnosing, prognosticating, or assessing the efficacy of a therapeutic/prophylactic treatment of a patient from whom the test sample was obtained. If the method further comprises assessing the efficacy of a therapeutic/prophylactic treatment of the patient from whom the test sample was obtained, the method optionally further comprises modifying the therapeutic/prophylactic treatment of the patient as needed to improve efficacy. The method can be adapted for use in an automated system or a semi-automated system.

With regard to the methods of assay (and kit therefor), it may be possible to employ commercially available anti-analyte antibodies or methods for production of anti-analyte as described in the literature. Commercial supplies of various antibodies include,

but are not limited to, Santa Cruz Biotechnology Inc. (Santa Cruz, CA), GenWay Biotech, Inc. (San Diego, CA), and R&D Systems (RDS; Minneapolis, MN).

Generally, a predetermined level can be employed as a benchmark against which to assess results obtained upon assaying a test sample for analyte or a fragment thereof, e.g., for detecting disease or risk of disease. Generally, in making such a comparison, the predetermined level is obtained by running a particular assay a sufficient number of times and under appropriate conditions such that a linkage or association of analyte presence, amount or concentration with a particular stage or endpoint of a disease, disorder or condition or with particular clinical indicia can be made. Typically, the predetermined level is obtained with assays of reference subjects (or populations of subjects). The analyte measured can include fragments thereof, degradation products thereof, and/or enzymatic cleavage products thereof.

In particular, with respect to a predetermined level as employed for monitoring disease progression and/or treatment, the amount or concentration of analyte or a fragment thereof may be "unchanged," "favorable" (or "favorably altered"), or "unfavorable" (or "unfavorably altered"). "Elevated" or "increased" refers to an amount or a concentration in a test sample that is higher than a typical or normal level or range (e.g., predetermined level), or is higher than another reference level or range (e.g., earlier or baseline sample). The term "lowered" or "reduced" refers to an amount or a concentration in a test sample that is lower than a typical or normal level or range (e.g., predetermined level), or is lower than another reference level or range (e.g., earlier or baseline sample). The term "altered" refers to an amount or a concentration in a sample that is altered (increased or decreased) over a typical or normal level or range (e.g., predetermined level), or over another reference level or range (e.g., earlier or baseline sample).

The typical or normal level or range for analyte is defined in accordance with standard practice. Because the levels of analyte in some instances will be very low, a so-called altered level or alteration can be considered to have occurred when there is any net change as compared to the typical or normal level or range, or reference level or range, that cannot be explained by experimental error or sample variation. Thus, the level measured in a particular sample will be compared with the level or range of levels

determined in similar samples from a so-called normal subject. In this context, a "normal subject" is an individual with no detectable disease, for example, and a "normal" (sometimes termed "control") patient or population is/are one(s) that exhibit(s) no detectable disease, respectively, for example. Furthermore, given that analyte is not routinely found at a high level in the majority of the human population, a "normal subject" can be considered an individual with no substantial detectable increased or elevated amount or concentration of analyte, and a "normal" (sometimes termed "control") patient or population is/are one(s) that exhibit(s) no substantial detectable increased or elevated amount or concentration of analyte. An "apparently normal subject" is one in which analyte has not yet been or currently is being assessed. The level of an analyte is said to be "elevated" when the analyte is normally undetectable (e.g., the normal level is zero, or within a range of from about 25 to about 75 percentiles of normal populations), but is detected in a test sample, as well as when the analyte is present in the test sample at a higher than normal level. Thus, inter alia, the disclosure provides a method of screening for a subject having, or at risk of having, a particular disease, disorder, or condition. The method of assay can also involve the assay of other markers and the like.

Accordingly, the methods described herein also can be used to determine whether or not a subject has or is at risk of developing a given disease, disorder or condition. Specifically, such a method can comprise the steps of:

(a) determining the concentration or amount in a test sample from a subject of analyte (or a fragment thereof) (e.g., using the methods described herein, or methods known in the art); and

(b) comparing the concentration or amount of analyte (or a fragment thereof) determined in step (a) with a predetermined level, wherein, if the concentration or amount of analyte determined in step (a) is favorable with respect to a predetermined level, then the subject is determined not to have or be at risk for a given disease, disorder or condition. However, if the concentration or amount of analyte determined in step (a) is unfavorable with respect to the predetermined level, then the subject is determined to have or be at risk for a given disease, disorder or condition.

Additionally, provided herein is method of monitoring the progression of disease in a subject. Optimally the method comprising the steps of:

(a) determining the concentration or amount in a test sample from a subject of analyte;

(b) determining the concentration or amount in a later test sample from the subject of analyte; and

(c) comparing the concentration or amount of analyte as determined in step (b) with the concentration or amount of analyte determined in step (a), wherein if the concentration or amount determined in step (b) is unchanged or is unfavorable when compared to the concentration or amount of analyte determined in step (a), then the disease in the subject is determined to have continued, progressed or worsened. By comparison, if the concentration or amount of analyte as determined in step (b) is favorable when compared to the concentration or amount of analyte as determined in step (a), then the disease in the subject is determined to have discontinued, regressed or improved.

Optionally, the method further comprises comparing the concentration or amount of analyte as determined in step (b), for example, with a predetermined level. Further, optionally the method comprises treating the subject with one or more pharmaceutical compositions for a period of time if the comparison shows that the concentration or amount of analyte as determined in step (b), for example, is unfavorably altered with respect to the predetermined level.

Still further, the methods can be used to monitor treatment in a subject receiving treatment with one or more pharmaceutical compositions. Specifically, such methods involve providing a first test sample from a subject before the subject has been administered one or more pharmaceutical compositions. Next, the concentration or amount in a first test sample from a subject of analyte is determined (e.g., using the methods described herein or as known in the art). After the concentration or amount of analyte is determined, optionally the concentration or amount of analyte is then compared with a predetermined level. If the concentration or amount of analyte as determined in the first test sample is lower than the predetermined level, then the subject is not treated

with one or more pharmaceutical compositions. However, if the concentration or amount of analyte as determined in the first test sample is higher than the predetermined level, then the subject is treated with one or more pharmaceutical compositions for a period of time. The period of time that the subject is treated with the one or more pharmaceutical compositions can be determined by one skilled in the art (for example, the period of time can be from about seven (7) days to about two years, preferably from about fourteen (14) days to about one (1) year).

During the course of treatment with the one or more pharmaceutical compositions, second and subsequent test samples are then obtained from the subject. The number of test samples and the time in which said test samples are obtained from the subject are not critical. For example, a second test sample could be obtained seven (7) days after the subject is first administered the one or more pharmaceutical compositions, a third test sample could be obtained two (2) weeks after the subject is first administered the one or more pharmaceutical compositions, a fourth test sample could be obtained three (3) weeks after the subject is first administered the one or more pharmaceutical compositions, a fifth test sample could be obtained four (4) weeks after the subject is first administered the one or more pharmaceutical compositions, etc.

After each second or subsequent test sample is obtained from the subject, the concentration or amount of analyte is determined in the second or subsequent test sample is determined (e.g., using the methods described herein or as known in the art). The concentration or amount of analyte as determined in each of the second and subsequent test samples is then compared with the concentration or amount of analyte as determined in the first test sample (e.g., the test sample that was originally optionally compared to the predetermined level). If the concentration or amount of analyte as determined in step (c) is favorable when compared to the concentration or amount of analyte as determined in step (a), then the disease in the subject is determined to have discontinued, regressed or improved, and the subject should continue to be administered the one or pharmaceutical compositions of step (b). However, if the concentration or amount determined in step (c) is unchanged or is unfavorable when compared to the concentration or amount of analyte as determined in step (a), then the disease in the subject is determined to have continued, progressed or worsened, and the subject should be treated with a higher concentration of the one or more pharmaceutical compositions administered to the subject in step (b) or

the subject should be treated with one or more pharmaceutical compositions that are different from the one or more pharmaceutical compositions administered to the subject in step (b). Specifically, the subject can be treated with one or more pharmaceutical compositions that are different from the one or more pharmaceutical compositions that the subject had previously received to decrease or lower said subject's analyte level.

Generally, for assays in which repeat testing may be done (e.g., monitoring disease progression and/or response to treatment), a second or subsequent test sample is obtained at a period in time after the first test sample has been obtained from the subject. Specifically, a second test sample from the subject can be obtained minutes, hours, days, weeks or years after the first test sample has been obtained from the subject. For example, the second test sample can be obtained from the subject at a time period of about 1 minute, about 5 minutes, about 10 minutes, about 15 minutes, about 30 minutes, about 45 minutes, about 60 minutes, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10 weeks, about 11 weeks, about 12 weeks, about 13 weeks, about 14 weeks, about 15 weeks, about 16 weeks, about 17 weeks, about 18 weeks, about 19 weeks, about 20 weeks, about 21 weeks, about 22 weeks, about 23 weeks, about 24 weeks, about 25 weeks, about 26 weeks, about 27 weeks, about 28 weeks, about 29 weeks, about 30 weeks, about 31 weeks, about 32 weeks, about 33 weeks, about 34 weeks, about 35 weeks, about 36 weeks, about 37 weeks, about 38 weeks, about 39 weeks, about 40 weeks, about 41 weeks, about 42 weeks, about 43 weeks, about 44 weeks, about 45 weeks, about 46 weeks, about 47 weeks, about 48 weeks, about 49 weeks, about 50 weeks, about 51 weeks , about 52 weeks, about 1.5 years, about 2 years, about 2.5 years, about 3.0 years, about 3.5 years, about 4.0 years, about 4.5 years, about 5.0 years, about 5.5. years, about 6.0 years, about 6.5 years, about 7.0 years, about 7.5 years, about 8.0 years, about 8.5 years, about 9.0 years, about 9.5 years or about 10.0 years after the first test sample from the subject is obtained.

When used to monitor disease progression, the above assay can be used to monitor the progression of disease in subjects suffering from acute conditions. Acute conditions, also known as critical care conditions, refer to acute, life-threatening diseases or other critical medical conditions involving, for example, the cardiovascular system or excretory system. Typically, critical care conditions refer to those conditions requiring acute medical intervention in a hospital-based setting (including, but not limited to, the emergency room, intensive care unit, trauma center, or other emergent care setting) or administration by a paramedic or other field-based medical personnel. For critical care conditions, repeat monitoring is generally done within a shorter time frame, namely, minutes, hours or days (e.g., about 1 minute, about 5 minutes, about 10 minutes, about 15 minutes, about 30 minutes, about 45 minutes, about 60 minutes, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days or about 7 days), and the initial assay likewise is generally done within a shorter timeframe, e.g., about minutes, hours or days of the onset of the disease or condition.

The assays also can be used to monitor the progression of disease in subjects suffering from chronic or non-acute conditions. Non-critical care or, non-acute conditions, refers to conditions other than acute, life-threatening disease or other critical medical conditions involving, for example, the cardiovascular system and/or excretory system. Typically, non-acute conditions include those of longer-term or chronic duration. For non-acute conditions, repeat monitoring generally is done with a longer timeframe, e.g., hours, days, weeks, months or years (e.g., about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10 weeks, about 11 weeks, about 12 weeks, about 13 weeks,

about 14 weeks, about 15 weeks, about 16 weeks, about 17 weeks, about 18 weeks, about 19 weeks, about 20 weeks, about 21 weeks, about 22 weeks, about 23 weeks, about 24 weeks, about 25 weeks, about 26 weeks, about 27 weeks, about 28 weeks, about 29 weeks, about 30 weeks, about 31 weeks, about 32 weeks, about 33 weeks, about 34 weeks, about 35 weeks, about 36 weeks, about 37 weeks, about 38 weeks, about 39 weeks, about 40 weeks, about 41 weeks, about 42 weeks, about 43 weeks, about 44 weeks, about 45 weeks, about 46 weeks, about 47 weeks, about 48 weeks, about 49 weeks, about 50 weeks, about 51 weeks , about 52 weeks, about 1.5 years, about 2 years, about 2.5 years, about 3.0 years, about 3.5 years, about 4.0 years, about 4.5 years, about 5.0 years, about 5.5. years, about 6.0 years, about 6.5 years, about 7.0 years, about 7.5 years, about 8.0 years, about 8.5 years, about 9.0 years, about 9.5 years or about 10.0 years), and the initial assay likewise generally is done within a longer time frame, e.g., about hours, days, months or years of the onset of the disease or condition.

Furthermore, the above assays can be performed using a first test sample obtained from a subject where the first test sample is obtained from one source, such as urine, serum or plasma. Optionally, the above assays can then be repeated using a second test sample obtained from the subject where the second test sample is obtained from another source. For example, if the first test sample was obtained from urine, the second test sample can be obtained from serum or plasma. The results obtained from the assays using the first test sample and the second test sample can be compared. The comparison can be used to assess the status of a disease or condition in the subject.

Moreover, the present disclosure also relates to methods of determining whether a subject predisposed to or suffering from a given disease, disorder or condition will benefit from treatment. In particular, the disclosure relates to analyte companion diagnostic methods and products. Thus, the method of "monitoring the treatment of disease in a subject" as described herein further optimally also can encompass selecting or identifying candidates for therapy.

Thus, in particular embodiments, the disclosure also provides a method of determining whether a subject having, or at risk for, a given disease, disorder or condition is a candidate for therapy. Generally, the subject is one who has experienced some symptom of a given disease, disorder or condition or who has actually been diagnosed as

having, or being at risk for, a given disease, disorder or condition, and/or who demonstrates an unfavorable concentration or amount of analyte or a fragment thereof, as described herein.

The method optionally comprises an assay as described herein, where analyte is assessed before and following treatment of a subject with one or more pharmaceutical compositions (e.g., particularly with a pharmaceutical related to a mechanism of action involving analyte), with immunosuppressive therapy, or by immunoabsorption therapy, or where analyte is assessed following such treatment and the concentration or the amount of analyte is compared against a predetermined level. An unfavorable concentration of amount of analyte observed following treatment confirms that the subject will not benefit from receiving further or continued treatment, whereas a favorable concentration or amount of analyte observed following treatment confirms that the subject will benefit from receiving further or continued treatment. This confirmation assists with management of clinical studies, and provision of improved patient care.

It goes without saying that, while certain embodiments herein are advantageous when employed to assess a given disease, disorder or condition as discussed herein, the assays and kits can be employed to assess analyte in other diseases, disorders and conditions. The method of assay can also involve the assay of other markers and the like.

The method of assay also can be used to identify a compound that ameliorates a given disease, disorder or condition. For example, a cell that expresses analyte can be contacted with a candidate compound. The level of expression of analyte in the cell contacted with the compound can be compared to that in a control cell using the method of assay described herein.

II. Kit

A kit for assaying a test sample for the presence, amount or concentration of an analyte (or a fragment thereof) in a test sample is also provided. The kit comprises at least one component for assaying the test sample for the analyte (or a fragment thereof) and instructions for assaying the test sample for the analyte (or a fragment thereof). The at least one component for assaying the test sample for the analyte (or a fragment thereof)

can include a composition comprising an anti-analyte DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof), which is optionally immobilized on a solid phase.

The kit can comprise at least one component for assaying the test sample for an analyte by immunoassay, e.g., chemiluminescent microparticle immunoassay, and instructions for assaying the test sample for an analyte by immunoassay, e.g., chemiluminescent microparticle immunoassay. For example, the kit can comprise at least one specific binding partner for an analyte, such as an anti-analyte, monoclonal/polyclonal antibody (or a fragment thereof that can bind to the analyte, a variant thereof that can bind to the analyte, or a fragment of a variant that can bind to the analyte) or an anti-analyte DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof), either of which can be detectably labeled. Alternatively or additionally, the kit can comprise detectably labeled analyte (or a fragment thereof that can bind to an anti-analyte, monoclonal/polyclonal antibody or an anti-analyte DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof)), which can compete with any analyte in a test sample for binding to an anti-analyte, monoclonal/polyclonal antibody (or a fragment thereof that can bind to the analyte, a variant thereof that can bind to the analyte, or a fragment of a variant that can bind to the analyte) or an anti-analyte DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof), either of which can be immobilized on a solid support. The kit can comprise a calibrator or control, e.g., isolated or purified analyte. The kit can comprise at least one container (e.g., tube, microtiter plates or strips, which can be already coated with a first specific binding partner, for example) for conducting the assay, and/or a buffer, such as an assay buffer or a wash buffer, either one of which can be provided as a concentrated solution, a substrate solution for the detectable label (e.g., an enzymatic label), or a stop solution. Preferably, the kit comprises all components, i.e., reagents, standards, buffers, diluents, etc., which are necessary to perform the assay. The instructions can be in paper form or computer-readable form, such as a disk, CD, DVD, or the like.

Any antibodies, such as an anti-analyte antibody or an anti-analyte DVD-Ig, or tracer can incorporate a detectable label as described herein, such as a fluorophore, a radioactive moiety, an enzyme, a biotin/avidin label, a chromophore, a chemiluminescent label, or the like, or the kit can include reagents for carrying out detectable labeling. The

antibodies, calibrators and/or controls can be provided in separate containers or pre-dispensed into an appropriate assay format, for example, into microtiter plates.

Optionally, the kit includes quality control components (for example, sensitivity panels, calibrators, and positive controls). Preparation of quality control reagents is well-known in the art and is described on insert sheets for a variety of immunodiagnostic products. Sensitivity panel members optionally are used to establish assay performance characteristics, and further optionally are useful indicators of the integrity of the immunoassay kit reagents, and the standardization of assays.

The kit can also optionally include other reagents required to conduct a diagnostic assay or facilitate quality control evaluations, such as buffers, salts, enzymes, enzyme co-factors, enzyme substrates, detection reagents, and the like. Other components, such as buffers and solutions for the isolation and/or treatment of a test sample (e.g., pretreatment reagents), also can be included in the kit. The kit can additionally include one or more other controls. One or more of the components of the kit can be lyophilized, in which case the kit can further comprise reagents suitable for the reconstitution of the lyophilized components.

The various components of the kit optionally are provided in suitable containers as necessary, e.g., a microtiter plate. The kit can further include containers for holding or storing a sample (e.g., a container or cartridge for a urine sample). Where appropriate, the kit optionally also can contain reaction vessels, mixing vessels, and other components that facilitate the preparation of reagents or the test sample. The kit can also include one or more instruments for assisting with obtaining a test sample, such as a syringe, pipette, forceps, measured spoon, or the like.

If the detectable label is at least one acridinium compound, the kit can comprise at least one acridinium-9-carboxamide, at least one acridinium-9-carboxylate aryl ester, or any combination thereof. If the detectable label is at least one acridinium compound, the kit also can comprise a source of hydrogen peroxide, such as a buffer, a solution, and/or at least one basic solution. If desired, the kit can contain a solid phase, such as a magnetic particle, bead, test tube, microtiter plate, cuvette, membrane, scaffolding molecule, film, filter paper, disc or chip.

III. Adaptation of Kit and Method

The kit (or components thereof), as well as the method of determining the presence, amount or concentration of an analyte in a test sample by an assay, such as an immunoassay as described herein, can be adapted for use in a variety of automated and semi-automated systems (including those wherein the solid phase comprises a microparticle), as described, e.g., in U.S. Patent Nos. 5,089,424 and 5,006,309, and as commercially marketed, e.g., by Abbott Laboratories (Abbott Park, IL) as ARCHITECT®.

Some of the differences between an automated or semi-automated system as compared to a non-automated system (e.g., ELISA) include the substrate to which the first specific binding partner (e.g., an anti-analyte, monoclonal/polyclonal antibody (or a fragment thereof, a variant thereof, or a fragment of a variant thereof) or an anti-analyte DVD-Ig (or a fragment thereof, a variant thereof, or a fragment of a variant thereof) is attached; either way, sandwich formation and analyte reactivity can be impacted), and the length and timing of the capture, detection and/or any optional wash steps. Whereas a non-automated format, such as an ELISA, may require a relatively longer incubation time with sample and capture reagent (e.g., about 2 hours), an automated or semi-automated format (e.g., ARCHITECT®, Abbott Laboratories) may have a relatively shorter incubation time (e.g., approximately 18 minutes for ARCHITECT®). Similarly, whereas a non-automated format, such as an ELISA, may incubate a detection antibody, such as the conjugate reagent, for a relatively longer incubation time (e.g., about 2 hours), an automated or semi-automated format (e.g., ARCHITECT®) may have a relatively shorter incubation time (e.g., approximately 4 minutes for the ARCHITECT®).

Other platforms available from Abbott Laboratories include, but are not limited to, AxSYM®, IMx® (see, e.g., U.S. Pat. No. 5,294,404, which is hereby incorporated by reference in its entirety), PRISM®, EIA (bead), and Quantum™ II, as well as other platforms. Additionally, the assays, kits and kit components can be employed in other formats, for example, on electrochemical or other hand-held or point-of-care assay systems. The present disclosure is, for example, applicable to the commercial Abbott Point of Care (i-STAT®, Abbott Laboratories) electrochemical immunoassay system that performs sandwich immunoassays. Immunosensors and their methods of manufacture

and operation in single-use test devices are described, for example in, U.S. Patent No. 5,063,081, U.S. Pat. App. Pub. No. 2003/0170881, U.S. Pat. App. Pub. No. 2004/0018577, U.S. Pat. App. Pub. No. 2005/0054078, and U.S. Pat. App. Pub. No. 2006/0160164, which are incorporated in their entireties by reference for their teachings regarding same.

In particular, with regard to the adaptation of an analyte assay to the I-STAT® system, the following configuration is preferred. A microfabricated silicon chip is manufactured with a pair of gold amperometric working electrodes and a silver-silver chloride reference electrode. On one of the working electrodes, polystyrene beads (0.2 mm diameter) with immobilized anti-analyte, monoclonal/polyclonal antibody (or a fragment thereof, a variant thereof, or a fragment of a variant thereof) or anti-analyte DVD-Ig (or a fragment thereof, a variant thereof, or a fragment of a variant thereof), are adhered to a polymer coating of patterned polyvinyl alcohol over the electrode. This chip is assembled into an I-STAT® cartridge with a fluidics format suitable for immunoassay. On a portion of the wall of the sample-holding chamber of the cartridge there is a layer comprising a specific binding partner for an analyte, such as an anti-analyte, monoclonal/polyclonal antibody (or a fragment thereof, a variant thereof, or a fragment of a variant thereof that can bind the analyte) or an anti-analyte DVD-Ig (or a fragment thereof, a variant thereof, or a fragment of a variant thereof that can bind the analyte), either of which can be detectably labeled. Within the fluid pouch of the cartridge is an aqueous reagent that includes p-aminophenol phosphate.

In operation, a sample suspected of containing an analyte is added to the holding chamber of the test cartridge, and the cartridge is inserted into the I-STAT® reader. After the specific binding partner for an analyte has dissolved into the sample, a pump element within the cartridge forces the sample into a conduit containing the chip. Here it is oscillated to promote formation of the sandwich. In the penultimate step of the assay, fluid is forced out of the pouch and into the conduit to wash the sample off the chip and into a waste chamber. In the final step of the assay, the alkaline phosphatase label reacts with p-aminophenol phosphate to cleave the phosphate group and permit the liberated p-aminophenol to be electrochemically oxidized at the working electrode. Based on the measured current, the reader is able to calculate the amount of analyte in the sample by means of an embedded algorithm and factory-determined calibration curve.

It further goes without saying that the methods and kits as described herein necessarily encompass other reagents and methods for carrying out the immunoassay. For instance, encompassed are various buffers such as are known in the art and/or which can be readily prepared or optimized to be employed, e.g., for washing, as a conjugate diluent, microparticle diluent, and/or as a calibrator diluent. An exemplary conjugate diluent is ARCHITECT® conjugate diluent employed in certain kits (Abbott Laboratories, Abbott Park, IL) and containing 2-(N-morpholino)ethanesulfonic acid (MES), a salt, a protein blocker, an antimicrobial agent, and a detergent. An exemplary calibrator diluent is ARCHITECT® human calibrator diluent employed in certain kits (Abbott Laboratories, Abbott Park, IL), which comprises a buffer containing MES, other salt, a protein blocker, and an antimicrobial agent. Additionally, as described in U.S. Patent Application No. 61/142,048 filed December 31, 2008, improved signal generation may be obtained, e.g., in an I-Stat cartridge format, using a nucleic acid sequence linked to the signal antibody as a signal amplifier.

Examples

Example 1: Design, Construction, and Analysis of a DVD-Ig

Example 1.1: Assays Used to Identify and Characterize Parent Antibodies and DVD-Ig

The following assays are used throughout the Examples to identify and characterize parent antibodies and DVD-Ig unless otherwise stated.

Example 1.1.1: Assays Used To Determine Binding and Affinity of Parent Antibodies and DVD-Ig for Their Target Antigen(s)

Example 1.1.1.A: ELISA ASSAY

Enzyme Linked Immunosorbent Assays (ELISA) to screen for antibodies that bind a desired target antigen are performed as follows.

Method 1

ELISA plates (Corning Costar, Acton, MA) are coated with 50µL/well of 5µg/ml goat anti-mouse IgG Fc specific (Pierce # 31170, Rockford, IL) in Phosphate Buffered Saline (PBS) overnight at 4°C. Plates are washed once with PBS containing 0.05%

Tween-20. Plates are blocked by addition of 200 µL/well blocking solution diluted to 2% in PBS (BioRad #170-6404, Hercules, CA.) for 1 hour at room temperature. Plates are washed once after blocking with PBS containing 0.05% Tween-20.

Fifty microliters per well of, e.g., mouse sera, hybridoma supernatants, or antibody or DVD-Ig preparations diluted in PBS containing 0.1% Bovine Serum Albumin (BSA) (Sigma, St. Louis, MO) is added to the ELISA plate prepared as described above and incubated for 1 hour at room temperature. Wells are washed three times with PBS containing 0.05% Tween-20. Fifty microliters of biotinylated recombinant purified target antigen diluted to 100ng/mL in PBS containing 0.1% BSA is added to each well and incubated for 1 hour at room temperature. Plates are washed 3 times with PBS containing 0.05% Tween-20. Streptavidin HRP (Pierce # 21126, Rockland, IL) is diluted 1:20000 in PBS containing 0.1% BSA; 50 µL/well is added and the plates incubated for 1 hour at room temperature. Plates are washed 3 times with PBS containing 0.05% Tween-20. Fifty microliters of TMB solution (Sigma # T0440, St. Louis, MO) is added to each well and incubated for 10 minutes at room temperature. The reaction is stopped by addition of 1N sulphuric acid. Plates are read spectrophotometrically at a wavelength of 450 nm.

Method 2

Enzyme linked immunosorbent assays to screen for anti-PGE_2 antibodies or anti-PGE_2 containing DVD-Ig molecules that bind prostaglandin E_2 were performed as follows. ELISA plates (Costar 3369, Corning, NY) were coated with 50 µl of anti-host Fc IgG (Sigma, St. Louis, MO) at 2 µg/ml in PBS (Invitrogen Carlsbad, CA). Following an overnight incubation at 4°C, the plate was blocked with 200 µl Superblock (Pierce #37535, Rockford, IL). The IgG or DVD-Ig containing samples were diluted to 1 µg/ml in Assay Buffer (10% Superblock in PBS containing 0.05% Surfactamps (Pierce #37535, Rockford, IL) and incubated on the plate at 50 µl/well for 1 hour at room temperature. Following the incubation, plates were washed four times with TTBS (Tween-Tris Buffered Solution). For PGE2 binding, PGE2-biotinamide (Cayman Chemicals, Ann Arbor, MI) was diluted to 30 nM and serially diluted in Assay Buffer. The titration curve was added to each IgG or DVD-Ig sample at a volume of 50 µl/well and incubated for 1 hour at room temperature. The plates were washed as previously described and 50 µl/well of 1:5000 dilution of streptavidin polyhrp40 (Fitzgerald Industries, Concord, MA)

in Assay Buffer was added and incubated for 45 minutes at room temperature. A final wash step was performed and the plates were developed using a single step TMB system (Sigma #T8665, St. Louis, MO) and 2N H_2SO_4. Plates were read at 450 nm on a Molecular Devices Spectramax plate reader (Sunnyvale, CA). EC_{50} was determined using GraphPad Prism 5 (GraphPad Software, La Jolla, CA) (Figure 2).

Method 3

Alternatively, prostaglandin binding screen for antibodies or DVD-Ig molecules can be determined using a ^{3}H-PGE_2 ELISA. Plates were coated at 50 µl/well with 5 µg/ml of goat anti-human IgG (Fc) (Thermo Scientific # 31170, Hudson, NH) or goat anti-mouse IgG (Fc) (Thermo Scientific # 31125, Hudson, NH) in PBS and incubated overnight at 4 °C. The following day plates were flicked and blotted dry. Plates were then blocked with 200 µL/well of Superblock (Thermo Scientific # 37515, Hudson, NH), for 1 hour at room temperature. Plates were flicked and blotted dry. Monoclonal antibodies were diluted to 0.04 µg/ml in PBST (Abbott Bioresearch Center, Worcester, MA) / 10% Superblock and 50 uL of each antibody or DVD-Ig was added to each well of the pre-blocked ELISA plate at 2ng/well and incubated for 1 hour at room temperature. Wells were washed 3 times with PBS + 0.1% Tween-20. A serial 3 fold titration of ^{3}H-PGE_2 (Perkin Elmer # NET-428, Waltham, MA) was prepared in PBST + 10% Superblock. Fifty microliters of the ^{3}H-PGE2 solution was then added to each well of the plate and incubated for 1 hour at room temperature. Wells were washed 6 times with PBST + 10% Superblock and 50 µL of scintillation fluid (Perkin Elmer # 6013621, Waltham, MA) added to each well. Plates were read using the TopCount reader (Perkin Elmer, Waltham, MA) with a 5 minutes count delay. EC_{50} number was determined using GraphPad Prism 5 (GraphPad Software, La Jolla, CA).

Example 1.1.1.B: Competition ELISA

Competition enzyme linked immunosorbent assays to determine prostaglandin binding specificity for anti-PGE_2 antibodies or anti-PGE_2 containing DVD-Ig molecules that bind prostaglandin E_2 were performed as follows.

Method 1

ELISA plates (Costar 3369, Corning, NY) were coated with 50 µl/well of anti-host Fc IgG (Sigma, St. Louis, MO) at 2 µg/ml in PBS (Invitrogen, Carlsbad, CA). Following an overnight incubation at 4°C, the plate was blocked with 200 µl Superblock (Pierce #37535, Rockford, IL). The IgG samples were diluted to 6 µg/ml in Assay Buffer (10% Superblock in PBS containing 0.05% Surfactamps (Pierce #37535, Rockford, IL). For PGE2 binding, the PGE_2-biotinamide was diluted to 3 nM in Assay Buffer. A titration curve in Assay Buffer was prepared for the prostaglandins PGA_2 (Cayman Chemicals, Ann Arbor, MI), PGD_2 (Cayman Chemicals, Ann Arbor, MI) and PGE_2 (Cayman Chemicals, Ann Arbor, MI) starting at 300 nM by a 1:10 serial dilution. The reagents were added to tubes at a volume of 50 µl each/well and preincubated for 1 hour at room temperature. Following the preincubation, the mix was transferred to the blocked plates and allowed to incubate for 1 hour at room temperature. Next, the plates were washed four times with Tween 20-Tris buffered solution (TTBS). Streptavidin polyhrp40 in Assay Buffer (Fitzgerald Industries, Concord, MA) at a 1:5000 dilution was then added to the wells and incubated for 45 minutes at room temperature. A final wash step was performed and the plates were developed using a single step TMB system (Sigma #T8665, Sigma, St. Louis, MO) and 2N H_2SO_4. Plates were read at 450 nm on a Molecular Devices Spectramax plate reader (Sunnyvale, CA). Wells in which unlabeled prostaglandins competed with the PGE_2-biotinamide for binding resulted in a decrease of signal. IC_{50} number was determined using GraphPad Prism 5 (GraphPad Software, La Jolla, CA). The cross reactivity index was then calculated by IC_{50} of PGE_2/IC_{50} of other prostaglandin(s).

Method 2

Alternatively, target selectivity was determined using a ^{3}H-PGE_2 competitition ELISA. Plates were coated with 50 uL/well of 5 µg/ml of goat anti-human IgG (Fc) (Thermo Scientific # 31170, Hudson, NH) or goat anti-mouse IgG (Fc) (Thermo Scientific # 31125, Hudson, NH) in PBS and incubated overnight at 4 °C. The following day plates were flicked and blotted dry. Plates were then blocked with 200 µL/well of Superblock (Thermo Scientific # 37515, Hudson, NH), 1 hour at room temperature. Plates were flicked and blotted dry. Monoclonal antibodies were diluted to 0.04 µg/ml in PBST (Abbott Bioresearch Center, Worcester, MA) + 10% Superblock and 50 µL of each was added to each well (2ng/well) of the pre-blocked ELISA plate and incubated for 1 hour at

room temperature. Wells were washed 3 times with PBS + 0.1% Tween-20. ^{3}H-PGE_2 (Perkin Elmer # NET-428, Waltham, MA) was diluted in PBST + 10% Superblock to 6nM (2X stock). Each prostaglandin (Cayman Chemicals, Ann Arbor, MI)was prepared in PBST + 10% Superblock at various concentrations ranging from 2000 µM (2X stock) to 0.00004 µM (2X). Equal volumes of the ^{3}H-PGE2 solution and of each prostaglandin dilution were mixed. Fifty microliters of this mixture was then added to each well of the plate and incubated for 1 hour at room temperature. Wells were washed manually 6 times with PBST / 10% Superblock and 50 µL of scintillation fluid (Perkin Elmer # 6013621, Waltham, MA) added to each well. Plates were read using a TopCount reader (Perkin Elmer, Waltham, MA) with a 5 minutes count delay. IC_{50} number was determined using GraphPad Prism 5 (GraphPad Software, La Jolla, CA). The cross reactivity index was then calculated by IC_{50} of PGE_2/IC_{50} of other prostaglandin(s)

Example 1.1.1.C: Affinity Determination Using BIAcore Assay

The BIACORE assay (BIAcore, Inc, Piscataway, NJ) determines the affinity of antibodies or DVD-Ig with kinetic measurements of on-rate and off-rate constants. Binding of antibodies or DVD-Ig to a target antigen (for example, a purified recombinant target antigen) is determined by surface plasmon resonance-based measurements with a BIAcore® 3000 instrument (BIAcore® AB, Uppsala, Sweden) using running HBS-EP (10 mM HEPES [pH 7.4], 150 mM NaCl, 3 mM EDTA, and 0.005% surfactant P20) at 25° C. All chemicals are obtained from BIAcore® AB (Uppsala, Sweden) or otherwise from a different source as described in the text. For example, approximately 5000 RU of goat anti-mouse IgG, (Fcγ), fragment specific polyclonal antibody (Pierce Biotechnology Inc, Rockford, IL) diluted in 10 mM sodium acetate (pH 4.5) is directly immobilized across a CM5 research grade biosensor chip using a standard amine coupling kit according to manufacturer's instructions and procedures at 25 µg/ml. Unreacted moieties on the biosensor surface are blocked with ethanolamine. Modified carboxymethyl dextran surface in flowcell 2 and 4 is used as a reaction surface. Unmodified carboxymethyl dextran without goat anti-mouse IgG in flow cell 1 and 3 is used as the reference surface. For kinetic analysis, rate equations derived from the 1:1 Langmuir binding model are fitted simultaneously to association and dissociation phases of all eight injections (using global fit analysis) with the use of Biaevaluation 4.0.1 software. Purified antibodies or DVD-Ig are diluted in HEPES-buffered saline for capture across

goat anti-mouse IgG specific reaction surfaces. Antibodies to be captured as a ligand (25 μg/ml) are injected over reaction matrices at a flow rate of 5 μl/min. The association and dissociation rate constants, k_{on} ($M^{-1}s^{-1}$) and k_{off} (s^{-1}) are determined under a continuous flow rate of 25 μl/min. Rate constants are derived by making kinetic binding measurements at ten different antigen concentrations ranging from 10 – 200 nM or alternatively from 1.25 to 1000mM. The equilibrium dissociation constant (M) of the reaction between antibodies or DVD-Igs and the target antigen is then calculated from the kinetic rate constants by the following formula: $K_D = k_{off}/k_{on}$. Binding is recorded as a function of time and kinetic rate constants are calculated. In this assay, on-rates as fast as $10^6 M^{-1}s^{-1}$ and off-rates as slow as $10^{-6} s^{-1}$ can be measured.

Example 1.1.2: Assays Used To Determine the Functional Activity Of Parent Antibodies And DVD-Ig

Example 1.1.2.A: EP4 Bioassay

The ability of anti-PGE_2 antibodies and anti-PGE_2 containing DVD-Ig molecules to inhibit the cellular response of PGE_2 was determined in a Ca++ flux assay in HEK293 Gα16 cells stably transfected with human EP4 receptor. Cells were plated in black/clear poly-D-lysine plates, (Corning #3667, Corning, NY) and incubated with Ca++-sensitive dye (Molecular Devices) for 90 minutes. Stock PGE_2 (in 200 proof ethanol) was diluted with FLIPR buffer (containing 1X HBSS (Invitrogen, Carlsbad, CA), 20 mM HEPES (Invitrogen, Carlsbad, CA), 0.1% BSA (Sigma, St. Louis, MO) and 2.5 mM Probenecid (Sigma, St. Louis, MO)). Anti- PGE_2 antibodies, DVD-Ig molecules or isotype matched control antibodies were also pre-diluted in FLIPR buffer. 25 μl of PGE_2 or pre-incubated PGE_2/antibody mixture or pre-incubated PGE_2/DVD-Ig molecule mixture was added to the wells pre-plated with cells. A dose response of PGE2 was done by a serial titration of PGE_2 and was determined using FLIPR1 or Tetra (Molecular Devices). EC50 was determined using GraphPad Prism 5 (GraftPad Software, La Jolla, CA). For testing antibodies and DVD-Ig molecules, PGE_2 at EC50 concentration was incubated with varying concentrations of test articles or isotype matched antibody (negative control) for 20 minutes, added to dye-loaded human EP4 in HEK293 Gα16 cells. Ca++ flux was monitored using FLIPR1 and data was analyzed using GraphPad Prism 5.

Example 1.1.2.B: Competitive Inhibition of PGE_2 binding to PGE_2 receptors by Anti Prostaglandin E_2 Antibodies Using ^{3}H-PGE_2

Competitive inhibition of PGE_2 binding to PGE_2 receptors, for example EP4 or EP3, by an anti-PGE_2 antibody are determined using a cell-based or membrane based receptor binding assay using ^{3}H-PGE_2 (ProstaglandinE2, [5,6,8,11,12,14,15-3H(N)], Perkin Elmer, Waltham, MA. Cat# NET428250UC).

Cells endogenously expressing or stably overexpressing EP4 receptor (i.e., HEK293-EP4 cells or HEK293-EP4-Gα16 cells used for EP4 bioassay) (10^5 cells/mL) are grown overnight in a 24-well plate in DMEM medium (Invitrogen, Carlsbad, CA) / 10% FCS (Sigma #T8665, Sigma, St. Louis, MO). The medium is removed and 100 µl binding buffer (medium without FCS) is added. The plate is placed on ice for 10 minutes. Non-radioactive PGE_2 (0-1 µM) is added together with tracer (40 pM of ^{3}H-PGE_2) in 100 µl volume. Equilibrium receptor binding is performed for 90 minutes at 4°C. The medium is removed and the cells are washed four times with 200 µl cold medium. The cells are harvested by adding 20 µl 0.5 M NaOH. The lysate is transferred to a liquid scintillation plate. 100 µl Aquasafe 500 (Zinsser Analytic, Frankfurt, Germany) plus LSC cocktail (Lumac LSC, Groningen, The Netherlands) is added to each well and mixed. The cell-bound radioactivity is determined by liquid scintillation counting. For most agonist-receptor interactions, it is assumed that receptor binding inhibition by agonist (PGE_2) follows a one-site model. The EC_{50}, K_i and K_d values are calculated using the GraphPad Prism 5 (GraphPad Software, La Jolla, CA).

Inhibition of an anti-PGE_2 antibody on the binding of ^{3}H-PGE_2 (ProstaglandinE2, [5,6,8,11,12,14,15-3H(N)], Perkin Elmer, Watham, MA. Cat# NET428250UC) to the EP3 receptor was performed using membrane preparations from cells that over-express the EP3 receptor (Millipore, Billerica, MA). Before the binding assay, 50 µl/well of 0.3% polyethyleneimine (PEI) (Sigma, St. Louis, MO) was added to a Unifilter-96 GF/B filter plate (Perkin Elmer, Watham, MA) and placed at 4°C for one hour until ready for use. A 1:3 dilution of antibody was prepared at 2X concentration in binding buffer (50 mM HEPES pH 7.0, 10 mM $MgCl_2$, 1mM EDTA, 0.2% BSA). ^{3}H-PGE_2 was also prepared at 2X concentration in binding buffer. 50 µl of a serial dilution of antibody was then added to each well containing 50 µl of 200 pM ^{3}H-PGE_2, mixed well and allowed to sit at room

temperature for 10 minutes. Frozen membranes were thawed and resuspended in binding buffer. 5µg of membrane was added to each well. Mixtures were incubated at room temperature for 60 minutes before filtering onto pretreated GF/B filtration plates using a Packard 96 well harvester. Plates were then dried for one hour before adding Microscint^{TM}20 (Perkin Elmer, Waltham, MA). Plates were then sealed and counted on the TopCount reader (Perkin Elmer, Waltham, MA). Non-specific binding was determined in the presence of 100 µM cold PGE_2. The measured radioactivity (cpm) was used to determine IC_{50} values using Graphpad Prism (GraphPad Software, La Jolla, CA).

Example 1.1.2.C: L929 Assay

The ability of anti-TNFα antibodies and anti-TNFα DVD-Ig molecules to inhibit the TNFα induced death of L929 cells (ATCC #CCL-1) was analyzed as follows. L929 cells were harvested and resuspended at 1 x 10^6 cells/ml in RPMI assay medium containing 4 µg/ml actinomycin. The cells were seeded on day one at 50 µl/well in a 96-well plate (Costar) at a final concentration of 5 x 10^4 cells/well. The DVD-Ig molecules were prepared for testing by serial dilution in RPMI assay medium without actinomycin. The diluted samples were then added to the 96-well plate at a volume of 50 µl/well. The murine TNFα (A-846899.0) was serially diluted to generate a standard curve and 50 µl was added to the standard curve wells. The final concentrations of the curve ranged 3 ng/ml – 0.0014 ng/ml. For neutralization, the murine TNFα was added to the DVD sample wells at 50 µl for a final concentration of 100 pg/ml. The wells were brought to a final volume of 200 µl/well and incubated for 18 hours at 37°C, 5% $C0_2$. After incubation, the plates were centrifuged at 1200 rpm and 100 µl removed from the wells. WST-1 (Roche) was added to the cells at 10 µl/well and the plates were incubated at 37°C, 5% $C0_2$ for 4 hours. The plates were centrifuged at 1200 rpm and 75 µl removed from the wells and transferred to an ELISA plate (Costar 3369) and read at 420 – 600 nm on a Molecular Devices Spectramax plate reader. Neutralizing DVDs protected the cells from death, resulting in a bright orange color.

Example 1.1.2.D: Cytokine Bioassay

The ability of an anti-cytokine parent antibody or DVD-Ig containing anti-cytokine sequences to inhibit or neutralize a target cytokine bioactivity is analyzed by determinating inhibitory potential of the antibody or DVD-Ig. For example, the ability of

an anti-IL-4 antibody to inhibit IL-4 mediated IgE production may be used. For example, human naive B cells are isolated from peripheral blood, respectively, buffy coats by Ficoll-paque density centrifugation, followed by magnetic separation with MACS beads (Miltenyi Biotech) specific for human sIgD FITC labeled goat F(ab)2 antibodies followed by anti-FITC MACS beads. Magnetically sorted naive B cells are adjusted to 3 x 10^5 cells per ml in XV15 and plated out in 100 µl per well of 96-well plates in a 6 x 6 array in the center of the plate, surrounded by PBS filled wells during the 10 days of culture at 37° C in the presence of 5% CO_2. One plate each is prepared per antibody to be tested, consisting of 3 wells each of un-induced and induced controls and quintuplicate repeats of antibody titrations starting at 7 µg/ml and running in 3-fold dilution down to 29 ng/ml final concentrations added in 50µl four times concentrated pre-dilution. To induce IgE production, rhIL-4 at 20 ng/ml plus anti-CD40 monoclonal antibody (Novartis) at 0.5 µg/ml final concentrations in 50 µl each are added to each well, and IgE concentrations are determined at the end of the culture period by a standard sandwich ELISA method.

Example 1.1.2.E: Cytokine Release Assay

The ability of a parent antibody or DVD-Ig to cause cytokine release is analyzed. Peripheral blood is withdrawn from three healthy donors by venipuncture into heparized vacutainer tubes. Whole blood is diluted 1:5 with RPMI-1640 medium and placed in 24-well tissue culture plates at 0.5 mL per well. The anti-cytokine antibodies (e.g., anti-IL-4) are diluted into RPMI-1640 and placed in the plates at 0.5 mL/well to give final concentrations of 200, 100, 50, 10, and 1 µg/mL. The final dilution of whole blood in the culture plates is 1:10. LPS and PHA are added to separate wells at 2µg/mL and 5µg/mL final concentration as a positive control for cytokine release. Polyclonal human IgG is used as negative control antibody. The experiment is performed in duplicate. Plates are incubated at 37°C at 5% CO_2. Twenty-four hours later the contents of the wells are transferred into test tubes and spun for 5 minutes at 1200 rpm. Cell-free supernatants are collected and frozen for cytokine assays. Cells left over on the plates and in the tubes are lysed with 0.5 mL of lysis solution, and placed at –20°C and thawed. 0.5 mL of medium is added (to bring the volume to the same level as the cell-free supernatant samples) and the cell preparations are collected and frozen for cytokine assays. Cell-free supernatants and cell lysates are assayed for cytokine levels by ELISA, for example, for levels of IL-8, IL-6, IL-1β, IL-1RA, or TNF-α.

Example 1.1.2.F: Cytokine Cross-Reactivity Study

The ability of an anti-cytokine parent antibody or DVD-Ig directed to a cytokine(s) of interest to cross react with other cytokines is analyzed. Parent antibodies or DVD-Ig are immobilized on a BIAcore biosensor matrix. An anti-human Fc mAb is covalently linked via free amine groups to the dextran matrix by first activating carboxyl groups on the matrix with 100mM N-hydroxysuccinimide (NHS) and 400mM N-Ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC). Approximately 50μL of each antibody or DVD-Ig preparation at a concentration of 25μg/mL, diluted in sodium acetate, pH4.5, is injected across the activated biosensor and free amines on the protein are bound directly to the activated carboxyl groups. Typically, 5000 Resonance Units (RU's) are immobilized. Unreacted matrix EDC-esters are deactivated by an injection of 1 M ethanolamine. A second flow cell is prepared as a reference standard by immobilizing human IgG1/K using the standard amine coupling kit. SPR measurements are performed using the CM biosensor chip. All antigens to be analyzed on the biosensor surface are diluted in HBS-EP running buffer containing 0.01% P20.

To examine the cytokine binding specificity, excess cytokine of interest (100nM, e.g., soluble recombinant human) is injected across the anti-cytokine parent antibody or DVD-Ig immobilized biosensor surface (5 minute contact time). Before injection of the cytokine of interest and immediately afterward, HBS-EP buffer alone flows through each flow cell. The net difference in the signals between the baseline and the point corresponding to approximately 30 seconds after completion of cytokine injection are taken to represent the final binding value. Again, the response is measured in Resonance Units. Biosensor matrices are regenerated using 10mM HCl before injection of the next sample where a binding event is observed, otherwise running buffer was injected over the matrices. Human cytokines (e.g., IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-22, IL-23, IL-27, TNF-α, TNF-β, and IFN-γ, for example) are also simultaneously injected over the immobilized mouse IgG1/K reference surface to record any nonspecific binding background. By preparing a reference and reaction surface, BIAcore can automatically subtract the reference surface data from the reaction surface data in order to eliminate the majority of the refractive index change and injection noise. Thus, it is possible to ascertain the true binding response attributed to an anti-cytokine antibody or DVD-Ig binding reaction.

When a cytokine of interest is injected across immobilized anti-cytokine antibody, significant binding is observed. 10mM HCl regeneration completely removes all non-covalently associated proteins. Examination of the sensorgram shows that immobilized anti-cytokine antibody or DVD-Ig binding to soluble cytokine is strong and robust. After confirming the expected result with the cytokine of interest, the panel of remaining recombinant human cytokines is tested, for each antibody or DVD-Ig separately. The amount of anti-cytokine antibody or DVD-Ig bound or unbound cytokine for each injection cycle is recorded. The results from three independent experiments are used to determine the specificity profile of each antibody or DVD-Ig. Antibodies or DVD-Ig with the expected binding to the cytokine of interest and no binding to any other cytokine are selected.

Example 1.1.2.G: Tissue Cross Reactivity

Tissue cross reactivity studies are done in three stages, with the first stage including cryosections of 32 tissues, second stage inluding up to 38 tissues, and the 3rd stage including additional tissues from 3 unrelated adults as described below. Studies are done typically at two dose levels.

Stage 1: Cryosections (about 5 µm) of human tissues (32 tissues (typically: Adrenal Gland, Gastrointestinal Tract, Prostate, Bladder, Heart, Skeletal Muscle, Blood Cells, Kidney, Skin, Bone Marrow, Liver, Spinal Cord, Breast, Lung, Spleen, Cerebellum, Lymph Node, Testes, Cerebral Cortex, Ovary, Thymus, Colon, Pancreas, Thyroid, Endothelium, Parathyroid, Ureter, Eye, Pituitary, Uterus, Fallopian Tube and Placenta) from one human donor obtained at autopsy or biopsy) are fixed and dried on object glass. The peroxidase staining of tissue sections is performed, using the avidin-biotin system.

Stage 2:Cryosections (about 5 µm) of human tissues 38 tissues (including adrenal, blood, blood vessel, bone marrow, cerebellum, cerebrum, cervix, esophagus, eye, heart, kidney, large intestine, liver, lung, lymph node, breast mammary gland, ovary, oviduct, pancreas, parathyroid, peripheral nerve, pituitary, placenta, prostate, salivary gland, skin, small intestine, spinal cord, spleen, stomach, striated muscle, testis, thymus, thyroid, tonsil, ureter, urinary bladder, and uterus) from 3 unrelated adults obtained at autopsy or

biopsy) are fixed and dried on object glass. The peroxidase staining of tissue sections is performed, using the avidin-biotin system.

Stage 3: Cryosections (about 5 μm) of cynomolgus monkey tissues (38 tissues (including adrenal, blood, blood vessel, bone marrow, cerebellum, cerebrum, cervix, esophagus, eye, heart, kidney, large intestine, liver, lung, lymph node, breast mammary gland, ovary, oviduct, pancreas, parathyroid, peripheral nerve, pituitary, placenta, prostate, salivary gland, skin, small intestine, spinal cord, spleen, stomach, striated muscle, testis, thymus, thyroid, tonsil, ureter, urinary bladder, and uterus) from 3 unrelated adult monkeys obtained at autopsy or biopsy) are fixed and dried on object glass. The peroxidase staining of tissue sections is performed, using the avidin-biotin system.

The antibody or DVD-Ig is incubated with the secondary biotinylated anti-human IgG and developed into immune complex. The immune complex at the final concentrations of 2 and 10 μg/mL of antibody or DVD-Ig is added onto tissue sections on object glass and then the tissue sections are reacted for 30 minutes with a avidin-biotin-peroxidase kit. Subsequently, DAB (3,3'-diaminobenzidine), a substrate for the peroxidase reaction, is applied for 4 minutes for tissue staining. Antigen-Sepharose beads are used as positive control tissue sections. Target antigen and human serum blocking studies serve as additional controls. The immune complex at the final concentrations of 2 and 10 μg/mL of antibody or DVD-Ig is pre-incubated with target antigen (final concentration of 100 μg/ml) or human serum (final concentration 10%) for 30 minutes, and then added onto the tissue sections on object glass and then the tissue sections are reacted for 30 minutes with a avidin-biotin-peroxidase kit. Subsequently, DAB (3,3'-diaminobenzidine), a substrate for the peroxidase reaction, is applied for 4 minutes for tissue staining.

Any specific staining is judged to be either an expected (e.g., consistent with antigen expression) or unexpected reactivity based upon known expression of the target antigen in question. Any staining judged specific is scored for intensity and frequency. The tissue staining between stage 2 (human tissue) and stage 3 (cynomolgus monkey tissue) is either judged to be similar or different.

Example 1.1.2.H: Tumoricidal Effect Of A Parent or DVD-Ig Antibody *In Vitro*

Parent antibodies or DVD-Ig that bind to target antigens on tumor cells may be analyzed for tumoricidal activity. Briefly, parent antibodies or DVD-Ig are diluted in D-PBS-BSA (Dulbecco's phosphate buffered saline with 0.1%BSA) and added to human tumor cells at final concentrations of 0.01 μg/mL to 100 μg/mL. The plates are incubated at 37 °C in a humidified, 5% CO_2 atmosphere for 3 days. The number of live cells in each well is quantified using MTS reagents according to the manufacturer's instructions (Promega, Madison, WI) to determine the percent of tumor growth inhibition. Wells without antibody treatment are used as controls of 0% inhibition whereas wells without cells are considered to show 100% inhibition.

For assessment of apoptosis, caspase-3 activation is determined by the following protocol: antibody-treated cells in 96 well plates are lysed in 120 μl of 1x lysis buffer (1.67mM Hepes, pH 7.4, 7mM KCl, 0.83mM $MgCl_2$, 0.11mM EDTA, 0.11mM EGTA, 0.57% CHAPS, 1mM DTT, 1x protease inhibitor cocktail tablet; EDTA-free; Roche Pharmaceuticals, Nutley, NJ) at room temperature with shaking for 20 minutes. After cell lysis, 80 μl of a caspase-3 reaction buffer (48mM Hepes, pH 7.5, 252mM sucrose, 0.1% CHAPS, 4mM DTT, and 20 μM Ac-DEVD-AMC substrate; Biomol Research Labs, Inc., Plymouth Meeting, PA) is added and the plates are incubated for 2 hours at 37^0C. The plates are read on a 1420 VICTOR Multilabel Counter (Perkin Elmer Life Sciences, Downers Grove, IL) using the following settings: excitation= 360/40, emission= 460/40. An increase of fluorescence units from antibody-treated cells relative to the isotype antibody control-treated cells is seen, which is indicative of apoptosis.

Example 1.1.2.I: Inhibition Of Receptor Activation By Antibodies or DVD-Ig *In Vitro*

Parent antibodies or DVD-Ig that bind to cell receptors or their ligands may be tested for inhibition of receptor activation. Parent antibodies or DVD-Ig diluted in D-PBS-BSA (Dulbecco's phosphate buffered saline with 0.1%BSA) are added to human carcinoma cells at final concentrations of 0.01 μg/mL to 100 μg/mL. The plates are incubated at 37 °C in a humidified, 5% CO_2 atmosphere for 1h. Growth factors (e.g., IGF1 or IGF2) at concentration of 1-100 ng/mL are added to the cells for 5-15 minutes to stimulate receptor (e.g., IGF1R) autophosphorylation. Wells without antibody treatment are used as controls of 0% inhibition whereas wells without growth factor stimulation are

considered to show 100% inhibition. Cell lysates are made by incubation with cell extraction buffer (10 mM Tris, pH 7.4, 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM NaF, 1 mM sodium orthovanadate, 1% Triton X-100, 10% Glycerol, 0.1% SDS, and protease inhibitor cocktail). Phospho-IGF1R in these cell lysates is determined using specific ELISA kits purchased from R&D System (Minneapolis, MN).

Example 1.1.2.J: Efficacy Of An Anti-Tumor Cell Antigen Antibody or DVD-Ig By Itself Or In Combination With Chemotherapy On The Growth Of Human Carcinoma Xenografts (Subcutaneous Flank, Orthotopic, Or Spontaneous Metastases)

Human cancer cells are grown *in vitro* to 99% viability, 85% confluence in tissue culture flasks. SCID female or male mice (Charles Rivers Labs) at 19-25 grams are ear tagged and shaved. Mice are then inoculated subcutaneously into the right flank with 0.2 ml of 2 x 10^6 human tumor cells (1:1 matrigel) on study day 0. Administration (IP, Q3D/ week) of vehicle (PBS), antibody or DVD-Ig, and/or chemotherapy is initiated after mice are size matched into separate cages of mice with mean tumor volumes of approximately 150 to 200 mm^3. The tumors are measured by a pair of calipers twice a week starting on approximately day 10 post inoculation and the tumor volumes calculated according to the formula $V = L \times W^2/2$ (V: volume, mm^3; L: length, mm. W: width, mm). Reduction in tumor volume is seen in animals treated with antibody or DVD-Ig alone or in combination with chemotherapy relative to tumors in animals that received only vehicle or an isotype control mAb.

Example 1.1.2.K: Binding of Monoclonal Antibodies to the Surface of Human Tumor Cell Lines as Assessed by Flow Cytometry

Stable cell lines overexpressing cell-surface antigen of interest or human tumor cell lines were harvested from tissue culture flasks and resuspended in phosphate buffered saline (PBS) containing 5% fetal calf serum (PBS/FCS). Prior to staining, human tumor cells were incubated on ice with human IgG at 200µg/ml in PBS/FCS. 1-5 x10^5 cells were incubated with antibody or DVD-Ig (1-2 µg/mL) in PBS/FCS for 30-60 minutes on ice. Cells were washed twice and 100µl of goat anti mouse IgG- phycoerythrin (1:300 dilution in PBS/BSA) (Jackson ImmunoResearch, West Grove, PA, Cat.#115-115-164) was added. After 30 minutes incubation on ice, cells were washed twice and resuspended

in PBS/FCS. Fluorescence was measured using a Becton Dickinson FACSCalibur (Becton Dickinson, San Jose, CA).

Example 1.2: Generation And Characterization Of Parent Monoclonal Antibodies to a Human Antigen of Interest

Parent mouse mAbs able to bind to and neutralize a human antigen of interest and a variant thereof are obtained as follows:

Example 1.2.1: Immunization Of Mice With a Human Antigen of Interest

Twenty micrograms of recombinant purified human antigen (e.g., IGF1,2) mixed with complete Freund's adjuvant or Immunoeasy adjuvant (Qiagen, Valencia, CA) is injected subcutaneously into five 6-8 week-old Balb/C, five C57B/6 mice, and five AJ mice on Day 1. On days 24, 38, and 49, twenty micrograms of recombinant purified human antigen variant mixed with incomplete Freund's adjuvant or Immunoeasy adjuvant is injected subcutaneously into the same mice. On day 84 or day 112 or day 144, mice are injected intravenously with 1 µg recombinant purified human antigen of interest.

Example 1.2.2: Generation of Hybridomas

Splenocytes obtained from the immunized mice described in Example 1.2.A are fused with SP2/O-Ag-14 cells at a ratio of 5:1 according to the established method described in Kohler, G. and Milstein (1975) Nature, 256:495 to generate hybridomas. Fusion products are plated in selection media containing azaserine and hypoxanthine in 96-well plates at a density of 2.5×10^6 spleen cells per well. Seven to ten days post fusion, macroscopic hybridoma colonies are observed. Supernatant from each well containing hybridoma colonies is tested by ELISA for the presence of antibody to the antigen of interest (as described in Example 1.2.A). Supernatants displaying antigen-specific activity are then tested for activity (as described in the assays of Example 1.1.2), for example, the ability to neutralize the antigen of interest in a bioassay such as that described in Example 1.1.2.A).

Example 1.2.3: Characterization Of Parent Monoclonal Antibodies to a Human Target Antigen of Interest

Example 1.2.3.1: Analyzing Parent Monoclonal Antibody Neutralizing Activity

Hybridoma supernatants are assayed for the presence of parent antibodies that bind an antigen of interest and are also capable of binding a variant of the antigen of interest ("antigen variant"). Supernatants with antibodies positive in both assays are then tested for their antigen neutralization potency, for example, in the cytokine bioassays of Example 1.1.2. The hybridomas producing antibodies with IC_{50} values in the bioassay less than 1000pM, in an embodiment, less than 100pM are scaled up and cloned by limiting dilution. Hybridoma cells are expanded into media containing 10% low IgG fetal bovine serum (Hyclone #SH30151, Logan, UT.). On average, 250 mL of each hybridoma supernatant (derived from a clonal population) is harvested, concentrated and purified by protein A affinity chromatography, as described in Harlow, E. and Lane, D. 1988 "Antibodies: A Laboratory Manual". The ability of purified mAbs to inhibit the activity of its target antigen is determined, for example, using the cytokine bioassays as described in Example 1.1.2.

Example 1.2.3.2: Analyzing Parent Monoclonal Antibody Cross-Reactivity To Cynomolgus Target Antigen Of Interest

To determine whether the selected mAbs described herein recognize cynomolgus antigen of interest, BIACORE analysis is conducted as described herein (Example 1.1.1.C) using recombinant cynomolgus target antigen. In addition, neutralization potencies of mAbs against recombinant cynomolgus antigen of interest may also be measured in the cytokine bioassay (Example 1.1.2). MAbs with good cyno cross-reactivity (in an embodiment, within 5-fold of reactivity for human antigen) are selected for future characterization.

Example 1.2.4: Determination Of The Amino Acid Sequence Of The Variable Region For Each Murine Anti-Human Monoclonal Antibody

Isolation of the cDNAs, expression and characterization of the recombinant anti-human mouse mAbs is conducted as follows. For each amino acid sequence determination, approximately 1 x 10^6 hybridoma cells are isolated by centrifugation and processed to isolate total RNA with Trizol (Gibco BRL/Invitrogen, Carlsbad, CA.) following manufacturer's instructions. Total RNA is subjected to first strand DNA

synthesis using the SuperScript First-Strand Synthesis System (Invitrogen, Carlsbad, CA) per the manufacturers instructions. Oligo(dT) is used to prime first-strand synthesis to select for poly(A)+ RNA. The first-strand cDNA product is then amplified by PCR with primers designed for amplification of murine immunoglobulin variable regions (Ig-Primer Sets, Novagen, Madison, WI). PCR products are resolved on an agarose gel, excised, purified, and then subcloned with the TOPO Cloning kit into pCR2.1-TOPO vector (Invitrogen, Carlsbad, CA) and transformed into TOP10 chemically competent E. coli (Invitrogen, Carlsbad, CA). Colony PCR is performed on the transformants to identify clones containing insert. Plasmid DNA is isolated from clones containing insert using a QIAprep Miniprep kit (Qiagen, Valencia, CA). Inserts in the plasmids are sequenced on both strands to determine the variable heavy or variable light chain DNA sequences using M13 forward and M13 reverse primers (Fermentas Life Sciences, Hanover MD). Variable heavy and variable light chain sequences of the mAbs are identified. In an embodiment, the selection criteria for a panel of lead mAbs for next step development (humanization) includes the following:

- The antibody does not contain any N-linked glycosylation sites (NXS), except from the standard one in CH2
- The antibody does not contain any extra cysteines in addition to the normal cysteines in every antibody
- The antibody sequence is aligned with the closest human germline sequences for VH and VL and any unusual amino acids should be checked for occurrence in other natural human antibodies
- N-terminal Glutamine (Q) is changed to Glutamic acid (E) if it does not affect the activity of the antibody. This will reduce heterogeneity due to cyclization of Q
- Efficient signal sequence cleavage is confirmed by Mass Spectrophotometry. This can be done with COS cell or 293 cell material
- The protein sequence is checked for the risk of deamidation of Asn that could result in loss of activity
- The antibody has a low level of aggregation
- The antibody has solubility >5-10 mg/ml (in research phase); >25 mg/ml
- The antibody has a normal size (5-6 nm) by Dynamic Light Scattering (DLS)
- The antibody has a low charge heterogeneity
- The antibody lacks cytokine release (see Example 1.1.2.E)

- The antibody has specificity for the intended cytokine (see Example 1.1.2.F)
- The antibody lacks unexpected tissue cross reactivity (see Example 1.1.2.G)
- The antibody has similarity between human and cynomolgus tissue cross reactivity (see Example 1.1.2.G)

Example 1.3: Generation and Characterization of Recombinant Humanized Parent Antibodies

Example 1.3.1: Construction And Expression Of Recombinant Chimeric Anti Human Parent Antibodies

The DNA encoding the heavy chain constant region of murine anti-human parent mAbs is replaced by a cDNA fragment encoding the human IgG1 constant region containing 2 hinge-region amino acid mutations by homologous recombination in bacteria. These mutations are a leucine to alanine change at position 234 (EU numbering) and a leucine to alanine change at position 235 (Lund et al., 1991, J. Immunol., 147:2657). The light chain constant region of each of these antibodies is replaced by a human kappa constant region. Full-length chimeric antibodies are transiently expressed in COS cells by co-transfection of chimeric heavy and light chain cDNAs ligated into the pBOS expression plasmid (Mizushima and Nagata, Nucleic Acids Research 1990, Vol 18, pg 5322). Cell supernatants containing recombinant chimeric antibody are purified by Protein A Sepharose chromatography and bound antibody is eluted by addition of acid buffer. Antibodies are neutralized and dialyzed into PBS.

The heavy chain cDNA encoding a chimeric mAb is co-transfected with its chimeric light chain cDNA (both ligated in the pBOS vector) into COS cells. Cell supernatant containing recombinant chimeric antibody is purified by Protein A Sepharose chromatography and bound antibody is eluted by addition of acid buffer. Antibodies are neutralized and dialyzed into PBS.

The purified chimeric anti-human parent mAbs are then tested for their ability to bind (by BIAcore) and for functional activity, e.g., to inhibit the cytokine induced production of IgE as described in Example 1.1. Chimeric mAbs that maintain the activity of the parental hybridoma mAbs are selected for future development.

Example 1.3.2: Construction And Expression Of Humanized Anti Human Parent Antibodies

Example 1.3.2.1: Selection Of Human Antibody Frameworks

Each murine variable heavy and variable light chain gene sequence is separately aligned against 44 human immunoglobulin germline variable heavy chain or 46 germline variable light chain sequences (derived from NCBI Ig Blast website at http://www.ncbi.nlm.nih.gov/igblast/retrieveig.html.) using Vector NTI software.

Humanization is based on amino acid sequence homology, CDR cluster analysis, frequency of use among expressed human antibodies, and available information on the crystal structures of human antibodies. Taking into account possible effects on antibody binding, VH- VL pairing, and other factors, murine residues are mutated to human residues where murine and human framework residues are different, with a few exceptions. Additional humanization strategies are designed based on an analysis of human germline antibody sequences, or a subgroup thereof, that possessed a high degree of homology, i.e., sequence similarity, to the actual amino acid sequence of the murine antibody variable regions.

Homology modeling is used to identify residues unique to the murine antibody sequences that are predicted to be critical to the structure of the antibody combining site, the CDRs. Homology modeling is a computational method whereby approximate three dimensional coordinates are generated for a protein. The source of initial coordinates and guidance for their further refinement is a second protein, the reference protein, for which the three dimensional coordinates are known and the sequence of which is related to the sequence of the first protein. The relationship among the sequences of the two proteins is used to generate a correspondence between the reference protein and the protein for which coordinates are desired, the target protein. The primary sequences of the reference and target proteins are aligned with coordinates of identical portions of the two proteins transferred directly from the reference protein to the target protein. Coordinates for mismatched portions of the two proteins, e.g., from residue mutations, insertions, or deletions, are constructed from generic structural templates and energy refined to insure consistency with the already transferred model coordinates. This computational protein structure may be further refined or employed directly in modeling studies. The quality of

the model structure is determined by the accuracy of the contention that the reference and target proteins are related and the precision with which the sequence alignment is constructed.

For the murine mAbs, a combination of BLAST searching and visual inspection is used to identify suitable reference structures. Sequence identity of 25% between the reference and target amino acid sequences is considered the minimum necessary to attempt a homology modeling exercise. Sequence alignments are constructed manually and model coordinates are generated with the program Jackal (see Petrey, D. et al. (2003) Proteins 53 (Suppl. 6): 430–435).

The primary sequences of the murine and human framework regions of the selected antibodies share significant identity. Residue positions that differ are candidates for inclusion of the murine residue in the humanized sequence in order to retain the observed binding potency of the murine antibody. A list of framework residues that differ between the human and murine sequences is constructed manually.

The likelihood that a given framework residue would impact the binding properties of the antibody depends on its proximity to the CDR residues. Therefore, using the model structures, the residues that differ between the murine and human sequences are ranked according to their distance from any atom in the CDRs. Those residues that fell within 4.5 Å of any CDR atom are identified as most important and are recommended to be candidates for retention of the murine residue in the humanized antibody (i.e., back mutation).

In silico constructed humanized antibodies are constructed using oligonucleotides. For each variable region cDNA, 6 oligonucleotides of 60-80 nucleotides each are designed to overlap each other by 20 nucleotides at the 5' and/or 3' end of each oligonucleotide. In an annealing reaction, all 6 oligonulceotides are combined, boiled, and annealed in the presence of dNTPs. DNA polymerase I, Large (Klenow) fragment (New England Biolabs #M0210, Beverley, MA.) is added to fill-in the approximately 40bp gaps between the overlapping oligonucleotides. PCR is performed to amplify the entire variable region gene using two outermost primers containing overhanging sequences complementary to the multiple cloning site in a modified pBOS vector (Mizushima, S. and Nagata, S. (1990) Nucleic Acids Res. 18: 17). The PCR products

derived from each cDNA assembly are separated on an agarose gel and the band corresponding to the predicted variable region cDNA size is excised and purified. The variable heavy region is inserted in-frame onto a cDNA fragment encoding the human IgG1 constant region containing 2 hinge-region amino acid mutations by homologous recombination in bacteria. These mutations are a leucine to alanine change at position 234 (EU numbering) and a leucine to alanine change at position 235 (Lund et al. (1991) J. Immunol. 147:2657). The variable light chain region is inserted in-frame with the human kappa constant region by homologous recombination. Bacterial colonies are isolated and plasmid DNA extracted. cDNA inserts are sequenced in their entirety. Correct humanized heavy and light chains corresponding to each antibody are co-transfected into COS cells to transiently produce full-length humanized anti-human antibodies. Cell supernatants containing recombinant chimeric antibody are purified by Protein A Sepharose chromatography and bound antibody is eluted by addition of acid buffer. Antibodies are neutralized and dialyzed into PBS.

Example 1.3.3: Characterization Of Humanized Antibodies

The ability of purified humanized antibodies to inhibit a functional activity is determined, e.g., using the cytokine bioassays as described in Examples 1.1.2. The binding affinities of the humanized antibodies to recombinant human antigen are determined using surface plasmon resonance (BIAcore®) measurement as described in Example 1.1.1.C. The IC_{50} values from the bioassays and the affinity of the humanized antibodies are ranked. The humanized mAbs that fully maintain the activity of the parental hybridoma mAbs are selected as candidates for future development. The top 2-3 most favorable humanized mAbs are further characterized.

Example 1.3.3.1: Pharmacokinetic Analysis Of Humanized Antibodies

Pharmacokinetic studies are carried out in Sprague-Dawley rats and cynomolgus monkeys. Male and female rats and cynomolgus monkeys are dosed intravenously or subcutaneously with a single dose of 4mg/kg mAb and samples are analyzed using antigen capture ELISA, and pharmacokinetic parameters are determined by noncompartmental analysis. Briefly, ELISA plates are coated with goat anti-biotin antibody (5 mg/ml, 4°C, overnight), blocked with Superblock (Pierce), and incubated with biotinylated human antigen at 50 ng/ml in 10% Superblock TTBS at room

temperature for 2 hours. Serum samples are serially diluted (0.5% serum, 10% Superblock in TTBS) and incubated on the plate for 30 minutes at room temperature. Detection is carried out with HRP-labeled goat anti human antibody and concentrations are determined with the help of standard curves using the four parameter logistic fit. Values for the pharmacokinetic parameters are determined by non-compartmental model using WinNonlin software (Pharsight Corporation, Mountain View, CA). Humanized mAbs with good pharmacokinetics profile (T1/2 is 8-13 days or better, with low clearance and excellent bioavailability 50-100%) are selected.

Example 1.3.3.2: Physicochemical And In Vitro Stability Analysis Of Humanized Monoclonal Antibodies

Example 1.3.3.2.A: Size Exclusion Chromatography

Antibodies are diluted to 2.5 mg/mL with water and 20 mL is analyzed on a Shimadzu HPLC system using a TSK gel G3000 SWXL column (Tosoh Bioscience, cat# k5539-05k). Samples are eluted from the column with 211 mM sodium sulfate, 92 mM sodium phosphate, pH 7.0, at a flow rate of 0.3 mL/minutes. The HPLC system operating conditions are the following:

Mobile phase: 211 mM Na_2SO_4, 92 mM $Na_2HPO_4*7H_2O$, pH 7.0
Gradient: Isocratic
Flow rate: 0.3 mL/minute
Detector wavelength: 280 nm
Autosampler cooler temp: 4°C
Column oven temperature: Ambient
Run time: 50 minutes

Example 1.3.3.2.B: SDS-PAGE

Antibodies are analyzed by sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) under both reducing and non-reducing conditions. Adalimumab lot AFP04C is used as a control. For reducing conditions, the samples are mixed 1:1 with 2X tris glycine SDS-PAGE sample buffer (Invitrogen, cat# LC2676, lot# 1323208) with 100 mM DTT, and heated at 60°C for 30 minutes. For non-reducing conditions, the samples are mixed 1:1 with sample buffer and heated at 100°C for 5 minutes. The reduced samples (10 mg per lane) are loaded on a 12% pre-cast tris-glycine

gel (Invitrogen, cat# EC6005box, lot# 6111021), and the non-reduced samples (10 mg per lane) are loaded on an 8%-16% pre-cast tris-glycine gel (Invitrogen, cat# EC6045box, lot# 6111021). SeeBlue Plus 2 (Invitrogen, cat#LC5925, lot# 1351542) is used as a molecular weight marker. The gels are run in a XCell SureLock mini cell gel box (Invitrogen, cat# EI0001) and the proteins are separated by first applying a voltage of 75 to stack the samples in the gel, followed by a constant voltage of 125 until the dye front reached the bottom of the gel. The running buffer used is 1X tris glycine SDS buffer, prepared from a 10X tris glycine SDS buffer (ABC, MPS-79-080106)). The gels are stained overnight with colloidal blue stain (Invitrogen cat# 46-7015, 46-7016) and destained with Milli-Q water until the background is clear. The stained gels are then scanned using an Epson Expression scanner (model 1680, S/N DASX003641).

Example 1.3.3.2.C: Sedimentation Velocity Analysis

Antibodies are loaded into the sample chamber of each of three standard two-sector carbon epon centerpieces. These centerpieces have a 1.2 cm optical path length and are built with sapphire windows. PBS is used for a reference buffer and each chamber contained 140 µL. All samples are examined simultaneously using a 4-hole (AN-60Ti) rotor in a Beckman ProteomeLab XL-I analytical ultracentrifuge (serial # PL106C01).

Run conditions are programmed and centrifuge control is performed using ProteomeLab (v5.6). The samples and rotor are allowed to thermally equilibrate for one hour prior to analysis (20.0 ± 0.1 °C). Confirmation of proper cell loading is performed at 3000 rpm and a single scan is recorded for each cell. The sedimentation velocity conditions are the following:

Sample Cell Volume: 420 mL
Reference Cell Volume: 420 mL
Temperature: 20°C
Rotor Speed: 35,000 rpm
Time: 8:00 hours
UV Wavelength: 280 nm
Radial Step Size: 0.003 cm
Data Collection: One data point per step without signal averaging.

Total Number of Scans: 100

Example 1.3.3.2.D: LC-MS Molecular Weight Measurement Of Intact Antibodies

Molecular weight of intact antibodies are analyzed by LC-MS. Each antibody is diluted to approximately 1 mg/mL with water. An 1100 HPLC (Agilent) system with a protein microtrap (Michrom Bioresources, Inc, cat# 004/25109/03) is used to desalt and introduce 5 mg of the sample into an API Qstar pulsar i mass spectrometer (Applied Biosystems). A short gradient is used to elute the samples. The gradient is run with mobile phase A (0.08% FA, 0.02% TFA in HPLC water) and mobile phase B (0.08% FA and 0.02% TFA in acetonitrile) at a flow rate of 50 mL/minute. The mass spectrometer is operated at 4.5 kvolts spray voltage with a scan range from 2000 to 3500 mass to charge ratio.

Example 1.3.3.2.E: LC-MS Molecular Weight Measurement Of Antibody Light And Heavy Chains

Molecular weight measurement of antibody light chain (LC), heavy chain (HC) and deglycosylated HC are analyzed by LC-MS. Antibody is diluted to 1 mg/mL with water and the sample is reduced to LC and HC with a final concentration of 10 mM DTT for 30 minutes at 37°C. To deglycosylate the antibody, 100 mg of the antibody is incubated with 2 mL of PNGase F, 5 mL of 10% N-octylglucoside in a total volume of 100 mL overnight at 37 °C. After deglycosylation the sample is reduced with a final concentration of 10 mM DTT for 30 minutes at 37°C. An Agilent 1100 HPLC system with a C4 column (Vydac, cat# 214TP5115, S/N 060206537204069) is used to desalt and introduce the sample (5 mg) into an API Qstar pulsar i mass spectrometer (Applied Biosystems). A short gradient is used to elute the sample. The gradient is run with mobile phase A (0.08% FA, 0.02% TFA in HPLC water) and mobile phase B (0.08% FA and 0.02% TFA in acetonitrile) at a flow rate of 50 mL/minute. The mass spectrometer is operated at 4.5 kvolts spray voltage with a scan range from 800 to 3500 mass to charge ratio.

Example 1.3.3.2.F: Peptide Mapping

Antibody is denatured for 15 minutes at room temperature with a final concentration of 6 M guanidine hydrochloride in 75 mM ammonium bicarbonate. The

denatured samples are reduced with a final concentration of 10 mM DTT at 37°C for 60 minutes, followed by alkylation with 50 mM iodoacetic acid (IAA) in the dark at 37°C for 30 minutes. Following alkylation, the sample is dialyzed overnight against four liters of 10 mM ammonium bicarbonate at 4°C. The dialyzed sample is diluted to 1 mg/mL with 10 mM ammonium bicarbonate, pH 7.8 and 100 mg of antibody is either digested with trypsin (Promega, cat# V5111) or Lys-C (Roche, cat# 11 047 825 001) at a 1:20 (w/w) trypsin/Lys-C:antibody ratio at 37°C for 4 hrs. Digests are quenched with 1 mL of 1 N HCl. For peptide mapping with mass spectrometer detection, 40 mL of the digests are separated by reverse phase high performance liquid chromatography (RPHPLC) on a C18 column (Vydac, cat# 218TP51, S/N NE9606 10.3.5) with an Agilent 1100 HPLC system. The peptide separation is run with a gradient using mobile phase A (0.02% TFA and 0.08% FA in HPLC grade water) and mobile phase B (0.02% TFA and 0.08% FA in acetonitrile) at a flow rate of 50 mL/minutes. The API QSTAR Pulsar i mass spectromer is operated in positive mode at 4.5 kvolts spray voltage and a scan range from 800 to 2500 mass to charge ratio.

Example 1.3.3.2.G: <u>Disulfide Bond Mapping</u>

To denature the antibody, 100 mL of the antibody is mixed with 300 mL of 8 M guanidine HCl in 100 mM ammonium bicarbonate. The pH is checked to ensure that it is between 7 and 8 and the samples are denatured for 15 minutes at room temperature in a final concentration of 6 M guanidine HCl. A portion of the denatured sample (100 mL) is diluted to 600 mL with Milli-Q water to give a final guanidine-HCl concentration of 1 M. The sample (220 mg) is digested with either trypsin (Promega, cat # V5111, lot# 22265901) or Lys-C (Roche, cat# 11047825001, lot# 12808000) at a 1:50 trypsin or 1:50 Lys-C: antibody (w/w) ratios (4.4 mg enzyme: 220 mg sample) at 37°C for approximately 16 hours. An additional 5 mg of trypsin or Lys-C is added to the samples and digestion is allowed to proceed for an additional 2 hours at 37°C. Digestions are stopped by adding 1 mL of TFA to each sample. Digested samples are separated by RPHPLC using a C18 column (Vydac, cat# 218TP51 S/N NE020630-4-1A) on an Agilent HPLC system. The separation is run with the same gradient used for peptide mapping using mobile phase A (0.02% TFA and 0.08% FA in HPLC grade water) and mobile phase B (0.02% TFA and 0.08% FA in acetonitrile) at a flow rate of 50 mL/minute. The HPLC operating conditions are the same as those used for peptide mapping. The API QSTAR Pulsar i

mass spectromer is operated in positive mode at 4.5 kvolts spray voltage and a scan range from 800 to 2500 mass-to-charge ratio. Disulfide bonds are assigned by matching the observed MWs of peptides with the predicted MWs of tryptic or Lys-C peptides linked by disulfide bonds.

Example 1.3.3.2.H: Free Sulfhydryl Determination

The method used to quantify free cysteines in an antibody is based on the reaction of Ellman's reagent, 5,5¢- dithio-bis (2-nitrobenzoic acid) (DTNB), with sulfhydryl groups (SH) which gives rise to a characteristic chromophoric product, 5-thio-(2-nitrobenzoic acid) (TNB). The reaction is illustrated in the formula:

DTNB + RSH ® RS-TNB + TNB- + H+

The absorbance of the TNB- is measured at 412 nm using a Cary 50 spectrophotometer. An absorbance curve is plotted using dilutions of 2 mercaptoethanol (b-ME) as the free SH standard and the concentrations of the free sulfhydryl groups in the protein are determined from absorbance at 412 nm of the sample.

The b-ME standard stock is prepared by a serial dilution of 14.2 M b-ME with HPLC grade water to a final concentration of 0.142 mM. Then standards in triplicate for each concentration are prepared. Antibody is concentrated to 10 mg/mL using an amicon ultra 10,000 MWCO centrifugal filter (Millipore, cat# UFC801096, lot# L3KN5251) and the buffer is changed to the formulation buffer used for adalimumab (5.57 mM sodium phosphate monobasic, 8.69 mM sodium phosphate dibasic, 106.69 mM NaCl, 1.07 mM sodium citrate, 6.45 mM citric acid, 66.68 mM mannitol, pH 5.2, 0.1% (w/v) Tween). The samples are mixed on a shaker at room temperature for 20 minutes. Then 180 mL of 100 mM Tris buffer, pH 8.1 is added to each sample and standard followed by the addition of 300 mL of 2 mM DTNB in 10 mM phosphate buffer, pH 8.1. After thorough mixing, the samples and standards are measured for absorption at 412 nm on a Cary 50 spectrophotometer. The standard curve is obtained by plotting the amount of free SH and OD_{412} nm of the b-ME standards. Free SH content of samples are calculated based on this curve after subtraction of the blank.

Example 1.3.3.2.I: Weak Cation Exchange Chromatography

Antibody is diluted to 1 mg/mL with 10 mM sodium phosphate, pH 6.0. Charge heterogeneity is analyzed using a Shimadzu HPLC system with a WCX-10 ProPac analytical column (Dionex, cat# 054993, S/N 02722). The samples are loaded on the column in 80% mobile phase A (10 mM sodium phosphate, pH 6.0) and 20% mobile phase B (10 mM sodium phosphate, 500 mM NaCl, pH 6.0) and eluted at a flow rate of 1.0 mL/minute.

Example 1.3.3.2.J: Oligosaccharide Profiling

Oligosaccharides released after PNGase F treatment of antibody are derivatized with 2-aminobenzamide (2-AB) labeling reagent. The fluorescent-labeled oligosaccharides are separated by normal phase high performance liquid chromatography (NPHPLC) and the different forms of oligosaccharides are characterized based on retention time comparison with known standards.

The antibody is first digested with PNGaseF to cleave N-linked oligosaccharides from the Fc portion of the heavy chain. The antibody (200 mg) is placed in a 500 mL Eppendorf tube along with 2 mL PNGase F and 3 mL of 10% N-octylglucoside. Phosphate buffered saline is added to bring the final volume to 60 mL. The sample is incubated overnight at 37°C in an Eppendorf thermomixer set at 700 RPM. Adalimumab lot AFP04C is also digested with PNGase F as a control.

After PNGase F treatment, the samples are incubated at 95°C for 5 minutes in an Eppendorf thermomixer set at 750 RPM to precipitate out the proteins, then the samples are placed in an Eppendorf centrifuge for 2 minutes at 10,000 RPM to spin down the precipitated proteins. The supernatent containing the oligosaccharides are transferred to a 500 mL Eppendorf tube and dried in a speed-vac at 65°C.

The oligosaccharides are labeled with 2AB using a 2AB labeling kit purchased from Prozyme (cat# GKK-404, lot# 132026). The labeling reagent is prepared according to the manufacturer's instructions. Acetic acid (150 mL, provided in kit) is added to the DMSO vial (provided in kit) and mixed by pipeting the solution up and down several times. The acetic acid/DMSO mixture (100 mL) is transferred to a vial of 2-AB dye (just prior to use) and mixed until the dye is fully dissolved. The dye solution is then added to a vial of reductant (provided in kit) and mixed well (labeling reagent). The labeling

reagent (5 mL) is added to each dried oligosaccharide sample vial, and mixed thoroughly. The reaction vials are placed in an Eppendorf thermomixer set at 65°C and 700-800 RPM for 2 hours of reaction.

After the labeling reaction, the excess fluorescent dye is removed using GlycoClean S Cartridges from Prozyme (cat# GKI-4726). Prior to adding the samples, the cartridges are washed with 1 mL of milli-Q water followed with 5 ishes of 1 mL 30% acetic acid solution. Just prior to adding the samples, 1 mL of acetonitrile (Burdick and Jackson, cat# AH015-4) is added to the cartridges.

After all of the acetonitrile passed through the cartridge, the sample is spotted onto the center of the freshly washed disc and allowed to adsorb onto the disc for 10 minutes. The disc is washed with 1 mL of acetonitrile followed by five ishes of 1 mL of 96% acetonitrile. The cartridges are placed over a 1.5 mL Eppendorf tube and the 2-AB labeled oligosaccharides are eluted with 3 ishes (400 mL each ish) of milli Q water.

The oligosaccharides are separated using a Glycosep N HPLC (cat# GKI-4728) column connected to a Shimadzu HPLC system. The Shimadzu HPLC system consisted of a system controller, degasser, binary pumps, autosampler with a sample cooler, and a fluorescent detector.

Example 1.3.3.2.K: Stability at Elevated Temperatures

The buffer of antibody is either 5.57 mM sodium phosphate monobasic, 8.69 mM sodium phosphate dibasic, 106.69 mM NaCl, 1.07 mM sodium citrate, 6.45 mM citric acid, 66.68 mM mannitol, 0.1% (w/v) Tween, pH 5.2; or 10 mM histidine, 10 mM methionine, 4% mannitol, pH 5.9 using Amicon ultra centrifugal filters. The final concentration of the antibodies is adjusted to 2 mg/mL with the appropriate buffers. The antibody solutions are then filter sterized and 0.25 mL aliquots are prepared under sterile conditions. The aliquots are left at either -80°C, 5°C, 25°C, or 40°C for 1, 2 or 3 weeks. At the end of the incubation period, the samples are analyzed by size exclusion chromatography and SDS-PAGE.

The stability samples are analyzed by SDS-PAGE under both reducing and non-reducing conditions. The procedure used is the same as described herein. The gels are stained overnight with colloidal blue stain (Invitrogen cat# 46-7015, 46-7016) and

destained with Milli-Q water until the background is clear. The stained gels are then scanned using an Epson Expression scanner (model 1680, S/N DASX003641). To obtain more sensitivity, the same gels are silver stained using silver staining kit (Owl Scientific) and the recommended procedures given by the manufacturer is used.

Example 1.3.3.2.L: Differential Scanning Calorimetry (DSC)

A protein in aqueous solution is in equilibrium between the native (folded) conformation and its denatured (unfolded) conformation. The stability of the native state is based on the magnitude of the Gibbs free energy (DG) of the system and the thermodynamic relationship between enthalpy (DH) and entropy (DS) changes. A positive DG indicates the native state is more stable than the denatured state – the more positive the DG, the greater the stability. For a protein to unfold, stabilizing forces need to be broken. Conformational entropy overcomes stabilizing forces allowing the protein to unfold at temperatures where entropy becomes dominant. Differential Scanning Calorimetry (DSC) measures DH of protein unfolding due to heat denaturation. As a general rule, the higher the transition midpoint (the Tm), the more stable the protein at lower temperatures. During the same experiment DSC also measures the change in heat capacity (DCp) for protein denaturation. Heat capacity changes associated with protein unfolding are primarily due to changes in hydration of side chains that were buried in the native state, but become solvent exposed in the denatured state. DSC is a predictor of liquid formulation stability for proteins and other biological macromolecules (Remmele, R.L. et al. (2000) *BioPharm* 13: 36-46; Remmele, R.L. et al. (1998) *Pharm. Res.* 15:200-208).

A different application of DSC is the characterization of protein structure, as demonstrated for a variety of monoclonal antibodies, e.g., Adalimumab (Humira), trastuzumab (Herceptin), bevacizumab (Avastin) and other antibodies (Ionescu, R. et al. (2008) J. Pharmaceut. Sci. 97(4):1414-1426). An IgG antibody typically shows three unfolding transitions (Tm): unfolding of the intact antibody is associated with the melting of the CH2 domain in the Fc fragment, melting of the CH3 domain in the Fc fragment, and melting of the Fab fragment (Ionescu, R. et al. (2008) J. Pharmaceut. Sci. 97(4):1414-1426) (Figure 5 and 7).

Prior to DSC analysis, proteins are dialized into a suitable buffer system using Slide-A-Lyzer Cassettes. This buffer system (10 mM phosphate, 10 mM citrate) is also used as a reference/blank for the DSC measurement. Both parent antibodies and the DVD-Ig molecule are analyzed at 2 mg/mL. An automated VP-DSC with Capillary Cell (Microcal) DSC instrument is used. Unfolding of the molecules is studied applying a 1°C/minute scan rate over a 25°C - 95°C

temperature range. Other measurement parameters are: Fitting period: 16 sec, pre-scan wait: 10 min, feedback mode: none.

Example 1.4: Generation of a DVD-Ig

DVD-Ig molecules capable of binding two antigens are constructed using two parent monoclonal antibodies, one against human antigen A, and the other against human antigen B, selected as described herein.

Example 1.4.1: Generation of a DVD-Ig Having Two Linker Lengths

A constant region containing γ1 Fc with mutations at 234, and 235 to eliminate ADCC/CDC effector functions is used. Four different anti-A/B DVD-Ig constructs are generated: 2 with short linker and 2 with long linker, each in two different domain orientations: V_A-V_B-C and V_B-V_A-C (see Table 3). The linker sequences, derived from the N-terminal sequence of human Cl/Ck or CH1 domain, are as follows:

For DVDAB constructs:

light chain (if anti-A has λ):Short linker: QPKAAP (SEQ ID NO: 15); Long linker: QPKAAPSVTLFPP (SEQ ID NO: 16)

light chain (if anti-A has κ):Short linker: TVAAP (SEQ ID NO: 13); Long linker: TVAAPSVFIFPP (SEQ ID NO: 14)

heavy chain (γ1): Short linker: ASTKGP (SEQ ID NO: 21); Long linker: ASTKGPSVFPLAP (SEQ ID NO: 22)

For DVDBA constructs:

light chain (if anti-B has λ):Short linker: QPKAAP (SEQ ID NO: 15); Long linker: QPKAAPSVTLFPP (SEQ ID NO: 16)

light chain (if anti-B has k):Short linker: TVAAP (SEQ ID NO: 13); Long linker: TVAAPSVFIFPP (SEQ ID NO: 14)

heavy chain (γ1): Short linker: ASTKGP (SEQ ID NO: 21); Long linker: ASTKGPSVFPLAP (SEQ ID NO: 22)

Heavy and light chain constructs are subcloned into the pBOS expression vector, and expressed in COS cells, followed by purification by Protein A chromatography. The purified materials are subjected to SDS-PAGE and SEC analysis.

The Table 3 below describes the heavy chain and light chain constructs used to express each anti-A/B DVD-Ig protein.

Table 3: Constructs To Express Anti-A/B DVD-Ig Proteins

DVD-Ig protein	Heavy chain construct	Light chain construct
DVDABSL	DVDABHC-SL	DVDABLC-SL
DVDABLL	DVDABHC-LL	DVDABLC-LL
DVDBASL	DVDBAHC-SL	DVDBALC-SL
DVDBALL	DVDBAHC-LL	DVDBALC-LL

Example 1.4.2: Molecular Cloning Of DNA Constructs For DVDABSL And DVDABLL

To generate heavy chain constructs DVDABHC-LL and DVDABHC-SL, a VH domain of A antibody is PCR amplified using specific primers (3' primers contain short/long liner sequence for SL/LL constructs, respectively); meanwhile VH domain of B antibody is amplified using specific primers (5' primers contains short/long liner sequence for SL/LL constructs, respectively). Both PCR reactions are performed according to standard PCR techniques and procedures. The two PCR products are gel-purified, and used together as overlapping template for the subsequent overlapping PCR reaction. The overlapping PCR products are subcloned into Srf I and Sal I double digested pBOS-hCγ1,z non-a mammalian expression vector (Abbott) by using standard homologous recombination approach.

To generate light chain constructs DVDABLC-LL and DVDABLC-SL, VL domain of A antibody is PCR amplified using specific primers (3' primers contain short/long liner sequence for SL/LL constructs, respectively); meanwhile VL domain of B antibody is amplified using specific primers (5' primers contains short/long liner sequence for SL/LL constructs, respectively). Both PCR reactions are performed according to standard PCR techniques and procedures. The two PCR products are gel-purified, and used together as overlapping template for the subsequent overlapping PCR reaction using standard PCR conditions. The overlapping PCR products are subcloned into Srf I and Not I double digested pBOS-hCk mammalian expression vector (Abbott) by using standard homologous recombination approach. Similar approach has been used to generate DVDBASL and DVDBALL as described below.

Example 1.4.3: Molecular Cloning Of DNA Constructs For DVDBASL And DVDBALL

To generate heavy chain constructs DVDBAHC-LL and DVDBAHC-SL, VH domain of antibody B is PCR amplified using specific primers (3' primers contain short/long liner sequence for SL/LL constructs, respectively); meanwhile VH domain of antibody A is amplified using specific primers (5' primers contains short/long liner sequence for SL/LL constructs, respectively). Both PCR reactions are performed

according to standard PCR techniques and procedures. The two PCR products are gel-purified, and used together as overlapping template for the subsequent overlapping PCR reaction using standard PCR conditions. The overlapping PCR products are subcloned into Srf I and Sal I double digested pBOS-hCγ1,z non-a mammalian expression vector (Abbott) by using standard homologous recombination approach.

To generate light chain constructs DVDBALC-LL and DVDBALC-SL, VL domain of antibody B is PCR amplified using specific primers (3' primers contain short/long liner sequence for SL/LL constructs, respectively); meanwhile VL domain of antibody A is amplified using specific primers (5' primers contains short/long liner sequence for SL/LL constructs, respectively). Both PCR reactions are performed according to standard PCR techniques and procedures. The two PCR products are gel-purified, and used together as overlapping template for the subsequent overlapping PCR reaction using standard PCR conditions. The overlapping PCR products are subcloned into Srf I and Not I double digested pBOS-hCk mammalian expression vector (Abbott) by using standard homologous recombination approach.

Example 1.4.4: Preparation Of DVD-Ig Vector Constructs

Parent antibody amino acid sequences for specific antibodies, which recognize specific antigens or epitopes thereof, for incorporation into a DVD-Ig can be obtained by preparation of hybridomas as described above or can be obtained by sequencing known antibody proteins or nucleic acids. In addition, known sequences can be obtained from the literature. The sequences can be used to synthesize nucleic acids using standard DNA synthesis or amplification technologies and assembling the desired antibody fragments into expression vectors, using standard recombinant DNA technology, for expression in cells.

For example, nucleic acid codons were determined from amino acids sequences and oligonucleotide DNA was synthesized by Blue Heron Biotechnology, Inc. (www.blueheronbio.com) Bothell, WA USA. The oligonucleotides were assembled into 300-2,000 base pair double-stranded DNA fragments, cloned into a plasmid vector and sequence-verified. Cloned fragments were assembled using an enzymatic process to yield the complete gene and subcloned into an expression vector. (See 7,306,914; 7,297,541; 7,279,159; 7,150,969; 20080115243; 20080102475; 20080081379; 20080075690; 20080063780; 20080050506;

20080038777; 20080022422; 20070289033; 20070287170; 20070254338; 20070243194; 20070225227; 20070207171; 20070150976; 20070135620; 20070128190; 20070104722; 20070092484; 20070037196; 20070028321; 20060172404; 20060162026; 20060153791; 20030215458; 20030157643).

A group of pHybE vectors (US Patent Application Serial No. 61/021,282) were used for parental antibody and DVD-Ig cloning. V1, derived from pJP183; pHybE-hCg1,z,non-a V2, was used for cloning of antibody and DVD heavy chains with a wildtype constant region. V2, derived from pJP191; pHybE-hCk V2, was used for cloning of antibody and DVD light chains with a kappa constant region. V3, derived from pJP192; pHybE-hCl V2, was used for cloning of antibody and DVDs light chains with a lambda constant region. V4, built with a lambda signal peptide and a kappa constant region, was used for cloning of DVD light chains with a lambda-kappa hybrid V domain. V5, built with a kappa signal peptide and a lambda constant region, was used for cloning of DVD light chains with a kappa-lambda hybrid V domain. V7, derived from pJP183; pHybE-hCg1,z,non-a V2, was used for cloning of antibody and DVD heavy chains with a (234,235 AA) mutant constant region.

Referring to Table 4, a number of vectors were used in the cloning of the parent antibodies and DVD-Ig VH and VL chains.

Table 4: Vectors Used to Clone Parent Antibodies and DVD-Igs

ID	Heavy chain vector	Light chain vector
AB003	V1	V2
AB014	V1	V2
AB011	V1	V2
AB010	V1	V3
AB020	V1	V2
AB004	V1	V2
AB029	V1	V2
AB032	V1	V2
AB043	V1	V2
AB040	V1	V2
AB048	V1	V2
AB044	V1	V2
AB045	V1	V2
AB046	V1	V2
AB049	V1	V2
AB052	V1	V2
AB054	V1	V2
AB033	V1	V2
DVD161	V1	V2
DVD162	V1	V2

ID	Heavy chain vector	Light chain vector
DVD163	V1	V2
DVD164	V1	V2
DVD155	V1	V2
DVD156	V1	V2
DVD157	V1	V2
DVD158	V1	V2
DVD249	V1	V2
DVD250	V1	V2
DVD227	V1	V2
DVD228	V1	V2
DVD199	V1	V2
DVD200	V1	V2
DVD213	V1	V2
DVD214	V1	V2
DVD241	V1	V2
DVD242	V1	V2
DVD153	V1	V2
DVD154	V1	V2
DVD251	V1	V2
DVD252	V1	V2
DVD151	V1	V2
DVD152	V1	V2
DVD311	V1	V2
DVD312	V1	V2
DVD313	V1	V2
DVD314	V1	V2
DVD315	V1	V2
DVD316	V1	V2
DVD317	V1	V5
DVD318	V1	V4
DVD319	V1	V2
DVD320	V7	V2

Example 1.4.4.2: Transfection And Expression In 293 Cells

The DVD-Ig vector constructs are tranfected into 293 cells for production of DVD-Ig protein. The 293 transient transfection procedure used is a modification of the methods published in Durocher et al. (2002) Nucleic Acids Res. 30(2):E9 and Pham et al. (2005) Biotech. Bioengineering 90(3):332-44. Reagents that were used in the transfection included:

- HEK 293-6E cells (human embryonic kidney cell line stably expressing EBNA1; obtained from National Research Council Canada) cultured in disposable Erlenmeyer flasks in a humidified incubator set at 130 rpm, 37°C and 5% CO_2.

- Culture medium: FreeStyle 293 Expression Medium (Invitrogen 12338-018) plus 25 µg/mL Geneticin (G418) (Invitrogen 10131-027) and 0.1% Pluronic F-68 (Invitrogen 24040-032).

- Transfection medium: FreeStyle 293 Expression Medium plus 10 mM HEPES (Invitrogen 15630-080).

- Polyethylenimine (PEI) stock: 1 mg/mL sterile stock solution, pH 7.0, prepared with linear 25kDa PEI (Polysciences) and stored at less than -15°C.

- Tryptone Feed Medium: 5% w/v sterile stock of Tryptone N1 (Organotechnie, 19554) in FreeStyle 293 Expression Medium.

Cell preparation for transfection: Approximately 2 - 4 hours prior to transfection, HEK 293-6E cells are harvested by centrifugation and resuspended in culture medium at a cell density of approximately 1 million viable cells per mL. For each transfection, 40 mL of the cell suspension is transferred into a disposable 250-mL Erlenmeyer flask and incubated for 2 - 4 hours.

Transfection: The transfection medium and PEI stock are prewarmed to room temperature (RT). For each transfection, 25µg of plasmid DNA and 50µg of polyethylenimine (PEI) are combined in 5 mL of transfection medium and incubated for 15 – 20 minutes at RT to allow the DNA:PEI complexes to form. For the BR3-Ig transfections, 25µg of BR3-Ig plasmid is used per transfection. Each 5-mL DNA:PEI complex mixture is added to a 40-mL culture prepared previously and returned to the humidified incubator set at 130 rpm, 37°C and 5% CO_2. After 20-28 hours, 5 mL of Tryptone Feed Medium is added to each transfection and the cultures are continued for six days.

Example 1.4.5: Characterization And Lead Selection Of A/B DVD Igs

The binding affinities of anti-A/B DVD-Igs are analyzed on BIAcore against both protein A and protein B. The tetravalent property of the DVD-Ig is examined by multiple binding studies on BIAcore. Meanwhile, the neutralization potency of the DVD-Igs for protein A and protein B are assessed by bioassays, respectively, as described herein. The DVD-Ig molecules that best retain the affinity and potency of the original parental mAbs

are selected for in-depth physicochemical and bio-analytical (rat PK) characterizations as described herein for each mAb. Based on the collection of analyses, the final lead DVD-Ig is advanced into CHO stable cell line development, and the CHO-derived material is employed in stability, pharmacokinetic and efficacy studies in cynomolgus monkey, and preformulation activities.

Example 2: Construction, Expression, And Purification Of Anti-Murine TNFα/ Anti-PGE_2 Dual Variable Domain Immunoglobulin (DVD-Igs)

A DVD-Ig capable of binding murine TNFα and PGE2 was generated. Briefly, parent mAbs include two high affinity murine Abs, anti-TNFα (clone 8C11) and anti-PGE2 (clone 2B5-8.0), respectively. The VL/VH genes of anti-TNFα monoclonal antibody clone 8C11 hybridoma were isolated by RT-PCR using the mouse Ig Primer Kit (Novagen, Madison, WI). The VL/VH protein sequences of 2B5-8.0 were obtained as described in Example 1.2.4 and were converted to DNA sequences. Different linkers were used between the two variable domains in both the light chain (e.g., ADAAP) and the heavy chain (e.g., AKTTPP). These linker sequences, selected from the N-termini of murine Ck and CH1, are a natural extension of the variable domains and exhibit a flexible conformation without significant secondary structures based on the analysis of several Fab crystal structures. The detailed procedure of the PCR cloning is described below:

Example 2.2.1: Molecular Cloning of Murine Anti-Murine TNFα/anti-PGE_2 DVD-Ig With Murine Linkers

Methods for making DVD-Ig molecules are described in US Patent Publication No. 20070071675. The amino acid sequence of VH and VL regions for various recombinant antibodies specific for PGE2 are shown in Table 5. Using overlapping PCR, six murine anti-murine TNFα/anti-PGE2 DVD-Ig molecules with either no mouse linker (mNL), murine short linker (mSL), or murine long linker (mLL) were generated and the protein sequences of the VH and the VL are listed in Table 6 below. The DNA encoding the VH of the DVD-Ig molecules was fused to mouse heavy chain IgG1 constant region, and the DNA encoding the VL of the DVD-Ig molecules was fused to mouse light chain k constant region, respectively. The protein expression and purification of DVD-Ig

molecules are essentially the same as that described in Example 1.3 and in US Patent Publication No. 20070071675.

Table 5: Recombinant Antibodies Specific to Prostaglandin E2

SEQ ID No.	Protein region		Sequence
			12345678901234567890123456789 0
80	VH 2B5-7.0		QVQLQQSGPELVRPGSSVKISCKAS**GYTFT** **KYWLG**WVKQRPGHGLEWIG**DIYPGYDYTHY** **NEKFKD**KATLTVDTSSSTAYMQLSSLTSED SAVYFCAR**SDGSSTY**WGQGTLVTVSA
81	VL 2B5-7.0		DVLMTQTPLSLPVSLGDQASISC**TSSQNIV** **HSNGNTYLE**WYLQRPGQSPKLLIY**KVSNRF** **SG**VPDRFSGSGSGTVFTLKISRVEAEDLGV YYC**FQVSHVPYT**FGGGTKLEIKR
82	VH 2B5-8.0		QVQLQQSGPELVRPGSSVKISCKAS**GYTFT** **KYWLG**WVKQRPGHGLEWIG**DIYPGYDYTHY** **NEKFKD**KATLTVDTSSSTAYMQLSSLTSED SAIYYCAR**SDGSSTY**WGQGTLVTVSA
83	VL 2B5-8.0		DVLMTQTPLSLPVSLGDQASISC**TSSQNIV** **HSNGNTYLE**WYLQRPGQSPKLLIY**KVSNRF** **SG**VPDRFSGSGSGTVFTLKISRVEAEDLGV YYC**FQVSHVPYT**FGGGTKLEIKR
84	VH 2B5-9.0		QVQLQQSGPELVRPGSSVKISCKAS**GYTFT** **KYWLG**WVKQRPGHGLEWIG**DIYPYGDYTHY** **NEKFKD**KATLTVDTSSSTAYMQLSSLTSED SAVYFCAR**SDGSSTY**WGQGTLVTVSA
85	VL 2B5-9.0		DVLMTQTPLSLPVSLGDQASISC**TSSQNIV** **HSNGNTYLE**WYLQRPGQSPKLLIY**KVSNRF** **SG**VPDRFSGSGSGTVFTLKISRVEAEDLGV YYC**FQVSHVPYTF**GGGTKLEIKR

Figure 2 shows binding of the antibodies in Table 5 to PGE2-biotin in a direct bind ELISA. Figure 3 shows inhibition of the cellular response to PGE2 in an EP4 binding assay.

Table 6: List Of Amino Acid Sequences Of VH And VL Regions Of Anti-Murine TNFα/Anti-PGE_2 DVD-Ig With Murine Linkers

SEQ ID No.	Protein region		Sequence
			12345678901234567890123456789O
86	**VH 8C11-mNL-2B5**		**EFQLQQSGPELVKPGASVRISCKASGYSFT DYNMNWVKQSNGKSLEWVGVINPNYGSSTY NQKFKGKATLTVDQSSSTAYMQLNSLTSED SAVYYCARKWGQLGRGFFDVWGTGTTVTVS** SQVQLQQSGPELVRPGSSVKISCKASGYTF TKYWLGWVKQRPGHGLEWIGDIYPGYDYTH YNEKFKDKATLTVDTSSSTAYMQLSSLTSE DSAIYYCARSDGSSTYWGQGTLVTVSA
87	VH 8C11	Residues 1-121 of SEQ ID NO.:86	**EFQLQQSGPELVKPGASVRISCKASGYSFT DYNMNWVKQSNGKSLEWVGVINPNYGSSTY NQKFKGKATLTVDQSSSTAYMQLNSLTSED SAVYYCARKWGQLGRGFFDVWGTGTTVTVS S**
	MNL FOR HC	No	
88	VH 2B5	Residues 122-237 of SEQ ID NO.:86	QVQLQQSGPELVRPGSSVKISCKASGYTFT KYWLGWVKQRPGHGLEWIGDIYPGYDYTHY NEKFKDKATLTVDTSSSTAYMQLSSLTSED SAIYYCARSDGSSTYWGQGTLVTVSA
			12345678901234567890123456789O
89	**VL 8C11-mNL-2B5**		**QIVLSQSPAILSASPGEKVTMTCRASSSVS YMHWFQQKPGSSPKPWIYATSNLASGVPAR FSGSGSGTSYSLTISRVEAEDAATYYCQQW SSSPLTFGAGTKLELKR**DVLMTQTPLSLPV SLGDQASISCTSSQNIVHSNGNTYLEWYLQ RPGQSPKLLIYKVSNRFSGVPDRFSGSGSG TVFTLKISRVEAEDLGVYYCFQVSHVPYTF GGGTKLEIKR
90	VL 8C11	Residues 1-107 of SEQ ID NO.:89	**QIVLSQSPAILSASPGEKVTMTCRASSSVS YMHWFQQKPGSSPKPWIYATSNLASGVPAR FSGSGSGTSYSLTISRVEAEDAATYYCQQW SSSPLTFGAGTKLELKR**
	MNL FOR LC	No	
91	VL 2B5	Residues 108-220 of SEQ ID NO.:89	DVLMTQTPLSLPVSLGDQASISCTSSQNIV HSNGNTYLEWYLQRPGQSPKLLIYKVSNRF SGVPDRFSGSGSGTVFTLKISRVEAEDLGV YYCFQVSHVPYTFGGGTKLEIKR
			12345678901234567890123456789O
92	**VH 8C11-mSL-2B5**		EFQLQQSGPELVKPGASVRISCKASGYSFT DYNMNWVKQSNGKSLEWVGVINPNYGSSTY NQKFKGKATLTVDQSSSTAYMQLNSLTSED SAVYYCARKWGQLGRGFFDVWGTGTTVTVS S**AKTTAP**QVQLQQSGPELVRPGSSVKISCK ASGYTFTKYWLGWVKQRPGHGLEWIGDIYP GYDYTHYNEKFKDKATLTVDTSSSTAYMQL SSLTSEDSAIYYCARSDGSSTYWGQGTLVT VSA
87	VH 8C11	Residues 1-121 of SEQ ID NO.:92	EFQLQQSGPELVKPGASVRISCKASGYSFT DYNMNWVKQSNGKSLEWVGVINPNYGSSTY NQKFKGKATLTVDQSSSTAYMQLNSLTSED SAVYYCARKWGQLGRGFFDVWGTGTTVTVS S

SEQ ID No.	Protein region		Sequence
			1234567890123456789012345678900
19	MSL FOR HC	Residues 122-127 of SEQ ID NO.:92	**AKTTAP**
88	VH 2B5	Residues 128-243 of SEQ ID NO.:92	QVQLQQSGPELVRPGSSVKISCKASGYTFT KYWLGWVKQRPGHGLEWIGDIYPGYDYTHY NEKFKDKATLTVDTSSSTAYMQLSSLTSED SAIYYCARSDGSSTYWGQGTLVTVSA
			1234567890123456789012345678900
93	**VL 8C11-mSL-2B5**		QIVLSQSPAILSASPGEKVTMTCRASSSVS YMHWFQQKPGSSPKPWIYATSNLASGVPAR FSGSGSGTSYSLTISRVEAEDAATYYCQQW SSSPLTFGAGTKLELKR**ADAAP**DVLMTQTP LSLPVSLGDQASISCTSSQNIVHSNGNTYL EWYLQRPGQSPKLLIYKVSNRFSGVPDRFS GSGSGTVFTLKISRVEAEDLGVYYCFQVSH VPYTFGGGTKLEIKR
90	VL 8C11	Residues 1-107 of SEQ ID NO.:93	QIVLSQSPAILSASPGEKVTMTCRASSSVS YMHWFQQKPGSSPKPWIYATSNLASGVPAR FSGSGSGTSYSLTISRVEAEDAATYYCQQW SSSPLTFGAGTKLELKR
11	MSL FOR LC	Residues 108-112 of SEQ ID NO.:93	**ADAAP**
91	VL 2B5	Residues 113-225 of SEQ ID NO.:93	DVLMTQTPLSLPVSLGDQASISCTSSQNIV HSNGNTYLEWYLQRPGQSPKLLIYKVSNRF SGVPDRFSGSGSGTVFTLKISRVEAEDLGV YYCFQVSHVPYTFGGGTKLEIKR
			1234567890123456789012345678900
94	**VH 8C11-mLL-2B5**		EFQLQQSGPELVKPGASVRISCKASGYSFT DYNMNWVKQSNGKSLEWVGVINPNYGSSTY NQKFKGKATLTVDQSSSTAYMQLNSLTSED SAVYYCARKWGQLGRGFFDVWGTGTTVTVS S**AKTTAPSVYPLAP**QVQLQQSGPELVRPGS SVKISCKASGYTFTKYWLGWVKQRPGHGLE WIGDIYPGYDYTHYNEKFKDKATLTVDTSS STAYMQLSSLTSEDSAIYYCARSDGSSTYW GQGTLVTVSA
87	VH 8C11	Residues 1-121 of SEQ ID NO.:94	EFQLQQSGPELVKPGASVRISCKASGYSFT DYNMNWVKQSNGKSLEWVGVINPNYGSSTY NQKFKGKATLTVDQSSSTAYMQLNSLTSED SAVYYCARKWGQLGRGFFDVWGTGTTVTVS S
20	MLL FOR HC	Residues 121-134 of SEQ ID NO.:94	**AKTTAPSVYPLAP**
88	VH 2B5	Residues 135-250 of SEQ ID NO.:94	QVQLQQSGPELVRPGSSVKISCKASGYTFT KYWLGWVKQRPGHGLEWIGDIYPGYDYTHY NEKFKDKATLTVDTSSSTAYMQLSSLTSED SAIYYCARSDGSSTYWGQGTLVTVSA
			1234567890123456789012345678900

SEQ ID No.	Protein region		Sequence
			1234567890123456789012345678900
95	VL 8C11-mLL-2B5		QIVLSQSPAILSASPGEKVTMTCRASSSVS YMHWFQQKPGSSPKPWIYATSNLASGVPAR FSGSGSGTSYSLTISRVEAEDAATYYCQQW SSSPLTFGAGTKLELKR**ADAAPTVSIFPP**D VLMTQTPLSLPVSLGDQASISCTSSQNIVH SNGNTYLEWYLQRPGQSPKLLIYKVSNRFS GVPDRFSGSGSGTVFTLKISRVEAEDLGVY YCFQVSHVPYTFGGGTKLEIKR
90	VL 8C11	Residues 1-107 of SEQ ID NO.:95	QIVLSQSPAILSASPGEKVTMTCRASSSVS YMHWFQQKPGSSPKPWIYATSNLASGVPAR FSGSGSGTSYSLTISRVEAEDAATYYCQQW SSSPLTFGAGTKLELKR
12	MLL FOR LC	Residues 108-119 of SEQ ID NO.:95	**ADAAPTVSIFPP**
91	VL 2B5	Residues 120-232 of SEQ ID NO.:95	DVLMTQTPLSLPVSLGDQASISCTSSQNIV HSNGNTYLEWYLQRPGQSPKLLIYKVSNRF SGVPDRFSGSGSGTVFTLKISRVEAEDLGV YYCFQVSHVPYTFGGGTKLEIKR
			1234567890123456789012345678900
96	VH 2B5-mNL-8C11		QVQLQQSGPELVRPGSSVKISCKASGYTFT KYWLGWVKQRPGHGLEWIGDIYPGYDYTHY NEKFKDKATLTVDTSSSTAYMQLSSLTSED SAIYYCARSDGSSTYWGQGTLVTVSA**EFQL QQSGPELVKPGASVRISCKASGYSFTDYNM NWVKQSNGKSLEWVGVINPNYGSSTYNQKF KGKATLTVDQSSSTAYMQLNSLTSEDSAVY YCARKWGQLGRGFFDVWGTGTTVTVSS**
88	VH 2B5	Residues 1-116 of SEQ ID NO.:96	QVQLQQSGPELVRPGSSVKISCKASGYTFT KYWLGWVKQRPGHGLEWIGDIYPGYDYTHY NEKFKDKATLTVDTSSSTAYMQLSSLTSED SAIYYCARSDGSSTYWGQGTLVTVSA
	MNL FOR HC	No	
87	VH 8C11	Residues 117-237 of SEQ ID NO.:96	**EFQLQQSGPELVKPGASVRISCKASGYSFT DYNMNWVKQSNGKSLEWVGVINPNYGSSTY NQKFKGKATLTVDQSSSTAYMQLNSLTSED SAVYYCARKWGQLGRGFFDVWGTGTTVTVS S**
			1234567890123456789012345678900
97	VL 2B5-mNL-8C11		DVLMTQTPLSLPVSLGDQASISCTSSQNIV HSNGNTYLEWYLQRPGQSPKLLIYKVSNRF SGVPDRFSGSGSGTVFTLKISRVEAEDLGV YYCFQVSHVPYTFGGGTKLEIKR**QIVLSQS PAILSASPGEKVTMTCRASSSVSYMHWFQQ KPGSSPKPWIYATSNLASGVPARFSGSGSG TSYSLTISRVEAEDAATYYCQQWSSSPLTF GAGTKLELKR**
91	VL 2B5	Residues 1-113 of SEQ ID NO.:97	DVLMTQTPLSLPVSLGDQASISCTSSQNIV HSNGNTYLEWYLQRPGQSPKLLIYKVSNRF SGVPDRFSGSGSGTVFTLKISRVEAEDLGV YYCFQVSHVPYTFGGGTKLEIKR
	MNL FOR LC	No	
90	VL 8C11	Residues 114-220 of SEQ ID NO.:97	**QIVLSQSPAILSASPGEKVTMTCRASSSVS YMHWFQQKPGSSPKPWIYATSNLASGVPAR FSGSGSGTSYSLTISRVEAEDAATYYCQQW SSSPLTFGAGTKLELKR**
			1234567890123456789012345678900

SEQ ID No.	Protein region		Sequence
			123456789**0**123456789**0**123456789**0**
98	**VH 2B5-mSL-8C11**		QVQLQQSGPELVRPGSSVKISCKASGYTFT KYWLGWVKQRPGHGLEWIGDIYPGYDYTHY NEKFKDKATLTVDTSSSTAYMQLSSLTSED SAIYYCARSDGSSTYWGQGTLVTVSA**AKTT APE**FQLQQSGPELVKPGASVRISCKASGYS FTDYNMNWVKQSNGKSLEWVGVINPNYGSS TYNQKFKGKATLTVDQSSSTAYMQLNSLTS EDSAVYYCARKWGQLGRGFFDVWGTGTTVT VSS
88	VH 2B5	Residues 1-116 of SEQ ID NO.:98	QVQLQQSGPELVRPGSSVKISCKASGYTFT KYWLGWVKQRPGHGLEWIGDIYPGYDYTHY NEKFKDKATLTVDTSSSTAYMQLSSLTSED SAIYYCARSDGSSTYWGQGTLVTVSA
19	MSL FOR HC	Residues 117-122 of SEQ ID NO.:98	**AKTTAP**
87	VH 8C11	Residues 123-243 of SEQ ID NO.:98	EFQLQQSGPELVKPGASVRISCKASGYSFT DYNMNWVKQSNGKSLEWVGVINPNYGSSTY NQKFKGKATLTVDQSSSTAYMQLNSLTSED SAVYYCARKWGQLGRGFFDVWGTGTTVTVS S
			123456789**0**123456789**0**123456789**0**
99	**VL 2B5-mSL-8C11**		DVLMTQTPLSLPVSLGDQASISCTSSQNIV HSNGNTYLEWYLQRPGQSPKLLIYKVSNRF SGVPDRFSGSGSGTVFTLKISRVEAEDLGV YYCFQVSHVPYTFGGGTKLEIKR**ADAAP**QI VLSQSPAILSASPGEKVTMTCRASSSVSYM HWFQQKPGSSPKPWIYATSNLASGVPARFS GSGSGTSYSLTISRVEAEDAATYYCQQWSS SPLTFGAGTKLELKR
91	VL 2B5	Residues 1-113 of SEQ ID NO.:99	DVLMTQTPLSLPVSLGDQASISCTSSQNIV HSNGNTYLEWYLQRPGQSPKLLIYKVSNRF SGVPDRFSGSGSGTVFTLKISRVEAEDLGV YYCFQVSHVPYTFGGGTKLEIKR
11	MSL FOR LC	Residues 114-118 of SEQ ID NO.:99	**ADAAP**
90	VL 8C11	Residues 119-225 of SEQ ID NO.:99	QIVLSQSPAILSASPGEKVTMTCRASSSVS YMHWFQQKPGSSPKPWIYATSNLASGVPAR FSGSGSGTSYSLTISRVEAEDAATYYCQQW SSSPLTFGAGTKLELKR
			123456789**0**123456789**0**123456789**0**
100	**VH 2B5-mLL-8C11**		QVQLQQSGPELVRPGSSVKISCKASGYTFT KYWLGWVKQRPGHGLEWIGDIYPGYDYTHY NEKFKDKATLTVDTSSSTAYMQLSSLTSED SAIYYCARSDGSSTYWGQGTLVTVSA**AKTT APSVYPLAP**EFQLQQSGPELVKPGASVRIS CKASGYSFTDYNMNWVKQSNGKSLEWVGVI NPNYGSSTYNQKFKGKATLTVDQSSSTAYM QLNSLTSEDSAVYYCARKWGQLGRGFFDVW GTGTTVTVSS
88	VH 2B5	Residues 1-116 of SEQ ID NO.:100	QVQLQQSGPELVRPGSSVKISCKASGYTFT KYWLGWVKQRPGHGLEWIGDIYPGYDYTHY NEKFKDKATLTVDTSSSTAYMQLSSLTSED SAIYYCARSDGSSTYWGQGTLVTVSA

SEQ ID No.	Protein region		Sequence
			123456789012345678901234567890
20	MLL FOR HC	Residues 117-129 of SEQ ID NO.:100	**AKTTAPSVYPLAP**
87	VH 8C11	Residues 130-250 of SEQ ID NO.:100	EFQLQQSGPELVKPGASVRISCKASGYSFT DYNMNWVKQSNGKSLEWVGVINPNYGSSTY NQKFKGKATLTVDQSSSTAYMQLNSLTSED SAVYYCARKWGQLGRGFFDVWGTGTTVTVS S
			123456789012345678901234567890
101	**VL 2B5-mLL-8C11**		DVLMTQTPLSLPVSLGDQASISCTSSQNIV HSNGNTYLEWYLQRPGQSPKLLIYKVSNRF SGVPDRFSGSGSGTVFTLKISRVEAEDLGV YYCFQVSHVPYTFGGGTKLEIKR**ADAAPTV SIFPP**QIVLSQSPAILSASPGEKVTMTCRA SSSVSYMHWFQQKPGSSPKPWIYATSNLAS GVPARFSGSGSGTSYSLTISRVEAEDAATY YCQQWSSSPLTFGAGTKLELKR
91	VL 2B5	Residues 1-113 of SEQ ID NO.:101	DVLMTQTPLSLPVSLGDQASISCTSSQNIV HSNGNTYLEWYLQRPGQSPKLLIYKVSNRF SGVPDRFSGSGSGTVFTLKISRVEAEDLGV YYCFQVSHVPYTFGGGTKLEIKR
12	MLL FOR LC	Residues 114-125 of SEQ ID NO.:101	**ADAAPTVSIFPP**
90	VL 8C11	Residues 126-232 of SEQ ID NO.:101	QIVLSQSPAILSASPGEKVTMTCRASSSVS YMHWFQQKPGSSPKPWIYATSNLASGVPAR FSGSGSGTSYSLTISRVEAEDAATYYCQQW SSSPLTFGAGTKLELKR
			123456789012345678901234567890

Example 2.2.2: Molecular Cloning of Murine Anti-Murine TNFα/anti-PGE2 DVD-Ig With Human Linkers

Methods for making DVD-Ig molecules is described in US Patent Publication No. 20070071675. Using overlapping PCR, six murine anti-murine TNFα/anti-PGE2 DVD-Ig molecules with no either human linker (hNL), human short linker (hSL), or human long linker (hLL) were generated and their protein sequences of the VH and the VL are listed in Table 7 below. The DNA encoding the VH of the DVD-Ig molecules was fused to human heavy chain IgG1 constant region and the DNA encoding the VL of the DVD-Ig molecules was fused to human light chain k constant region, repectively. The protein expression and purification of DVD-Ig molecules are essentially the same as that described in Example 1.3 and in US Patent Publication No. 20070071675.

Table 7: List Of Amino Acid Sequences Of VH And VL Regions Of Anti-Murine TNFα/Anti-PGE2 DVD-Ig With Human Linkers

SEQ ID No.	Protein region		Sequence
			12345678901234567890123456789 0
86	VH 8C11-hNL-2B5		**EFQLQQSGPELVKPGASVRISCKASGYSFT DYNMNWVKQSNGKSLEWVGVINPNYGSSTY NQKFKGKATLTVDQSSSTAYMQLNSLTSED SAVYYCARKWGQLGRGFFDVWGTGTTVTVS S**QVQLQQSGPELVRPGSSVKISCKASGYTF TKYWLGWVKQRPGHGLEWIGDIYPGYDYTH YNEKFKDKATLTVDTSSSTAYMQLSSLTSE DSAIYYCARSDGSSTYWGQGTLVTVSA
87	VH 8C11	Residues 1-121 of SEQ ID NO.:86	**EFQLQQSGPELVKPGASVRISCKASGYSFT DYNMNWVKQSNGKSLEWVGVINPNYGSSTY NQKFKGKATLTVDQSSSTAYMQLNSLTSED SAVYYCARKWGQLGRGFFDVWGTGTTVTVS S**
	HNL FOR HC	No	
88	VH 2B5	Residues 122-237 of SEQ ID NO.:86	QVQLQQSGPELVRPGSSVKISCKASGYTFT KYWLGWVKQRPGHGLEWIGDIYPGYDYTHY NEKFKDKATLTVDTSSSTAYMQLSSLTSED SAIYYCARSDGSSTYWGQGTLVTVSA
			12345678901234567890123456789 0
89	VL 8C11-hNL-2B5		**QIVLSQSPAILSASPGEKVTMTCRASSSVS YMHWFQQKPGSSPKPWIYATSNLASGVPAR FSGSGSGTSYSLTISRVEAEDAATYYCQQW SSSPLTFGAGTKLELKR**DVLMTQTPLSLPV SLGDQASISCTSSQNIVHSNGNTYLEWYLQ RPGQSPKLLIYKVSNRFSGVPDRFSGSGSG TVFTLKISRVEAEDLGVYYCFQVSHVPYTF GGGTKLEIKR
90	VL 8C11	Residues 1-107 of SEQ ID NO.:89	**QIVLSQSPAILSASPGEKVTMTCRASSSVS YMHWFQQKPGSSPKPWIYATSNLASGVPAR FSGSGSGTSYSLTISRVEAEDAATYYCQQW SSSPLTFGAGTKLELKR**
	HNL FOR LC	No	
91	VL 2B5	Residues 108-220 of SEQ ID NO.:89	DVLMTQTPLSLPVSLGDQASISCTSSQNIV HSNGNTYLEWYLQRPGQSPKLLIYKVSNRF SGVPDRFSGSGSGTVFTLKISRVEAEDLGV YYCFQVSHVPYTFGGGTKLEIKR
			12345678901234567890123456789 0
102	VH 8C11-hSL-2B5		EFQLQQSGPELVKPGASVRISCKASGYSFT DYNMNWVKQSNGKSLEWVGVINPNYGSSTY NQKFKGKATLTVDQSSSTAYMQLNSLTSED SAVYYCARKWGQLGRGFFDVWGTGTTVTVS S**ASTKGP**QVQLQQSGPELVRPGSSVKISCK ASGYTFTKYWLGWVKQRPGHGLEWIGDIYP GYDYTHYNEKFKDKATLTVDTSSSTAYMQL SSLTSEDSAIYYCARSDGSSTYWGQGTLVT VSA
87	VH 8C11	Residues 1-121 of SEQ ID NO.:102	EFQLQQSGPELVKPGASVRISCKASGYSFT DYNMNWVKQSNGKSLEWVGVINPNYGSSTY NQKFKGKATLTVDQSSSTAYMQLNSLTSED SAVYYCARKWGQLGRGFFDVWGTGTTVTVS S

SEQ ID No.	Protein region		Sequence
			123456789**0**123456789**0**1234567890
21	HSL FOR HC	Residues 122-127 of SEQ ID NO.:102	**ASTKGP**
88	VH 2B5	Residue 128-243 of SEQ ID NO.:102	QVQLQQSGPELVRPGSSVKISCKASGYTFT KYWLGWVKQRPGHGLEWIGDIYPGYDYTHY NEKFKDKATLTVDTSSSTAYMQLSSLTSED SAIYYCARSDGSSTYWGQGTLVTVSA
			123456789**0**123456789**0**1234567890
103	VL 8C11-hSL-2B5		QIVLSQSPAILSASPGEKVTMTCRASSSVS YMHWFQQKPGSSPKPWIYATSNLASGVPAR FSGSGSGTSYSLTISRVEAEDAATYYCQQW SSSPLTFGAGTKLELKR**TVAAP**DVLMTQTP LSLPVSLGDQASISCTSSQNIVHSNGNTYL EWYLQRPGQSPKLLIYKVSNRFSGVPDRFS GSGSGTVFTLKISRVEAEDLGVYYCFQVSH VPYTFGGGTKLEIKR
90	VL 8C11	Residues 1-107 of SEQ ID NO.:103	QIVLSQSPAILSASPGEKVTMTCRASSSVS YMHWFQQKPGSSPKPWIYATSNLASGVPAR FSGSGSGTSYSLTISRVEAEDAATYYCQQW SSSPLTFGAGTKLELKR
13	HSL FOR LC	Residues 108-112 of SEQ ID NO.:103	**TVAAP**
91	VL 2B5	Residues 113-225 of SEQ ID NO.:103	DVLMTQTPLSLPVSLGDQASISCTSSQNIV HSNGNTYLEWYLQRPGQSPKLLIYKVSNRF SGVPDRFSGSGSGTVFTLKISRVEAEDLGV YYCFQVSHVPYTFGGGTKLEIKR
			123456789**0**123456789**0**1234567890
104	VH 8C11-hLL-2B5		EFQLQQSGPELVKPGASVRISCKASGYSFT DYNMNWVKQSNGKSLEWVGVINPNYGSSTY NQKFKGKATLTVDQSSSTAYMQLNSLTSED SAVYYCARKWGQLGRGFFDVWGTGTTVTVS S**ASTKGPSVFPLAP**QVQLQQSGPELVRPGS SVKISCKASGYTFTKYWLGWVKQRPGHGLE WIGDIYPGYDYTHYNEKFKDKATLTVDTSS STAYMQLSSLTSEDSAIYYCARSDGSSTYW GQGTLVTVSA
87	VH 8C11	Residues 1-121 of SEQ ID NO.:104	EFQLQQSGPELVKPGASVRISCKASGYSFT DYNMNWVKQSNGKSLEWVGVINPNYGSSTY NQKFKGKATLTVDQSSSTAYMQLNSLTSED SAVYYCARKWGQLGRGFFDVWGTGTTVTVS S
22	HLL FOR HC	Residues 121-134 of SEQ ID NO.:104	**ASTKGPSVFPLAP**
88	VH 2B5	Residues 135-250 of SEQ ID NO.:104	QVQLQQSGPELVRPGSSVKISCKASGYTFT KYWLGWVKQRPGHGLEWIGDIYPGYDYTHY NEKFKDKATLTVDTSSSTAYMQLSSLTSED SAIYYCARSDGSSTYWGQGTLVTVSA
			123456789**0**123456789**0**1234567890

SEQ ID No.	Protein region		Sequence
			1234567890123456789012345678901234567890
105	VL 8C11-hLL-2B5		QIVLSQSPAILSASPGEKVTMTCRASSSVS YMHWFQQKPGSSPKPWIYATSNLASGVPAR FSGSGSGTSYSLTISRVEAEDAATYYCQQW SSSPLTFGAGTKLELKR**TVAAPSVFIFPP**D VLMTQTPLSLPVSLGDQASISCTSSQNIVH SNGNTYLEWYLQRPGQSPKLLIYKVSNRFS GVPDRFSGSGSGTVFTLKISRVEAEDLGVY YCFQVSHVPYTFGGGTKLEIKR
90	VL 8C11	Residues 1-107 of SEQ ID NO.:105	QIVLSQSPAILSASPGEKVTMTCRASSSVS YMHWFQQKPGSSPKPWIYATSNLASGVPAR FSGSGSGTSYSLTISRVEAEDAATYYCQQW SSSPLTFGAGTKLELKR
14	HLL FOR LC	Residues 108-119 of SEQ ID NO.:105	**TVAAPSVFIFPP**
91	VL 2B5	Residues 120-232 of SEQ ID NO.:105	DVLMTQTPLSLPVSLGDQASISCTSSQNIV HSNGNTYLEWYLQRPGQSPKLLIYKVSNRF SGVPDRFSGSGSGTVFTLKISRVEAEDLGV YYCFQVSHVPYTFGGGTKLEIKR
			1234567890123456789012345678901234567890
96	VH 2B5-hNL-8C11		QVQLQQSGPELVRPGSSVKISCKASGYTFT KYWLGWVKQRPGHGLEWIGDIYPGYDYTHY NEKFKDKATLTVDTSSSTAYMQLSSLTSED SAIYYCARSDGSSTYWGQGTLVTVSA**EFQL QQSGPELVKPGASVRISCKASGYSFTDYNM NWVKQSNGKSLEWVGVINPNYGSSTYNQKF KGKATLTVDQSSSTAYMQLNSLTSEDSAVY YCARKWGQLGRGFFDVWGTGTTVTVSS**
68	VH 2B5	Residues 1-116 of SEQ ID NO.:96	QVQLQQSGPELVRPGSSVKISCKASGYTFT KYWLGWVKQRPGHGLEWIGDIYPGYDYTHY NEKFKDKATLTVDTSSSTAYMQLSSLTSED SAIYYCARSDGSSTYWGQGTLVTVSA
	HNL FOR HC	No	
87	VH 8C11	Residues 117-237 of SEQ ID NO.:96	**EFQLQQSGPELVKPGASVRISCKASGYSFT DYNMNWVKQSNGKSLEWVGVINPNYGSSTY NQKFKGKATLTVDQSSSTAYMQLNSLTSED SAVYYCARKWGQLGRGFFDVWGTGTTVTVS S**
			1234567890123456789012345678901234567890
97	VL 2B5-hNL-8C11		DVLMTQTPLSLPVSLGDQASISCTSSQNIV HSNGNTYLEWYLQRPGQSPKLLIYKVSNRF SGVPDRFSGSGSGTVFTLKISRVEAEDLGV YYCFQVSHVPYTFGGGTKLEIKR**QIVLSQS PAILSASPGEKVTMTCRASSSVSYMHWFQQ KPGSSPKPWIYATSNLASGVPARFSGSGSG TSYSLTISRVEAEDAATYYCQQWSSSPLTF GAGTKLELKR**
91	VL 2B5	Residues 1-113 of SEQ ID NO.:97	DVLMTQTPLSLPVSLGDQASISCTSSQNIV HSNGNTYLEWYLQRPGQSPKLLIYKVSNRF SGVPDRFSGSGSGTVFTLKISRVEAEDLGV YYCFQVSHVPYTFGGGTKLEIKR
	HNL FOR LC	No	
90	VL 8C11	Residues 114-220 of SEQ ID NO.:97	**QIVLSQSPAILSASPGEKVTMTCRASSSVS YMHWFQQKPGSSPKPWIYATSNLASGVPAR FSGSGSGTSYSLTISRVEAEDAATYYCQQW SSSPLTFGAGTKLELKR**
			1234567890123456789012345678901234567890

SEQ ID No.	Protein region		Sequence
			123456789012345678901234567890
106	**VH 2B5-hSL-8C11**		QVQLQQSGPELVRPGSSVKISCKASGYTFT KYWLGWVKQRPGHGLEWIGDIYPGYDYTHY NEKFKDKATLTVDTSSSTAYMQLSSLTSED SAIYYCARSDGSSTYWGQGTLVTVSA**ASTK GP**EFQLQQSGPELVKPGASVRISCKASGYS FTDYNMNWVKQSNGKSLEWVGVINPNYGSS TYNQKFKGKATLTVDQSSSTAYMQLNSLTS EDSAVYYCARKWGQLGRGFFDVWGTGTTVT VSS
88	VH 2B5	Residues 1-116 of SEQ ID NO.:106	QVQLQQSGPELVRPGSSVKISCKASGYTFT KYWLGWVKQRPGHGLEWIGDIYPGYDYTHY NEKFKDKATLTVDTSSSTAYMQLSSLTSED SAIYYCARSDGSSTYWGQGTLVTVSA
21	HSL FOR HC	Residues 117-122 of SEQ ID NO.:106	**ASTKGP**
87	VH 8C11	Residues 123-243 of SEQ ID NO.:106	EFQLQQSGPELVKPGASVRISCKASGYSFT DYNMNWVKQSNGKSLEWVGVINPNYGSSTY NQKFKGKATLTVDQSSSTAYMQLNSLTSED SAVYYCARKWGQLGRGFFDVWGTGTTVTVS S
			123456789012345678901234567890
107	**VL 2B5-hSL-8C11**		DVLMTQTPLSLPVSLGDQASISCTSSQNIV HSNGNTYLEWYLQRPGQSPKLLIYKVSNRF SGVPDRFSGSGSGTVFTLKISRVEAEDLGV YYCFQVSHVPYTFGGGTKLEIKR**TVAAP**QI VLSQSPAILSASPGEKVTMTCRASSSVSYM HWFQQKPGSSPKPWIYATSNLASGVPARFS GSGSGTSYSLTISRVEAEDAATYYCQQWSS SPLTFGAGTKLELKR
91	VL 2B5	Residues 1-113 of SEQ ID NO.:107	DVLMTQTPLSLPVSLGDQASISCTSSQNIV HSNGNTYLEWYLQRPGQSPKLLIYKVSNRF SGVPDRFSGSGSGTVFTLKISRVEAEDLGV YYCFQVSHVPYTFGGGTKLEIKR
13	MSL FOR LC	Residues 114-118 of SEQ ID NO.:107	**TVAAP**
90	VL 8C11	Residues 119-225 of SEQ ID NO.:107	QIVLSQSPAILSASPGEKVTMTCRASSSVS YMHWFQQKPGSSPKPWIYATSNLASGVPAR FSGSGSGTSYSLTISRVEAEDAATYYCQQW SSSPLTFGAGTKLELKR
			123456789012345678901234567890
108	**VH 2B5-hLL-8C11**		QVQLQQSGPELVRPGSSVKISCKASGYTFT KYWLGWVKQRPGHGLEWIGDIYPGYDYTHY NEKFKDKATLTVDTSSSTAYMQLSSLTSED SAIYYCARSDGSSTYWGQGTLVTVSA**ASTK GPSVFPLAP**EFQLQQSGPELVKPGASVRIS CKASGYSFTDYNMNWVKQSNGKSLEWVGVI NPNYGSSTYNQKFKGKATLTVDQSSSTAYM QLNSLTSEDSAVYYCARKWGQLGRGFFDVW GTGTTVTVSS
88	VH 2B5	Residues 1-116 of SEQ ID NO.:108	QVQLQQSGPELVRPGSSVKISCKASGYTFT KYWLGWVKQRPGHGLEWIGDIYPGYDYTHY NEKFKDKATLTVDTSSSTAYMQLSSLTSED SAIYYCARSDGSSTYWGQGTLVTVSA

SEQ ID No.	Protein region		Sequence
			123456789012345678901234567890
22	HLL FOR HC	Residues 117-129 of SEQ ID NO.:108	**ASTKGPSVFPLAP**
87	VH 8C11	Residues 130-250 of SEQ ID NO.:108	EFQLQQSGPELVKPGASVRISCKASGYSFT DYNMNWVKQSNGKSLEWVGVINPNYGSSTY NQKFKGKATLTVDQSSSTAYMQLNSLTSED SAVYYCARKWGQLGRGFFDVWGTGTTVTVS S
			123456789012345678901234567890
109	VL 2B5-hLL-8C11		DVLMTQTPLSLPVSLGDQASISCTSSQNIV HSNGNTYLEWYLQRPGQSPKLLIYKVSNRF SGVPDRFSGSGSGTVFTLKISRVEAEDLGV YYCFQVSHVPYTFGGGTKLEIKR**TVAAPSV FIFPP**QIVLSQSPAILSASPGEKVTMTCRA SSSVSYMHWFQQKPGSSPKPWIYATSNLAS GVPARFSGSGSGTSYSLTISRVEAEDAATY YCQQWSSSPLTFGAGTKLELKR
91	VL 2B5	Residues 1-113 of SEQ ID NO.:109	DVLMTQTPLSLPVSLGDQASISCTSSQNIV HSNGNTYLEWYLQRPGQSPKLLIYKVSNRF SGVPDRFSGSGSGTVFTLKISRVEAEDLGV YYCFQVSHVPYTFGGGTKLEIKR
14	HLL FOR LC	Residues 114-125 of SEQ ID NO.:109	**TVAAPSVFIFPP**
90	VL 8C11	Residues 126-232 of SEQ ID NO.:109	QIVLSQSPAILSASPGEKVTMTCRASSSVS YMHWFQQKPGSSPKPWIYATSNLASGVPAR FSGSGSGTSYSLTISRVEAEDAATYYCQQW SSSPLTFGAGTKLELKR
			123456789012345678901234567890

Example 2.2.3: Characterization of Murine Anti-Murine TNFα/anti-PGE$_2$ DVD-Ig With Human Linkers

Example 2.2.3.1: Assays To Identify PGE$_2$ Binding Activity Of Anti-Mouse TNFα/Anti-PGE$_2$ DVD-Ig Molecules With Human Linkers

Assays used to identify and characterize PGE$_2$ binding activity of anti-murine TNFα/anti-PGE$_2$ DVD-Igs with human linkers, including the ^{3}H- PGE$_2$ ELISA are described in Example 1 unless otherwise stated. 2B5-8.0, 2B5-huSL-8C11 and 2B5-hLL-8C11 and PGE$_2$ at K_D values of 334, 238, 383 pM, respectively.

Example 2.2.3.2: Assays To Identify Anti-PGE$_2$ Neutralization Activity Of Anti-Mouse TNFα/Anti-PGE$_2$ DVD-Ig Molecules With Human Linkers

Assays used to identify and characterize PGE$_2$ neutralization activity of anti-murine TNFα/anti-PGE2 DVD-Igs with human linkers, including the EP4 assay, are

described in Example 1 unless otherwise stated. 2B5-8.0, 2B5-huSL-8C11 and 2B5-hLL-8C11 neutralized PGE_2 at IC_{50} values of 20, 20, 64 pM, respectively.

Example 2.2.3.3: Assays To Identify Anti- TNFα Binding Activity Of Mouse TNFα/Anti-PGE_2 DVD-Ig Molecules With Human Linkers

Assays used to identify and characterize TNFα binding activity of anti-murine TNFα/anti-PGE_2 DVD-Igs with human linkers, including ELISA, are described in Example 1 unless otherwise stated. 8C11, 2B5-huSL-8C11 and 2B5-hLL-8C11 neutralized PGE_2 at IC_{50} values of 201, 234, 256 pM, respectively

Example 2.2.3.4: Assays To Identify Anti- TNFα Neutralization Activity Of Mouse TNFα/Anti-PGE_2 DVD-Ig Molecules With Human Linkers

Assays used to identify and characterize TNFα neutralization activity of anti-murine TNFα/anti-PGE_2 DVD-Igs with human linkers, including the L929 assay are described in Example 1 unless otherwise stated. 8C11, 2B5-huSL-8C11 and 2B5-hLL-8C11 neutralized PGE_2 at IC_{50} values of 5852, 4389, and 88 pM, respectively. Although the exact reason for why 2B5-hLL-8C11 has much more potent activity to neutralize murine TNFα is unclear, it may be related to the kinetics and the size of complex formation of DVD-Ig molecules with murine TNFα.

Example 2.3: Construction and Characterization Of Anti-Human TNFα/Anti-PGE_2 DVD-Ig Molecules

A dual variable domain immunoglobulin (DVD-Ig) molecule was designed such that two different light chain variable domains (VL) from the two different parent mAbs are linked in tandem directly or via a short linker by recombinant DNA techniques, followed by the light chain constant domain. Similarly, the heavy chain comprises two different heavy chain variable domains (VH) linked in tandem, followed by the constant domain CH1 and Fc region using the methods disclosed in US Patent Publication No. 20070071675. The constant region of a human DVD-Ig molecule can either be a human IgG1, IgG2, IgG3, IgG4.

Using the human anti-TNFα, D2E7, and HU2B5.7 as building blocks, four anti-TNFα/PGE_2 DVD-Ig molecules were constructed: two variants with either the TNFα- or

PGE_2-binding domains at the N-termini, and each with short (SL) or long (LL) linkers for bridging the heavy and light chains of the two antigen binding domains.

The VH and VL domains of two parental antibodies used to generate a DVD-Ig capable of binding human TNFα and PGE2 is listed in Table 8. Briefly, parent mAbs include two high affinity murine Abs, anti-human TNFα (D2E7) and humanized anti-PGE2 (HU2B5.7), respectively. The VL/VH genes of anti-TNFα monoclonal antibody D2E7 were amplified by PCR from plasmids encoding DNA sequence of D2E7, as described in US Patent No. 6,258,562. The VL/VH genes of HU2B5.7 were from plasmids encoding DNA sequence of HU2B5.7, as illustrated in Example 2.3. The all DVD2-Ig molecules were constructed similarly, except that they had a linker between the two variable domains in both the light chain and the heavy chain. These linker sequences, selected from the N-termini of human Ck and CH1, are a natural extension of the variable domains and exhibit a flexible conformation without significant secondary structures based on the analysis of several Fab crystal structures. The detailed procedures of the PCR cloning is described below:

Example 2.3.1: Molecular Cloning Of Human Anti-Human TNFα/Anti-PGE2 DVD-Ig

The general construction of DVD-Ig molecules is described in US Patent Publication No. 20070071675. Using overlapping PCR, four human anti-human TNFα/anti-PGE2 DVD-Ig molecules with human short linker (SL) and human long linker (LL) were generated and their protein sequences of VH and VL were listed in Table 8 below. The DNA encoding VH of DVD-Ig molecules was fused to human heavy chain IgG1 constant region, and the DNA encoding VL of DVD-Ig molecules was fused to human light chain k constant region repectively. The protein expression and purification of DVD-Ig molecules are essentially the same as that described in Example 1.3 and US Patent Publication No. 20070071675.

Table 8: List Of Amino Acid Sequences Of VH And VL Regions Of Anti-Human TNFα/Anti-PGE2 DVD-Ig With Human Linkers

SEQ ID No.	Protein region		Sequence
			123456789012345678901234567890
110	VH HU2B5.7		**EVQLVQSGAEVKKPGASVKVSCKASGYTFT KYWLGWVRQAPGQGLEWMGDIYPGYDYTHY NEKFKDRVTLTTDTSTSTAYMELRSLRSDD TAVYYCARSDGSSTYWGQGTLVTVSS**
111	VL HU2B5.7		**DVLMTQTPLSLPVTPGEPASISCTSSQNIV HSNGNTYLEWYLQKPGQSPQLLIYKVSNRF SGVPDRFSGSGSGTDFTLKISRVEAEDVGV YYCFQVSHVPYTFGGGTKVEIKR**
			123456789012345678901234567890
112	VH D2E7		**EVQLVESGGGLVQPGRSLRLSCAASGFTFD DYAMHWVRQAPGKGLEWVSAITWNSGHIDY ADSVEGRFTISRDNAKNSLYLQMNSLRAED TAVYYCAKVSYLSTASSLDYWGQGTLVTVS S**
			123456789012345678901234567890
113	VL D2E7		**DIQMTQSPSSLSASVGDRVTITCRASQGIR NYLAWYQQKPGKAPKLLIYAASTLQSGVPS RFSGSGSGTDFTLTISSLQPEDVATYYCQR YNRAPYTFGQGTKVEIKR**
			123456789012345678901234567890
114	**DVD VH** HU2B5.7SLD2E7		EVQLVQSGAEVKKPGASVKVSCKASGYTFT KYWLGWVRQAPGQGLEWMGDIYPGYDYTHY NEKFKDRVTLTTDTSTSTAYMELRSLRSDD TAVYYCARSDGSSTYWGQGTLVTVSS**ASTK GP**EVQLVESGGGLVQPGRSLRLSCAASGFT FDDYAMHWVRQAPGKGLEWVSAITWNSGHI DYADSVEGRFTISRDNAKNSLYLQMNSLRA EDTAVYYCAKVSYLSTASSLDYWGQGTLVT VSS
110	VH HU2B5.7	Residues 1-116 of SEQ ID NO.:114	EVQLVQSGAEVKKPGASVKVSCKASGYTFT KYWLGWVRQAPGQGLEWMGDIYPGYDYTHY NEKFKDRVTLTTDTSTSTAYMELRSLRSDD TAVYYCARSDGSSTYWGQGTLVTVSS
21	SL FOR HC	Residues 117-122 of SEQ ID NO.:114	**ASTKGP**
112	VH D2E7	Residues 123-243 of SEQ ID NO.:114	EVQLVESGGGLVQPGRSLRLSCAASGFTFD DYAMHWVRQAPGKGLEWVSAITWNSGHIDY ADSVEGRFTISRDNAKNSLYLQMNSLRAED TAVYYCAKVSYLSTASSLDYWGQGTLVTVS S
			123456789012345678901234567890
115	**DVD VL** HU2B5.7SLD2E7		DVLMTQTPLSLPVTPGEPASISCTSSQNIV HSNGNTYLEWYLQKPGQSPQLLIYKVSNRF SGVPDRFSGSGSGTDFTLKISRVEAEDVGV YYCFQVSHVPYTFGGGTKVEIKR**TVAAP**DI QMTQSPSSLSASVGDRVTITCRASQGIRNY LAWYQQKPGKAPKLLIYAASTLQSGVPSRF SGSGSGTDFTLTISSLQPEDVATYYCQRYN RAPYTFGQGTKVEIKR

SEQ ID No.	Protein region		Sequence
			12345678901234567890123456789**0**
111	VL HU2B5.7	Residues 1-113 of SEQ ID NO.:115	DVLMTQTPLSLPVTPGEPASISCTSSQNIV HSNGNTYLEWYLQKPGQSPQLLIYKVSNRF SGVPDRFSGSGSGTDFTLKISRVEAEDVGV YYCFQVSHVPYTFGGGTKVEIKR
13	SL FOR LC	Residues 114-118 of SEQ ID NO.:115	**TVAAP**
113	VL D2E7	Residues 119-226 of SEQ ID NO.:115	DIQMTQSPSSLSASVGDRVTITCRASQGIR NYLAWYQQKPGKAPKLLIYAASTLQSGVPS RFSGSGSGTDFTLTISSLQPEDVATYYCQR YNRAPYTFGQGTKVEIKR
			12345678901234567890123456789**0**
116	**DVD VH** HU2B5.7LLD2E7		EVQLVQSGAEVKKPGASVKVSCKASGYTFT KYWLGWVRQAPGQGLEWMGDIYPGYDYTHY NEKFKDRVTLTTDTSTSTAYMELRSLRSDD TAVYYCARSDGSSTYWGQGTLVTVSS**ASTK GPSVFPLAP**EVQLVESGGGLVQPGRSLRLS CAASGFTFDDYAMHWVRQAPGKGLEWVSAI TWNSGHIDYADSVEGRFTISRDNAKNSLYL QMNSLRAEDTAVYYCAKVSYLSTASSLDYW GQGTLVTVSS
110	VH HU2B5.7	Residues 1-116 of SEQ ID NO.:116	EVQLVQSGAEVKKPGASVKVSCKASGYTFT KYWLGWVRQAPGQGLEWMGDIYPGYDYTHY NEKFKDRVTLTTDTSTSTAYMELRSLRSDD TAVYYCARSDGSSTYWGQGTLVTVSS
22	LL FOR HC	Residues 117-129 of SEQ ID NO.:116	**ASTKGPSVFPLAP**
112	VH D2E7	Residues 130-250 of SEQ ID NO.:116	EVQLVESGGGLVQPGRSLRLSCAASGFTFD DYAMHWVRQAPGKGLEWVSAITWNSGHIDY ADSVEGRFTISRDNAKNSLYLQMNSLRAED TAVYYCAKVSYLSTASSLDYWGQGTLVTVS S
			12345678901234567890123456789**0**
117	**DVD VL** HU2B5.7LLD2E7		DVLMTQTPLSLPVTPGEPASISCTSSQNIV HSNGNTYLEWYLQKPGQSPQLLIYKVSNRF SGVPDRFSGSGSGTDFTLKISRVEAEDVGV YYCFQVSHVPYTFGGGTKVEIKR**TVAAPSV FIFPP**DIQMTQSPSSLSASVGDRVTITCRA SQGIRNYLAWYQQKPGKAPKLLIYAASTLQ SGVPSRFSGSGSGTDFTLTISSLQPEDVAT YYCQRYNRAPYTFGQGTKVEIKR
111	VL HU2B5.7	Residues 1-113 of SEQ ID NO.:117	DVLMTQTPLSLPVTPGEPASISCTSSQNIV HSNGNTYLEWYLQKPGQSPQLLIYKVSNRF SGVPDRFSGSGSGTDFTLKISRVEAEDVGV YYCFQVSHVPYTFGGGTKVEIKR
14	LL FOR LC	Residues 114-125 of SEQ ID NO.:117	**TVAAPSVFIFPP**
113	VL D2E7	Residues 126-233 of SEQ ID NO.:117	DIQMTQSPSSLSASVGDRVTITCRASQGIR NYLAWYQQKPGKAPKLLIYAASTLQSGVPS RFSGSGSGTDFTLTISSLQPEDVATYYCQR YNRAPYTFGQGTKVEIKR
			12345678901234567890123456789**0**

SEQ ID No.	Protein region		Sequence
			12345678901234567890123456789O
118	**DVD VH** D2E7SLHU2B5.7		EVQLVESGGGLVQPGRSLRLSCAASGFTFD DYAMHWVRQAPGKGLEWVSAITWNSGHIDY ADSVEGRFTISRDNAKNSLYLQMNSLRAED TAVYYCAKVSYLSTASSLDYWGQGTLVTVS S**ASTKGP**EVQLVQSGAEVKKPGASVKVSCK ASGYTFTKYWLGWVRQAPGQGLEWMGDIYP GYDYTHYNEKFKDRVTLTTDTSTSTAYMEL RSLRSDDTAVYYCARSDGSSTYWGQGTLVT VSS
112	VH D2E7	Residues 1-121 of SEQ ID NO.:118	EVQLVESGGGLVQPGRSLRLSCAASGFTFD DYAMHWVRQAPGKGLEWVSAITWNSGHIDY ADSVEGRFTISRDNAKNSLYLQMNSLRAED TAVYYCAKVSYLSTASSLDYWGQGTLVTVS S
21	SL FOR HC	Residues 122-127 of SEQ ID NO.:118	**ASTKGP**
110	VH HU2B5.7	Residues 128-243 of SEQ ID NO.:118	EVQLVQSGAEVKKPGASVKVSCKASGYTFT KYWLGWVRQAPGQGLEWMGDIYPGYDYTHY NEKFKDRVTLTTDTSTSTAYMELRSLRSDD TAVYYCARSDGSSTYWGQGTLVTVSS
			12345678901234567890123456789O
119	**DVD VL** D2E7SLHU2B5.7		DIQMTQSPSSLSASVGDRVTITCRASQGIR NYLAWYQQKPGKAPKLLIYAASTLQSGVPS RFSGSGSGTDFTLTISSLQPEDVATYYCQR YNRAPYTFGQGTKVEIKR**TVAAP**DVLMTQT PLSLPVTPGEPASISCTSSQNIVHSNGNTY LEWYLQKPGQSPQLLIYKVSNRFSGVPDRF SGSGSGTDFTLKISRVEAEDVGVYYCFQVS HVPYTFGGGTKVEIKR
113	VL D2E7	Residues 1-108 of SEQ ID NO.:119	DIQMTQSPSSLSASVGDRVTITCRASQGIR NYLAWYQQKPGKAPKLLIYAASTLQSGVPS RFSGSGSGTDFTLTISSLQPEDVATYYCQR YNRAPYTFGQGTKVEIKR
13	MSL FOR LC	Residues 109-113 of SEQ ID NO.:119	**TVAAP**
111	VL Hu2B5.7	Residues 114-226 of SEQ ID NO.:119	DVLMTQTPLSLPVTPGEPASISCTSSQNIV HSNGNTYLEWYLQKPGQSPQLLIYKVSNRF SGVPDRFSGSGSGTDFTLKISRVEAEDVGV YYCFQVSHVPYTFGGGTKVEIKR
			12345678901234567890123456789O
120	**DVD VH** D2E7LLHU2B5.7		EVQLVESGGGLVQPGRSLRLSCAASGFTFD DYAMHWVRQAPGKGLEWVSAITWNSGHIDY ADSVEGRFTISRDNAKNSLYLQMNSLRAED TAVYYCAKVSYLSTASSLDYWGQGTLVTVS S**ASTKGPSVFPLAP**EVQLVQSGAEVKKPGA SVKVSCKASGYTFTKYWLGWVRQAPGQGLE WMGDIYPGYDYTHYNEKFKDRVTLTTDTST STAYMELRSLRSDDTAVYYCARSDGSSTYW GQGTLVTVSS
112	VH D2E7	Residues 1-121 of SEQ ID NO.:120	EVQLVESGGGLVQPGRSLRLSCAASGFTFD DYAMHWVRQAPGKGLEWVSAITWNSGHIDY ADSVEGRFTISRDNAKNSLYLQMNSLRAED TAVYYCAKVSYLSTASSLDYWGQGTLVTVS S

SEQ ID No.	Protein region		Sequence
			12345678901234567890123456789**0**
22	LL FOR HC	Residues 122-134 of SEQ ID NO.:120	**ASTKGPSVFPLAP**
110	VH HU2B5.7	Residues 135-250 of SEQ ID NO.:120	EVQLVQSGAEVKKPGASVKVSCKASGYTFT KYWLGWVRQAPGQGLEWMGDIYPGYDYTHY NEKFKDRVTLTTDTSTSTAYMELRSLRSDD TAVYYCARSDGSSTYWGQGTLVTVSS
			12345678901234567890123456789**0**
121	**DVD VL** D2E7LLHU2B5.7		DIQMTQSPSSLSASVGDRVTITCRASQGIR NYLAWYQQKPGKAPKLLIYAASTLQSGVPS RFSGSGSGTDFTLTISSLQPEDVATYYCQR YNRAPYTFGQGTKVEIKR**TVAAPSVFIFPP** DVLMTQTPLSLPVTPGEPASISCTSSQNIV HSNGNTYLEWYLQKPGQSPQLLIYKVSNRF SGVPDRFSGSGSGTDFTLKISRVEAEDVGV YYCFQVSHVPYTFGGGTKVEIKR
113	VL D2E7	Residues 1-108 of SEQ ID NO.:121	DIQMTQSPSSLSASVGDRVTITCRASQGIR NYLAWYQQKPGKAPKLLIYAASTLQSGVPS RFSGSGSGTDFTLTISSLQPEDVATYYCQR YNRAPYTFGQGTKVEIKR
14	LL FOR LC	Residues 109-120 of SEQ ID NO.:121	**TVAAPSVFIFPP**
111	VL HU2B5.7	Residues 121-233 of SEQ ID NO.:121	DVLMTQTPLSLPVTPGEPASISCTSSQNIV HSNGNTYLEWYLQKPGQSPQLLIYKVSNRF SGVPDRFSGSGSGTDFTLKISRVEAEDVGV YYCFQVSHVPYTFGGGTKVEIKR
			12345678901234567890123456789**0**

Table 9: Sequence Of Human IgG Heavy Chain Constant Domain And Light Chain Constant Domain

Protein	Sequence Identifier	Sequence
		12345678901234567890123456789012 3
Ig gamma-1 constant region	SEQ ID NO.:122	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
Ig gamma-1 constant region mutant	SEQ ID NO.:123	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE PKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
Ig kappa constant region	SEQ ID NO.:124	TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC
Ig Lambda constant region	SEQ ID NO.:125	QPKAAPSVTLFPPSSEELQANKATLVCLISDFY PGAVTVAWKADSSPVKAGVETTTPSKQSNNKYA ASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTV APTECS

Example 2.3.2: Assays To Characterize Anti-Human TNFα/Anti-PGE_2 DVD-Ig Molecules

Assays used to identify and characterize anti-human TNFα/anti-PGE2 DVD-Ig molecules, including ELISA, competition ELISA, ^{3}H-**PGE_2** ELISA and/or ^{3}H-**PGE_2** competition ELISA, receptor blockade assay, are described in Example 1.1 unless otherwise stated.

Affinity for PGE_2 was measured using ^{3}H-PGE_2 ELISA. D2E7-SL-Hu2B5.7,.D2E7-LL-Hu2B5.7 and Hu2B5.7-SL-D2E7-SL molecules maintained comparable binding affinities PGE_2 (Table 10).

Table 10: In Vitro Characterization of Anti-TNFα/PGE_2 DVD-Ig Molecules

Antibody or DVD-Ig™	TNFα		PGE_2	
	Affinity K_D (pM)	Cellular Potency IC50 (pM)	Affinity K_D (pM)	Cellular Potency IC50 (pM)
2B5	-	-	446	118
D2E7	27	41	-	-
D2E7-SL-HU2B5.7	50	37	210	45
D2E7-LL-HU2B5.7	12	14	253	191
HU2B5.7-SL-D2E7	806	> 1000	261	118
HU2B5.7-LL-D2E7	431	>1000	-	69

The prostaglandin binding specificity of anti-TNFα/PGE_2 DVD-Ig molecules was evaluated using a ^{3}H- PGE_2 competition ELISA and was summarized in Table 11.

Table 11: Prostaglandin Binding Selectivity of D2E7-SL-Hu2B5.7 calculated as CRI (%)*

Prostaglandins		A-1067775
PGE_2		100
PGE_1		6.6
13,14-dihydroxyl-15-keto PGE2		0.017
15-keto PGE2		0.023
19R-hydroxy PGE2		0.07
PGA_2		0.04
PGB_2		0.0003
PGD_2		$< 1x\ 10^{-4}$
$PGF_{2\alpha}$		0.05
6-keto $PGF_{1\alpha}$		0.145
8-iso PGF2α		0.11
2,3-dinor-6-keto-PGF1α		0.25
PGI_2 & its stable analogues	PGI_2	0.13
	Iloprost	0.01
	Carboprostacyclin	0.17
TXA_2 stable analogues & metabolites	Pinane TXA_2	4.8×10^{-4}
	15R-Pinane TXA_2	2.9×10^{-3}
	Carbocyclic TXA_2	1.7×10^{-4}
TXB_2		9.2×10^{-4}
LTE4		0.0032
5(S)-HETE		<0.01
Arachidonic acid		<0.01

*CRI (Cross Reactivity Index) = 100 x IC_{50} of PGE_2/IC_{50} of other prostaglandins in a competition RIA. This number reflects the percentage of cross-reactivity.

Example 2.3.3: Functional Activity anti-PGE2 Activity Of Human TNFα/Anti-PGE2 DVD-Ig Molecules

Assays used to characterize the functional activity of the anti-human TNFα/anti-PGE2 DVD-Ig molecules, including the EP4 bioassay, are described in Example 1.1 unless otherwise stated. All the four DVD-Ig molecules maintained comparable binding neutralization potencies in cell assays for PGE_2 (Table 10).

Example 2.3.4: Affinity of Anti-Human TNFα/Anti-PGE2 DVD-Ig Molecules for TNFα

Assays used to identify and characterize the anti-TNF activity of anti-human TNFα/anti-PGE2 DVD-Ig molecules, including ELISA and BIAcore assay are essentially the same as those described in Example 1.1 unless otherwise stated. The DVD-Ig molecules, D2E7-SL-HU2B5.7 and D2E7-LL-HU2B5.7, with D2E7 at the N-termini maintained comparable TNFα binding affinity, whereas HU2B5.7-SL-D2E7 and HU2B5.7-SL-D2E7 with D2E7 at the C-termini had significantly reduced TNFα binding affinity (Table 10).

The TNFα binding kinetics of D2E7-SL-Hu2B5.7 for TNFα from various species was summarized in Table 12.

Table 12: TNFα binding affinity of D2E7-SL-Hu2B5.7

Antigen	k_{on} (M^{-1} s^{-1})	k_{off} (s^{-1})	K_D (pM)	Potency (IC_{50}, pM)
Human TNFα	1.6×10^6	9.2×10^5	64	56
Cynomolgus TNFα	1.4×10^6	1×10^6	73	35
Canine TNFα	6.3×10^5	1.5×10^4	241	402
Murine TNFα	4×10^5	2×10^3	4990	13,000
Rat TNFα	-	-	-	Below detection limit
PGE_2	-	-	210	45

Note: - not tested.

Example 2.3.5: Functional Activity Of Anti-TNFα Antibodies And Anti-TNFα Activity Of Anti-TNFα/Anti-PGE2 DVD-Ig Molecules

The L929 assay used to characterize the functional anti-human TNFα activity of anti-human TNFα/anti-PGE2 DVD-Ig molecules is essentially the same as that described in the Example 1.1.2.C unless otherwise stated. The DVD-Ig molecules, D2E7-SL-HU2B5.7 and D2E7-LL-HU2B5.7, with D2E7 at the N-termini maintained comparable TNFα neutralization activity, whereas HU2B5.7-SL-D2E7 and HU2B5.7-SL-D2E7 with D2E7 at the C-termini significantly reduced TNFα neutralization activity (Table 10).

The TNFα neutralization activity of D2E7-SL-Hu2B5.7 to TNFα from various species was summarized in Table 12.

Example 2.3.6: Mutual Interference of Antigen Binding Of Anti-TNFα/Anti-PGE2 DVD-Ig Molecules

To determine whether D2E7-SL-HU2B5.7 can neutralize TNFα and PGE_2 simultaneously, D2E7-SL-HU2B5.7 was pre-saturated with one antigen first, and then tested for the neutralization of the second antigen. D2E7-SL-HU2B5.7 did not show any decrease in neutralization potency for PGE_2 in the EP4 assay when pre-bound to TNFα, nor was there any decrease in the neutralization potency for TNFα in the L929 assay when pre-bound to PGE_2.

Example 2.3.7: EP3 Receptor Competitive Inhibition Assay

The EP3 receptor competitive inhibition assay used to characterize the functional activity of anti-human anti-PGE_2 activity of anti-human TNFα/anti-PGE_2 DVD-Ig molecules are described in the Example 1 unless otherwise stated. The IC_{50} value for D2E7-SL-HU2B5.7 to block 3H- PGE_2 binding to EP receptor was in the range of 70-120 pM.

Example 2.3.8: Human Cytokine Cross-Reactivity

The cytokine selectivity of D2E7-SL-HU2B5.7 was assessed by ELISA as described in Example 1. A panel of 26 soluble human cytokines was tested for competition with D2E7-SL-HU2B5.7 for binding to immobilized biotinylated TNFα. Specific competitive binding occurred only with TNFα; no other cytokines showed

competition with D2E7-SL-HU2B5.7 for binding to biotinylated TNFα. This data suggests that D2E7-SL-HU2B5.7 is highly specific for TNFα.

Example 2.3.9: Incubation of D2E7-SL-HU2B5.7 With Human Blood

The interaction of D2E7-Hu-2B5.7 with human blood cells was investigated by assays to determine cytokine release and cell surface binding as described in Example 1. These assays help to evaluate whether D2E7-Hu-2B5.7 causes activation of inflammatory cells or binds to unintended cell surface proteins in blood. Incubation of CHO cell-derived D2E7-Hu-2B5.7 with human peripheral blood cells did not induce any cytokine release above control values, suggesting it does not cause activation of inflammatory cells.

Example 2.3.10: In Vivo Efficacy Of Anti-PGE_2 Activity Of Human TNFα/Anti-PGE_2 DVD-Ig Molecules In a Carrageenan-Induced Footpad Edema Model

Carrageenan-Induced Footpad Edema is an acute rodent model of innate immune function. Intradermal (ID) injection of an inflammatory agent causes a rapid influx of neutrophils and fluid edema, which peaks at approximately 4 hours, followed by an influx of macrophages and monocytes, which peaks at approximately 48 hours. C57.BL/6 mice (8-10-week-old, Jackson Laboratories, Bar Harbor, ME) were injected ID in the rear footpad with 30μL of either PBS (left) or λ-carrageenen (Sigma Aldrich, St. Louis, MO) in PBS (right) at a concentration of 5.0 mg/mL (150μg/mouse). Rear footpad thickness was measured by the Dyer spring caliper model #310-119 at baseline (time=0), and 4 hours post carrageenan challenge. Significant difference for paw thickness was determined by comparing mean paw swelling for each treatment group to vehicle in a Student's two-tailed t-test.

Mice were given a dose titration of either a human anti-TNFα/PGE_2 DVD-Ig molecule D2E7-SL-Hu2B5.7 or an anti-PGE_2 antibody (2B5-8.0) intraperitonially (IP) 18 hours prior to carrageenan challenge, or Indomethacin, PO 2 hours prior to challenge. The endpoint measured was the difference in paw swelling (edema) between right and left paws 4 hours after treatment. The anti-PGE_2 antibody inhibited paw edema dose-dependently, and provided a maximal 40-50% inhibition of paw swelling at 10 mg/kg, comparable to the maximal inhibition achieved by indomethacin. Two independent

experiments with D2E7-SL-Hu2B5.7 demonstrated very similar inhibitions. Serum concentrations of D2E7-SL-Hu2B5.7 that provided maximal inhibition (at 10 mg/kg) was ~50 µg/mL at 22 hours post-dose, and this level was similar to that observed for anti-PGE_2 mAb. These results indicated that the anti-PGE_2 binding domain of D2E7-SL-Hu2B5.7 DVD-Ig was fully functional *in vivo* (Table 13).

Table 13: Paw Swelling in Mouse Carrageenan-Induced Footpad Edema after Ab or DVD-Ig Treatment

	Vehicle (PBS)	**Anti-PGE_2 (10 mg/kg, i.p.)**	**D2E7-SL-Hu2B5.7 (12.5 mg/kg i.p.)**	**Indomethacin (3 mg/kg, p.o.)**
Δ Paw Swelling (mm)	0.857 ± 0.06	0.529 ± 0.04	0.600 ± 0.00	0.486 ± 0.03
% Inhibition	NA	38 ± 4.6	30 ± 0.0	57 ± 3.5

Example 2.3.11: Human TNFα/Anti-PGE_2 DVD-Ig Molecules Rescue LPS-D-Galactosamine-Induced Lethality

D2E7-SL-Hu2B5.7 was prepared in sterile saline (Baxter lot C755694) and injected i.p. at 100, 30, 10, 3, 1 mg/kg into female C57BL6/N mice (n=10) (8-10-week-old, Jackson Laboratories, Bar Harbor, ME) 18 hours prior to LPS challenge. Saline alone was used as a negative control. Another group received mg/kg 8C11-1E10, an anti-mouse TNF monoclonal antibody, as a positive control.

At the time of challenge, the mice were injected i.p. with 500µL dose volume of saline containing 100ng/mouse LPS (Sigma L4130, lot 095K4056, St. Louis, MO) and 20mg/mouse D-Galactosamine (Sigma G-0500 , lot 048K1547, St. Louis, MO). The mice were then monitored for lethality twice a day for 48 hours. At 48 hours the remaining surviving mice were sacrificed and bled for plasma antibody levels.

The results are shown in Table 14. The D2E7-SL-Hu2B5.7 at 100 mg/kg protected 90% of the mice from lethality. The protection was achieved at an average (mean ± SD) exposure of 307.7 ± 26.1μg/ml. All other dose groups provided little or no protection. The mouse anti-mouse anti-TNF-antibody (8C11-1E10) at 12 mg/kg was used as a positive control and provided 100% protection from LPS/D-gal-induced lethality. This efficacy with the 8C11 antibody was achieved at an average (mean ± SD) exposure of 65 ± 4.0 μg/ml.

Table 14: Ability of D2E7-SL-Hu2B5.7 to Rescue LPS-D-Galactosidase-Iduced Lethality

	% Survival	Mean Plasma Ab Concentration (μg/mL)
Saline	0	NA
D2E7-SL-Hu2B5.7 @ 1 mg/kg	0	NA
D2E7-SL-Hu2B5.7 @ 3 mg/kg	10	26.6
D2E7-SL-Hu2B5.7 @ 10 mg/kg	20	91.7
D2E7-SL-Hu2B5.7 @ 30 mg/kg	10	184.0
D2E7-SL-Hu2B5.7 @ 100 mg/kg	90	307.7
D2E7-SL-Hu2B5.7 @ 12 mg/kg IP	100	65.2

Example 2.3.12: In Vivo Efficacy Of Anti-Human TNFα Activity Of Human TNFα/Anti-PGE2 DVD-Ig Molecules In A Human TNFα Transgenic Induced Arthritis Model

Example 2.3.12.1: Human TNFα Transgenic Induced Arthritis Model

Human TNFα transgenic B6.Cg(SJL)-Tg(*TNF*) N21+ mice (40 male and 40 females) (Taconic, Hudson, NY) at 4 weeks age were divided into 10 groups of 8 mice (4 male and 4 females / group). Each week, starting from week 5 until week 12 of age the

morphology of the rear ankle joints of the mice were evaluated and scored for signs and symptoms of arthritis. Arthritic scores were assigned as follows.

0 = no arthritis (normal appearance and flexion)

1 = mild arthritis (joint distortion)

2 = moderate arthritis (swelling, joint deformation); and

3 = severe arthritis (ankylosis detected on flexion and severely impaired movement)

Animals were randomized to achieve approximately similar mean arthritic score (MAS) for all groups at week 9 at first signs of symptoms. Animals were then treated i.p. twice a week starting from week 10 of age until week 12 of age. Mice in group 1 served as untreated control were administered PBS. Group 2 and 3 animals received anti- TNFα (D2E7) mAb treatment at 30 and 10 mg/kg, respectively, while group 4 and 5 animals received anti-PGE_2 (2B5-8.0) mAb treatment 30 and 10 mg/kg, respectively. Groups 6 and 7 animals were treated with both anti- TNFα (D2E7) and anti-PGE_2 (2B5-8.0) mAb treatment with each mAb at 30 and 10 mg/kg respectively. Groups 8, 9 and 10 animals were treated with D2E7-SL-Hu2B5.7 DVD-Ig at 40, 13.3 and 4 mg/kg respectively. Figure 4 shows the mean arthritic score of animals treated with 2B5-8.0, D2E7, or a combination 2B5-8.0 and D2E7.

Example 2.3.12.2: In Vivo Efficacy In A Human TNFα Transgenic Induced Arthritis Model

Unlike the collagen-induced arthritis model, in which disease is driven by multiple mechanisms, disease in the human TNFα transgenic mouse model is driven primarily by over-expression of human TNFα. Symptoms of disease are typically evident at 9-10 weeks and fully develop by 20 weeks of age. These mice were treated with equivalent doses of D2E7-SL-Hu2B5.7, D2E7, or anti-PGE_2 mAb 2B5-8.0 twice weekly between weeks 10 and 12. Disease was scored weekly starting from week 9 and was expressed as the inhibition of AUC of the mean arthritis score. D2E7-SL-Hu2B5.7 reduced arthritis in a dose-dependent manner in the 3 mg/kg (30%) and 10 mg/kg (63%) groups. The maximal efficacy achieved at 10 mg/kg (63%) and 30 mg/kg (60%) equivalent doses was comparable to the maximal efficacy (59%) of the anti-human TNFα

mAb D2E7 at 30 mg/kg. There was no significant effect on disease progression in this model from PGE_2 blockade with 2B5-8.0, because arthritis in this model is primarily driven by human TNFα. Overall, these results demonstrated the *in vivo* activity of the anti-TNFα binding domain.

Example 2.3.13: Pharmacokinetic Analysis of D2E7-SL-hu2B5.7-DVD-Ig

Pharmacokinetic studies with D2E7-SL-hu2B5.7-DVD-Ig were carried out in Sprague Dawley rats and cynomolgus monkeys. Male and female rats were dosed intravenously or subcutaneously with a single dose of 4 mg/kg (rats) or 5 mg/kg (monkeys) of D2E7-SL-hu2B5.7-DVD-Ig and serum samples were analyzed using antigen capture based chemiluminescent MSD (Meso Scale Discovery) method. Pharmacokinetic parameters were calculated by non-compartmental analysis using WinNonlin.

Example 2.3.13.1: Assay used to quantitate D2E7-SL-hu2B5.7-DVD-Ig in PK serum samples

The following MSD assay was used to measure DVD-Ig concentrations in rat. MSD streptavidin plates (Meso Scale Discovery) were washed with phosphate buffered saline containing 0.05% Tween-20 (diluted from 10X PBS, Abbott Bioresearch Center, Media Room, Worcester, MA and Tween-20, Sigma, St. Louis, MO). Plates were blocked with 250 µL/well blocking solution (MSD Block, Meso Scale Discovery, diluted to 3% final concentration in PBS) for 1 hour, covered, with shaking (600rpm) at room temperature.

Prior to analysis, rat samples were thawed on ice, mixed gently, and centrifuged at 14,000rpm for 3 minutes at 4°C in an eppendorf centrifuge. Standard curve and control samples were prepared in rat serum. Study samples, standard curve samples, blanks, and quality control samples were incubated in solution in a separate 2mL deep well 96-well plate (Corning, Corning, NY) 1:1:1 = V:V:V with biotinylated TNFα (A-923884.0 Lot no. 1346920, Abbott Bioresearch Center Biologics Pharmacy, Worcester, MA; 0.1ug/mL in assay buffer) and sulfo-tagged goat anti-human IgG (Meso Scale Discovery, 1 ug/mL in assay buffer) for 1 hour at room temperature. The samples were then transferred to the MSD plates and incubated for an additional hour with shaking (600 rpm) at room

temperature. The MSD plates were washed and developed with 2x Read Buffer (Meso Scale Discovery). Chemilumeniscence was measured within ten minutes on the MSD Sector Imager 6000.

Standard curves were analyzed using four-parameter logistic fit and sample concentrations were calculated by XLfit4 software version 2.2.1 Build 16, (Microsoft Corporation, Redmond, WA). Pharmacokinetic parameters were calculated for each animal using Winonlin software version 5.0.1 (Pharsight Corporation, Mountain View, CA) by noncompartmental analysis (Table 15).

Example 2.3.13.2: Pharmacokinetic Studies carried out in SD rat

Surgically altered (jugular vein cannulated, JVC) and regular male and female Sprague-Dawley Rats (approximately seven weeks old, weighing 240-390 grams) were purchased from Charles River Laboratories (Wilmington, MA). The animals were housed in rooms maintained at constant temperature and humidity under 12hour light/dark cycle, fed with normal rodent chow and were allowed food and water ad libitum. Hydration and clinical conditions of the animals were monitored daily.

Blood samples were collected (0.2 mL from the rats and 0.5 mL from the monkeys) at various timepoints, allowed to clot for 30 minutes at room temperature, centrifuged for 8 minutes at 13,200 rpm, the serum transferred to eppendorf tubes and stored frozen at -80°C.

Following intravenous administration in rat, D2E7-SL-2B5.7 DVD-Ig exhibited bi-exponential decay, typical of antibodies. D2E7-SL-2B5.7 DVD-Ig clearance and volumes of distributions were low, with a long half-life of 10 days that is comparable to other therapeutic human monoclonal antibodies. Following subcutaneous administration in rat, absorption was slow, with high Cmax of about 25-31 ug/ml reached by 3 days. In the animals without ADA, the half-life was long (T1/2 ~ 12 days), and the bioavailability was high (F: 87-98 %) (Table 15).

Table 15: Main Pharmacokinetic Parameters Of D2E7-SL-Hu2B5.7-DVD-Ig In Sprague-Dawley Rat And In Cynomolgus Monkey

IV							
Species /dose		T½ (day)	CL (mL/hr/kg)	Vz (mL/kg)	Vss (mL/kg)	$AUC_{0-\infty}$ (mg•hr/mL)	MRT (day)
Rat	M (N = 10)	10.1 ± 2.1	0.34 ± 0.06	116 ± 15.9	107 ± 14	12.1 ± 2.3	13.5 ± 2.6
(4 mg/kg)	F (N = 10)	9.4 ± 2.5	0.31 ± 0.04	98 ± 21.8	96 ± 19.9	13.4 ± 2.3	13.4 ± 3.4
SC							
Species		C_{max} (μg/mL)	Tmax (day)	T½ (day)	MRT (day)	$AUC_{0-\infty}$ (mg•hr/mL)	F (%)
Rat	M (N = 8)	25.2 ± 2.3	2.8 ± 0.5	12.0*	18.1*	11.9*	98.3*
(4 mg/kg)	F (N = 10)	30.6 ± 7.5	3.5 ± 2.6	11.2 ± 3.7[&]	17.0 ± 4.0[&]	11.1 ± 2.1[&]	86.9 ± [&]

* n=2; [&] n= 6

Example 2.4: Physicochemical Analysis Of Anti-TNFα/Anti-PGE_2 DVD-Ig

Example2.4.1: Size Exclusion Chromatography

D2E7-SL-Hu2B5.7 DVD-Ig samples were diluted to 1 mg/mL with PBS and 20 μL was injected onto Shimadzu HPLC system with a TSK gel G3000 SWXL column (Tosoh Bioscience, cat# 08541). Samples were eluted from the column with 100 mM sodium sulfate, 100 mM sodium phosphate, 1 mM sodium azide, pH 6.8, at a flow rate of 0.5 mL/minutes. The HPLC system operating conditions were the following.

Mobile phase: 100 mM sodium phosphate, 100 mM sodium sulfate, 1 mM sodium azide, pH 6.8,

Gradient: Isocratic

Flow rate: 0.5 mL/minute

Detector wavelength: 280 nm

Autosampler cooler temp: 4°C

Column oven temperature: Ambient

Run time: 30 minutes

The main peak eluted at around 16 minutes. The peak eluted at around 13 minutes was associated with the aggregates. The monomer peak was 99.7%, and the aggregate peak was 0.3%.

Example 2.4.2.B: SDS-PAGE

D2E7-SL-Hu2B5.7 DVD-Ig samples are analyzed by sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) under both reducing and non-reducing conditions. For reducing conditions, the samples are mixed 1:1 with 2X Tris-Glycine SDS sample buffer (Invitrogen, cat# LC2676) with 200 mM DTT, and heated to 70°C for 15 minutes. For non-reducing conditions, the samples are mixed 1:1 with 2X Tris-Glycine SDS sample buffer and heated at 75°C for 15 minutes. Both reduced and non-reduced samples were loaded onto 8%-16% pre-cast Tris-Glycine gels (Invitrogen, cat# EC6045box). Mark 12 unstained standard (Invitrogen, cat# LC5677) was used as a molecular weight marker. The gels were run in an XCell SureLock mini cell gel box (Invitrogen, cat# EI0001) and the proteins were separated by applying a voltage of 125 v until the dye front reached the bottom of the gel. The running buffer used was 1X Tris-Glycine SDS buffer, prepared from a 10X Tris-Glycine SDS buffer (ABC, MPS-79-080106)). The gels were stained overnight with colloidal blue stain (Invitrogen cat# 46-7015, 46-7016) and destained with Milli-Q water until the background was clear. The stained gels were then scanned using a flatbed densitometer (Bio-Rad, cat# GS-800).

Under non-reducing conditions a single major band consistent with the expected molecular weight (~200KDa) of the D2E7-SL-Hu2B5.7 DVD-Ig was observed. This major band migrated higher than a typical IgG1, which is consistent with the additional domains in a D2E7-SL-Hu2B5.7 DVD-Ig. Under reducing conditions two major bands consistent with the expected molecular weight for heavy chain (62.5 KDa) and light chain (37.5KDa) respectively are observed.

Example 2.4.3: Sedimentation Velocity Analysis

D2E7-SL-Hu2B5.7 DVD-Ig samples were dialyzed against PBS overnight. The samples are diluted in PBS to yield approximate absorbance values of 1.0 at 280 nm in the 1.2 cm path-length cells in the analytical centrifuge. This dilution resulted in a final concentration of ~0.5 mg/mL. PBS was used in the reference cell. Solution density, viscosity, and v-bar values were calculated using SEDINTERP (v1.09). Each DVD-Ig sample was analyzed independently and three identical replicates were run for each sample. Sample solutions and reference blanks were loaded into standard two-sector cells with a 1.2 cm optical path lengths, sapphire windows, and carbon epon centerpieces. All samples were examined simultaneously using a 4-hole (AN-60Ti) rotor in a Beckman ProteomeLab XL-I analytical ultracentrifuge (serial # PL106C01).

Run conditions were programmed and centrifuge control was performed using ProteomeLab (v5.6). Absorbance data was used for analysis.

The samples and rotor were allowed to thermally equilibrate for greater than two hours prior to starting the run (20.0 ± 0.1°C). Confirmation of proper cell loading was performed at 3000 rpm and a single scan is recorded for each cell. The sedimentation velocity conditions were the following:

Sample Cell Volume: 420 µL

Reference Cell Volume: 420 µL

Temperature: 20°C

Rotor Speed: 35,000 rpm

Time: 8:00 hours

UV Wavelength: 280 nm

Radial Step Size: 0.003 cm

Data Collection: One data point per step without signal averaging

Total Number of Scans: 100

The results from sedimentation velocity analytical ultracentrifugation demonstrate that the D2E7-SL-Hu2B5.7 DVD-Ig is predominately monomeric (99.58%) in solution with a low percentage of aggregate. The sedimentation coefficient of the monomer (7.5 S) was greater than the typical value of 6 - 6.5 S measured for a conventional human IgG 1 molecule. This larger sedimentation coefficient measured for the D2E7-SL-Hu2B5.7 DVD-Ig is the result of the larger hydrodynamic size resulting from the dual-variable domain. Additionally, the frictional ratio (1.64) is greater than what is typically observed for recombinant humanized IgGs. Most antibodies have frictional ratio of 1.4 - 1.5.

Example 2.4.4: SDS-CE

Both non-reduced and reduced SDS-CE of the D2E7-SL-Hu2B5.7 DVD-Ig were obtained. The D2E7-SL-Hu2B5.7 DVD-Ig samples were diluted to 2 mg/mL with Milli-Q water and mixed 1:1 with SDS-CE sample buffer. For non-reduced samples, 0.5 M Iodoacetamide (Sigma A3221-1VL) in the volume of 1/10 of the protein sample was added. For reduced samples, 1/10 protein volume of β-mercaptoethanol (Sigma M-7154) was added instead. Samples were vortexed and transferred to 70°C water bath. Samples were incubated for 15 minutes then stopped in a room temperature water bath. Sample vials were centrifuged at low speed briefly and vortexed to collect sample liquid in the bottom. Samples were transferred to PCR vials and loaded in vial racks for analysis. ProteomeLab PA800 capillary electrophoresis system (Beckman Coulter) and uncoated bare fused-silica capillary (Beckman Coulter, part of P/N 10663) were used for the SDS-CE analysis.

Non-reduced SDS-CE showed a main peak consistent with the intact D2E7-SL-Hu2B5.7 DVD-Ig. The peak area percentage of this main peak was 95.3%. There were minor peaks migrating earlier than the main peak. These minor peaks were likely fragments of the DVD-Ig molecule, some of which were caused by the heat treatment during sample preparation. Reduced SDS-CE showed two major peaks corresponding to light chain (36.1%) and heavy chain (60.8%) respectively. The minor peaks migrating after the heavy chain peak (0.5%) were most likely caused by incomplete reduction. Reduced SDS-CE also showed a small peak (2.2%) in front of the heavy chain peak. This peak was related to the non-glycosylated heavy chain.

Example 2.4.5: Dynamic Light Scattering

D2E7-SL-Hu2B5.7 DVD-Ig samples and the two parent molecules D2E7 and Hu2B5.7 were diluted to 2 mg/mL with PBS (GIBCO 20012). Samples were centrifuged at 10,000 RPM for 10 minutes before measured on DynaPro-801 Dynamic light scattering system. The hydrodynamic radius of the D2E7-SL-Hu2B5.7 DVD-Ig was 6.43nm. The average hydrodynamic radius of D2E7 and Hu2B5.7 was 5.46nm and 5.36nm, respectively. These results demonstrate that the D2E7-SL-Hu2B5.7 DVD-Ig has a larger hydrodynamic radius than both of the two parent molecules, which is consistent with the fact that the D2E7-SL-Hu2B5.7 DVD-Ig has additional domains than a typical IgG1 molecule.

Example 2.4.6: LC-MS Molecular Weight Measurement

The intact molecular weight and deglycosylated molecular weight of D2E7-SL-Hu2B5.7 DVD-Ig sample was analyzed by LC-MS. Each sample was diluted to approximately 1 mg/mL with water. 0.5 µL of the diluted sample was injected onto the Agilent 6510 Q-TOF LC/MS system (Agilent G6510A, serial no. US65020122) with a Poroshell 300SB-C3 column (Agilent, cat# 661750-909). A short gradient (Table 16) was used to elute the samples. The gradient was run with mobile phase A (0.1% formic acid in water, J. T. Baker, cat# 9834-03) and mobile phase B (0.1% formic acid in acetonitrile, J. T. Baker, cat# 9832-03) at a flow rate of 50 µL/minute. The mass spectrometer was operated at 5 kvolts spray voltage and scan range is from 600 to 3200 mass to charge ratio. To deglycosylated the D2E7-SL-Hu2B5.7 DVD-Ig, 50 µg of DVD-Ig sample is mixed with 2.5 µL of 10% N-octylglucoside (Sigma, cat# O8001) and 1 µL of PNGase F (Prozyme, cat# GKE5006A). Milli-Q water was added to each sample to make the total volume 50 µL. The sample was incubated at 37°C for 17 hours. The same HPLC and mass spectrometry conditions used to acquire the intact molecular weight was used to obtain the deglycosylated molecular weight (Table 16).

Table 16: HPLC Gradient For Intact Molecular Weight Analysis

Time (min)	% Buffer B
0	5
5	5
5.5	95
10	95
10.5	5
15	5

The intact molecular weight of the D2E7-SL-Hu2B5.7 DVD-Ig was 200,574 Dalton, with 5 Dalton difference from the theoretical value of 200,569 based on the amino acid derived from c-DNA sequence, with the addition of the two N-linked oligosaccharide modification of NGA2F. Molecular weight of 200,736 Dalton and 200,894 Dalton were also observed, corresponding to different oligosaccharide modifications. After deglycosylation, the molecular weight of the DVD-Ig was determined as 197,684 Dalton, with 5 Dalton difference from the theoretical value of 197,679. Low level of peaks with molecular weight 197,810 Dalton and 197,936 Dalton were also observed, which correspond to deglycosylated molecular weight with C-terminal lysines.

Example 2.4.7: LC-MS Molecular Weight Measurement of Light Chain and Heavy Chain

Molecular weight measurements of D2E7-SL-Hu2B5.7 DVD-Ig light chain (LC), heavy chain (HC) and deglycosylated HC were analyzed by LC-MS. A DVD-Ig sample was diluted to 1 mg/mL with water and the sample was reduced to LC and HC with a final concentration of 25 mM DTT for 30 minutes at 37°C. 0.5 µL of the diluted sample was injected onto the Agilent 6510 Q-TOF LC/MS system (Agilent G6510A, serial no. US65020122) with a Poroshell 300SB-C3 column (Agilent, cat# 661750-909). A gradient (Table 17) was run with mobile phase A (0.1% formic acid in water, J. T. Baker, cat# 9834-03) and mobile phase B (0.1% formic acid in acetonitrile, J. T. Baker, cat# 9832-03) at a flow rate of 50 µL/minute. The mass spectrometer was operated at 5 kvolts spray voltage and scan range was from 600 to 3200 mass to charge ratio. The deglycosylated DVD-Ig was reduced with a final concentration of 25 mM DTT for 30

minutes at 37°C. The deglycosylated heavy chain molecular weight was acquired using the same conditions as above.

Table 17: HPLC Gradient For Reduced Molecular Weight Analysis

Time (min)	% Buffer B
0	5
5	35
20	65
21	95
25	95
26	5

The light chain molecular weight of the DVD-Ig was determined as 36,193 Dalton, matching the theoretical value of 36,193 Dalton. The heavy chain molecular weight was 64,097 Dalton, with 1 Dalton difference from the theoretical value of 64,096 Dalton based on the amino acid sequence and the addition of N-linked oligosaccharide NGA2F. Molecular weights of 64,260 Dalton and 64,423 Dalton were also observed, corresponding to the heavy chain with the N-linked oligosaccharide modifications of NA1F and NA2F, respectively. After deglycosylation, the light chain molecular weight does not change, indicating the absence of oligosaccharide modification on the light chain. The heavy chain molecular weight was 62,653 Dalton, with 2 Dalton difference from the theoretical value of 62,651. Low level of C-terminal lysine variant is also observed as a molecular weight of 62,781 Dalton.

Example 2.4.8: Peptide Mapping

Peptide mapping of D2E7-SL-Hu2B5.7 DVD-Ig was performed as followings: 200 µg DVD-Ig sample was diluted to 1 mg/mL with 50 mM ammonium bicarbonate (Mallinckrodt cat# 0155), reduced and denatured in 10 mM DTT (EM Sciences, cat# 3810) and 6 M Guanidine HCl (Pierce, produce no. 24115) at 37°C for 30 minutes. 10 µL 1 M Iodoacetic acid (Sigma cat# I4386-100G) was added and the sample was incubated in dark at 37°C for 30 minutes. The reduced, denatured and alkylated sample was first dialyzed using a slide-A-lyzer (Pierce, product no 66333) against 4 L 50 mM ammonium bicarbonate at 4°C for 30 minutes, then dialyzed against 4 L 50 mM ammonium bicarbonate overnight. The sample was recovered and digested with trypsin (Promega, cat# V511A) at a 1:20 enzyme to protein ratio (w/w), at 37°C for 4 hours.

Digestion was stopped by addition of 2 µL of TFA (J. T. Baker, cat# 9470-01). The sample was transferred to an HPLC vial for LC/MS analysis. 5 µL sample was injected and the sample is separated by reverse phase HPLC using a Vydac C18 column (Vydac, cat# 218MS51) on an Agilent 1200 capillary HPLC system. The column heater was set at 60°C. The separation was run with a gradient shown in Table 18 using buffer A (0.1% FA in water, J. T. Baker, cat# 9834-03) and buffer B (0.1% FA in acetonitrile, J. T. Baker, cat# 9832-03) at a flow rate of 50 µL/minute. The Agilent 6510 Q-TOF LC/MS system (Agilent cat# G6510A, serial no. US65020122) is operated in positive electrospray mode. The mass spectrometer is operated at 5.0 kvolts spray voltage and scan range from 200 to 3000 mass to charge ratio.

Table 18: HPLC Gradient For LC-MS Peptide Mapping (Lot 1582940)

Time (minutes)	Mobile phase A (%)	Mobile phase B (%)
0	98	2
10	98	2
160	65	35
180	50	50
200	2	98
210	2	98
212	98	2
222	98	2
222.1	Stop	

Theoretically, the D2E7-SL-Hu2B5.7 DVD-Ig molecule has 332 amino acids in its light chain and 573 amino acids in its heavy chain. Peptide map by LC-MS reports observed amino acids and their sequences. Tables 20-22 show the LC/MS results of light chain and heavy chain peptides. All of the DVD-Ig light chain and heavy chain amino acids were detected. The observed mass all matched well with the theoretical mass, with difference being less than 0.02 Dalton. The overall sequence recovery was 100% for the DVD-Ig. Although C-terminal lysine residues were coded for the heavy chains, they could be removed post-translationally by endogenous carboxypeptidase B expressed by the host cells. LC-MS peptide map demonstrates that although the C-terminal lysine was present, the majority of the C-terminal peptide was without lysine.

Table 19: D2E7-SL-HuB5.7 DVD-Ig Light and Heavy Chain Sequences

DVD-Ig Chain	SEQ ID NO	Amino Acid Sequence 12345678901234567890123456789012345678901234567890
Light	126	DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKP GKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISSLQP EDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPDVLMTQT PLSLPVTPGEPASISCTSSQNIVHSNGNTYLEWYLQKPGQ SPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAED VGVYYCFQVSHVPYTFGGGTKVEIKRTVAAPSVFIFPPSD EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLS SPVTKSFNRGEC
Heavy	127	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQA PGKGLEWVSAITWNSGHIDYADSVEGRFTISRDNAKNSLY LQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLVTVS SASTKGPEVQLVQSGAEVKKPGASVKVSCKASGYTFTKYW LGWVRQAPGQGLEWMGDIYPGYDYTHYNEKFKDRVTLTTD TSTSTAYMELRSLRSDDTAVYYCARSDGSSTYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS RDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK

Table 20: LC/MS Results of DVD-Ig Light Chain

Peptide	Amino Acid Sequence (from - to)	Theoretical Mass	Observed Mass
1	1-18[a]	1877.88	1877.88
2	19-24	749.39	749.40
3	25-30	630.34	630.34
4	31-42	1494.76	1494.76
5	43-45	314.20	314.20
6	46-61	1674.93	1674.93
7	62-90	3187.43	3187.43
8	91-93	451.22	451.22
9	94-103	1068.52	1068.52
10	104-107	487.30	487.3
11	108-108[b]	174.11	643.40 theo. 643.40 observed
12	109-168	6540.29	6539.30
13	169-172	474.26	474.28
14	173-179	776.38	776.38
15	180-192	1302.61	1302.61
16	193-195	374.23	374.23
17	196-221	2909.32	2909.32
18	222-225	487.30	487.30
19	226-226[C]	174.11	2101.12 theo. 2101.12 observed
20	227-244	1945.02	1945.02
21	245-260	1797.87	1797.87
22	261-263	346.19	346.19
23	264-267	559.31	559.31
24	268-287	2134.96	2134.96
25	288-301	1501.75	1501.75
26	302-306	624.28	624.28
27	307-308	283.16	283.16
28	309-325	1875.90	1874.92
29	326-329	522.26	522.28
30	330-332[d]	365.09	869.33 theo. 869.33 observed

a. Column 2 shows the amino acid number starting from the N-terminus
b. Peptide 11 was observed as incomplete digest of sequence 104-108
c. Peptide 19 was observed as incomplete digest of sequence 226-244
d. Peptide 30 was observed as incomplete digest of sequence 326-332

Table 21: LC/MS Results of DVD-Ig Heavy Chain

Peptide	Amino Acid Sequence (from - to)	Theoretical Mass	Observed Mass
1	1-16[a]	1623.86	1623.86
2	17-19	374.23	374.23
3	20-38	2233.96	2233.96
4	39-43	499.28	499.28
5	44-67	2661.25	2661.24
6	68-72	622.34	622.34
7	73-76[b]	446.21	3265.58 theo. 3265.58 observed
8	77-87	1337.68	1337.68
9	88-98	1290.54	1289.56
10	99-125	2807.39	2807.39
11	126-139	1439.76	1439.76
12	140-146	685.41	685.41
13	147-150	493.22	492.24
14	151-158	873.42	873.42
15	159-165	978.51	978.51
16	166-190	2931.28	2931.28
17	191-192	293.17	293.17
18	193-194	289.14	289.14
19	195-211	1888.91	1888.91
20	212-214	374.23	374.23
21	215-225	1320.53	1319.55
22	226-247	2218.04	2218.04
23	248-259	1185.64	1185.64
24	260-273	1321.65	1320.67
25	274-336	6713.29	6713.30
26	337-339	360.20	360.20
27	340-340[c]	146.11	599.36 theo. 599.36 observed
28	341-344	471.27	471.27
29	345-348	509.18	509.18
30	349-374	2845.42	2845.42

a. Column 2 shows the amino acid number starting from the N-terminus
b. Peptide 7 was observed as incomplete digest of sequence 44-72
c. Peptide 27 was observed as incomplete digest of sequence 340-344

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Table 22: LC/MS Results of DVD-Ig Heavy Chain (continued)

Peptide	Amino Acid Sequence (from - to)	Theoretical Mass	Observed Mass
31	375-381[a]	834.43	834.43
32	382-400	2139.00	2139.00
33	401-414	1676.79	1676.79
34	415-418	500.31	500.31
35	419-427[b]	N/A	2633.04
36	428-443	1807.00	1807.00
37	444-446	438.21	438.21
38	447-448	307.12	307.12
39	449-452	446.25	446.25
40	453-460	837.50	837.50
41	461-464	447.27	447.27
42	465-466	217.14	217.14
43	467-470	456.24	456.24
44	471-481	1285.67	1285.67
45	482-486	604.31	604.31
46	487-496	1161.61	1161.61
47	497-518	2543.12	2543.13
48	519-535	1872.91	1872.91
49	536-540	574.33	574.33
50	541-542	261.14	261.14
51	543-565	2801.24	2801.24
52	566-572	659.35	659.35
52[c]	566-573	787.44	787.44

a. Column 2 shows the amino acid number starting from the N-terminus.
b. Peptide 35 was observed as peptide with N-linked oligosaccharide modification.
c. Low level of C-terminal lysine was observed.

Example 2.4.9: Disulfide Bond Mapping

The disulfide bond mapping was performed by peptide map of the D2E7-SL-Hu2B5.7 DVD-Ig under non-reducing conditions. Two aliquot of DVD-Ig samples were diluted to 0.5 mg/mL with 50 mM ammonium bicarbonate and denatured in 0.1% RapiGest SF (Waters Corporation, part no. 186001861) at 65°C for 1 hour. The total volume of each sample was 100 µL. The samples were digested with trypsin (Promega, cat# V511A) and Lys-C (Roche Diagnostics, cat#. 11 047 825 001), respectively. The trypsin digestion was performed at an enzyme to protein ratio (w/w) of 1:6.25 and the Lys-C digestion was performed at an enzyme to protein ratio of 1:20 (w/w), both at 37°C for approximately 17 hours. Digestions were stopped by addition of 1 µL of TFA. The

samples were incubated at 37°C for 30 minutes. Slight cloudiness was observed. The samples were then centrifuged at 13,000 RPM for 10 minutes. The hydrolytic RapiGest SF by-products were water immiscible, and some precipitation was observed. The supernatant was transferred to a sample vial for LC/MS analysis. The samples were separated by reverse phase HPLC using a Vydac C18 column on an Agilent 1200 capillary HPLC system. The column heater was set at 60°C. The separation was run with the same gradient used for peptide map shown in Table 18, using buffer A (0.1% FA water) and buffer B (0.1% FA in acetonitrile) at a flow rate of 50 µL/minutes. The Agilent 6510 Q-TOF LC/MS system was operated in positive electrospray mode. The mass spectrometer was operated at 5.0 kvolts spray voltage and scan range from 200 to 3000 mass to charge ratio. Disulfide bonds were assigned by matching the observed molecular weights of the peptides with the predicted molecular weights of tryptic or Lys-C peptides linked by disulfide bonds.

The D2E7-SL-Hu2B5.7 DVD-Ig has an additional variable domain on both light chains and heavy chains compared to a typical IgG1 molecule. The experimental results of the disulfide bond mapping of the DVD-Ig are summarized in Table 23. When combining the results of trypsin digestion and Lys-C digestion, all the theoretical disulfide linked peptides were observed.

Table 23: Disulfide Bond Mapping of the D2E7-SL-Hu2B5.7 DVD-Ig

		Trypsin		Lys-C		
LC (cys)	LC (cys)	Theoretical	Observed	Theoretical	Observed	Confirmed
23	88	3818.78	3818.77	10935.37	N/A	Yes
136	206	9331.58	9331.84	9843.91	N/A	Yes
252	312	3555.75	3555.75	3883.92	3883.93	Yes
HC (cys)	HC (cys)	Theoretical	Observed	Theoretical	Observed	Confirmed
22	97	3406.47	3406.48	7169.47	7169.47	Yes
151	227	1695.73	1695.73	6394.00	6393.96	Yes
276	332	7916.92	7916.80	7916.92	7916.85	Yes
360-363	360-363	5454.78	5454.78	5454.78	5454.75	Yes
395	457	2328.10	2328.10	3144.52	3144.50	Yes
503	563	3844.82	3844.83	4087.95	4087.95	Yes
LC (cys)	HC (cys)	Theoretical	Observed	Theoretical	Observed	Confirmed
332	352	756.25	756.25	1260.49	1260.48	Yes

Example 2.4.10: Free Sulfhydryl Determination

The method used to quantify free cysteines in the D2E7-SL-Hu2B5.7 DVD-Ig was based on the reaction of Ellman's reagent, 5,5¢- dithio-bis (2-nitrobenzoic acid) (DTNB), with sulfhydryl groups (SH), which gives rise to a characteristic chromophoric product, 5-thio-(2-nitrobenzoic acid) (TNB). The reaction is illustrated in the formula:

DTNB + RSH ® RS-TNB + TNB- + H+

The absorbance of the TNB- was measured at 412 nm using a SPECTRA max Plus 384 (Molecular Devices) plate reader. An absorbance curve is plotted using dilutions of 2 mercaptoethanol (β-ME) as the free SH standard and the concentrations of the free sulfhydryl groups in the protein were determined from absorbance at 412 nm of the sample.

The β-ME (Pierce, cat# 35602) standard stock was prepared by a serial dilution of 14.2 M β-ME with HPLC grade water to a final concentration of 0.142 mM. Then standards in triplicate for each concentration were prepared. The samples were mixed on a shaker at room temperature for 20 minutes (Table 24). After thorough mixing, the samples and standards were mixed with 54 µL 100 mM Tris, pH 8.1, and 90 µL 2 mM DTNB (Sigma, D8130). The OD was measured for absorption at 412 nm. The standard curve was obtained by plotting the amount of free SH and OD_{412} nm of the β-ME standards. Free SH content of samples were calculated based on this curve after subtraction of the blank.

Table 24: Sample and Control Preparation Scheme

Sample (prepared 3xs)	sample volume, µL	20% SDS, µ L	Milli-Q water, µ L	Final volume, µ L	nmol sample	mg/mL sample	mix in shaker in room temp for	add µL of 100 mM Tris, pH 8.1	add µL of 2mM DTNB	total µL pre-OD reading
Blank	90	18	72	180	0.00	0.0	20 minutes	54	90	324
DVD sample	90	18	72	180	10.94	24.3		54	90	324

The standard curve for the determination of free sulfhydryl concentration covered an absorbance range at 412 nm from 0.002 to 1.347 OD. The correlation coefficient for the fitted data was 0.994. Two duplicate samples of the DVD-Ig were prepared and

analyzed. The free sulfhydryl levels of the two duplicate samples were 0.35% and 0.63% respectively.

Example 2.4.10: Weak Cation Exchange Chromatography

The D2E7-SL-Hu2B5.7 DVD-Ig sample was diluted to 1 mg/mL in buffer A (10 mM Sodium phosphate dibasic, pH 7.5), and analyzed by a weak cation exchange HPLC method using the ProPac WCX-10 column (Dionex, ProPac WCX-10, product no. 054993, 4 mm × 250 mm). Table 25 shows the HPLC system operating conditions for WCX-10 analysis. The results showed that DVD-Ig has 19.3% acidic species, 65.2% main peak, and 15.5% basic species.

Table 25: HPLC System Operation Conditions for WCX-10 Analysis

Item	Description/ Operating Conditions	
Column	Dionex ProPac WCX-10, 4 mm × 250 mm	
Guard column	Dionex ProPac WCX-10G, 4 mm × 50 mm	
Mobile phase A	10 mM Sodium phosphate, pH 7.5	
Mobile phase B	10 mM Sodium phosphate, 500 mM sodium chloride, pH 5.5	
Gradient	Time (minute)	Mobile phase B (%)
	0	8
	34	25
	36	100
	41	100
	43	8
	48	8
	49	Stop
Flow rate	1.0 mL/min	
Autosampler temperature	4°C	
Column oven temperature	Ambient	
Sample load	30 µL of 1.0 mg/mL sample diluted in Buffer A	
Run time	49 minutes	
UV detection	280 nm	

Example 2.4.11: Capillary Zone Electrophoresis

Capillary zone electrophoresis is a capillary electrophoresis method in which the capillary is only filled with buffer. The separation mechanism was based on differences in electrophoretic mobility of the analyte. The electrophoretic mobility of a molecule was related to the charge-to-size ratio. Beckman-Coulter ProteomeLab PA 800 was used for

the CZE analysis. A neutral capillary (eCAP neutral, 56 cm total length, 50μm I. D. Beckman-Coulter, P/N 477441) was used. The method uses a 30.2 cm capillary with a detection window 20.2 cm from the sample introduction inlet. The running buffer was prepared by mixing 5 mL Citrate/MES buffer (Beckman Coulter, cat# 477443) with 0.5 mL 1% MC solution (Convergent Bioscience, cat# 101876) and centrifuging at 10, 000 RPM for 2 minutes.

The results show two basic peaks migrating before the main peak. The peaks corresponded to the lysine variants. The main peak showed a tailing on the acidic side, which is caused by acidic species that are not well resolved from the main peak.

Example 2.4.12: <u>Oligosaccharide Profiling</u>

Oligosaccharides released after PNGase F treatment of antibody were derivatized with a 2-aminobenzamide (2-AB) labeling reagent. The fluorescent-labeled oligosaccharides were separated by normal phase high performance liquid chromatography (NPHPLC) and the different forms of oligosaccharides were characterized based on retention time comparison with known standards.

The D2E7-SL-Hu2B5.7 DVD-Ig was first digested with PNGase F to cleave N-linked oligosaccharides from the Fc portion of the heavy chain. 200 μg of DVD-Ig was digested with 3 μL PNGase F (Prozyme: cat#. GKE5006D) in the presence of 2.5 μL 10% N-octylglucoside (Sigma, cat# O8001). Milli-Q water was added to bring the final volume to 50 μL. The sample was incubated in a thermomixer set at 37°C and 700-800 RPM for 17 hours.

After deglycosylation, the protein was precipitated at 95°C for 6 minutes. The sample tube was centrifuged at 10,000 RPM for 5 minutes. The supernatant was transferred into a microcentrifuge tube and placed into a speed vacuum centrifuge. The solution was brought to dryness. The temperature was set to room temperature or up to 30°C to speed up the process.

The 2-AB labeling kit from Prozyme was used for the labeling step. To prepare the reagent, 150 μL of acetic acid (vial C) was added to a vial of DMSO (vial B) and mixed well by vortexing. 100 μL of the acetic acid/DMSO mixture was then added to a

vial of 2-AB dye (vial A) and vortexed until the dye was dissolved. The entire content of this vial was then added to the reductant vial (vial D). The vial was heated at 65°C till dissolved, and then mixed by vortexing until the contents fully dissolved. This was the labeling reagent. 10 µL of the labeling reagent was added to each dried oligosaccharide sample. The sample tubes were vortexed to get the dried oligosaccharides into solution. The tubes were centrifuged quickly to collect the entire volume to the bottom. They were then placed in a thermomixer set at 65°C and 750 RPM for 2 hours.

After the sample was labeled, the free fluorescent reagent was removed using GlycoClean S cartridges. Each cartridge was washed with 1 mL Milli-Q water and allowed to drain completely. The water wash was followed by 5 x 1 mL of 30% acetic acid and allowed to drain completely. The cartridge was washed with 2 x 1 mL acetonitrile and allowed to drain completely.

After labeling the oligosaccharides, each tube was centrifuged quickly to collect all volume at the bottom of the tube. All tubes were allowed to cool to room temperature. Each sample was spotted onto the freshly washed membrane of the GlycoClean S cartridge, with the sample spreading over the entire membrane surface. Each sample tube was rinsed with 20 µL acetonitrile and the volume was added to the corresponding cartridge membrane. Each GlycoClean S cartridge was left at room temperature for a minimum of 15 minutes to allow the labeled oligosaccharides to adsorb onto the membrane.

Each cartridge was washed with 1 mL of 100% acetonitrile and allowed to drain completely. 5 x 1 mL of 96% acetonitrile solution is added, allowing each aliquot to drain before the next one is applied. The flow through was discarded to waste. The cartridge dead volume was removed from the base of the cartridge before sample elution. Each cartridge was placed over an appropriately labeled 1.5 mL microcentrifuge tube. The 2-AB labeled oligosaccharides are eluted with 3 x 400 µL of Milli-Q water.

Each sample was diluted by a factor of 3.3 x with acetonitrile. 150 µL of eluted 2-AB labeled oligosaccharide sample was mixed with 350 µL of acetonitrile. 200 µL was injected for HPLC analysis.

The oligosaccharides were separated using a Glycosep N HPLC (cat# GKI-4728) column connected to a Shimadzu HPLC system. The Shimadzu HPLC system consisted of a system controller, degasser, binary pumps, autosampler with a sample cooler, and a fluorescent detector.

The oligosaccharide profiling results of the D2E7-SL-Hu2B5.7 DVD-Ig is shown in Table 26.

Table26: HPLC Conditions For The N-Linked Oligosaccharide Analysis

Item	**Description/ Operating Conditions**	
Guard column	GlycoSep™ N Guard, 4.6 x 10 mm	
Column	GlycoSep™ N, 4.6 x 250 mm	
Mobile phase A	100% acetonitrile	
Mobile phase B	50 mM ammonium formate, pH 4.4	
Column storage	75% acetonitrile/water	
Gradient	Time (minute)	Mobile phase B%
	0.01	35
	5	35
	75	40
	95	40
	100	100
	105	100
	107	35
	127	35
	127.1	Stop
Flow rate	0.40 mL/min	
Excitation wavelength	330 nm	
Emission wavelength	420 nm	
Gain	4	
Sensitivity	Medium	
Response	1.5 sec	
Autosampler temperature	Nominal 4°C	
Column oven temperature	30°C	
Sample load	150 µL	
Run time	127.1 minutes	

Table27: Summary of Oligosaccharide Profiling Results

	Gal 0	Gal 1	Gal 2	Man 5	Man 6	Man 7	Man 8	Gal 0-GlcNAc	Gal 1-GlcNAc	Mannose total
DVD-Ig	55.8	22.6	2.9	6.5	2.4	1.8	1.1	5.7	2.0	11.8

Example 2.4.13: Circular Dichroism

D2E7-SL-Hu2B5.7 DVD-Ig sample was diluted to 0.3 mg/mL with 5 mM sodium phosphate pH 6.8. 500 µL of the sample was first dialyzed against 5 mM sodium phosphate pH 6.8 for 20 minutes at 4°C, and then dialyzed against the same buffer overnight at 4°C. After the sample was recovered from the Slide-A-Lyzer cassettes, the A_{280} protein absorbance was measured. The samples were further diluted to 0.15 mg/mL with the dialysate and the final A_{280} value is to 0.221 OD. 5 mM sodium phosphate buffer, pH 6.8 was used as reference blank for background subtraction.

Far-UV spectra were acquired on a Jasco J-810 spectropolarimeter using a thermostatically controlled housing at 20°C. Each CD measurement represented a corrected average of three wavelength scans from 184-260 nm. The cuvette chamber was continuously flushed with nitrogen throughout the experiment. Detector voltages HT[V] were kept below 600 volts to avoid PMT saturation.

Differences in protein secondary structure were measured based on distinctive circular dichroism spectra in the far-ultraviolet region. α-Helical structure was characterized by a strong positive band at approximately 190 nm and negative bands at approximately 208 nm and 222 nm. β-sheet structure was characterized by a positive band at approximately 200 nm and a negative band at about 216 nm. Random coil has a negative band at approximately 195 nm and a positive band at about 220 nm. The CD spectra of the DVD-Ig shows a positive band at approximately 200 nm and a negative band at about 216 nm, which is the characteristic signature of β-sheet structure. The results demonstrated that the DVD-Ig has a β-sheet secondary structure. There is a small positive ellipticity between 230 nm and 240 nm. This positive ellipticity is also observed in Hu2B5.7, one of the parent molecules, but not in D2E7, the other parent molecule. It is possible that this positive ellipticity is related to the folding of the anti-PGE-2 portion of the molecule.

Example 2.4.14: Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) was performed on D2E7 and the D2E7-SL-Hu2B5.7 DVD-Ig according to the methods of Example 1.3.3.2.L. In contrast to conventional antibodies, DVD-Igs contained one additional domain that theoretically can unfold, i.e., one additional VH1 and VL1 domain. No overlap of the unfolding transition temperatures (Tm) of the various domains assumed, 4 unfolding transitions may be expected that can be characterized by DSC analysis. D2E7-SL-Hu2B5.7 DVD-Ig had 4 transitions, which can be determined at 58.8°C, 68.6°C, 75.0°C, and 83.4°C (Figure 6). Furthermore, these data demonstrated that the DVD-Ig molecule has good intrinsic conformational stability which makes it a very good candidate for successful product development, as no domain of the DVD-molecule unfolds before 58.8°C, which is much higher than the recommended storage temperature of biologics products (compared to Tm values of Adalimumab of 56.9°C, 67.4°C, and 76.75°C).

Example 3: Formulation Analysis Development Of Anti-TNF/anti-PGE_2 DVD-Ig

Example 3.1: Methods Applied For Biophysico-Chemical Characterization And Formulation Development

Criteria tested ranged from general quality parameters (e.g., protein content, pH), parameters indicating intrinsic stability (DSC), physical stability (e.g., particle contamination and insoluble aggregate and precipitate formation monitored by visual inspection and light obscuration particle counting, purity including fragmentation and aggregation monitoring by SEC), and parameters indicating protein chemical stability (e.g., IEC for deamidation, oxidation, general chemical stability; SEC). Additionally, characteristics important for a superior biologics dosage form such as viscosity of high-concentration solutions were determined at various temperatures (cone-plate viscometer).

Subvisible particles were monitored by the light blockage method according to United States Pharmacopeia (USP). In addition, the physicochemical stability of the formulations was assessed by SEC which allows detection of fragments and aggregates. To monitor chemical stability, size exclusion high pressure liquid chromatography (SE-HPLC) (detection of fragments and hydrolysis specimen) and CEX-HPLC (cation exchange HPLC) were applied. CEX-HPLC resolves different lysine isoforms and

degradation products (e.g., deamidated and oxidized species) that may have formed during storage.

Example 3.1.1: Size Exclusion Chromatography (SEC)

Size exclusion chromatography was used to separate proteins based on size. Proteins are carried in an aqueous mobile phase and through a porous stationary phase resin packed in a column. The retention time in the column is a function of the hydrodynamic size of the protein and the size of the pores in the packed resin bed. Smaller molecules can penetrate into smaller pores in the resin and are retained longer than larger molecules. Upon elution from the column the proteins are detected by UV absorbance. The SEC method used a TSK gel guard (TOSOH Biosciences, Montgomeryville, PA, cat. no. 08543) and a TSK gel G3000SWxL (TOSOH Biosciences, Montgomeryville, PA, cat. no. 08541). The mobile phase was 100 mM Na2HPO4, 200 mM Na2SO4, pH 7.0. The flow rate was 0.3 mL/minute. Injection volume was 20 µL of 1 mg/mL sample. The column temperture was room temperature. The autosampler temperature was 2-8°C. The total run time was 50 minutes. The detection was based on UV absorbance at 214 nm wavelength, with band width set at 8 nm, using reference wavelength at 360 nm with band width 100 nm.

Example 3.1.2: Ion Exchange Chromatography (IEC)

Dionex Propac WCX-10 4x250mm column, p/n 054993; 4x50mM guard, p/n 054994. Buffer A: 10mM Na2HPO4, pH 7.5, buffer B 10mM Na2HPO4, 500 mM NaCl, pH 5.5. Detection at 280 nm, column temperature 35°C, flow rate 1 mL/min.

Table 28: IEC Gradient

Time (min)	%B
5	8
39	25
41	100
46	100
48	8
52	8
52.10	Stop

Example 3.1.3: Measurement of Subvisible Particle Levels

The number of subvisible particles was measured on a KLOTZ light obscuration particle counter in alignment with United States Pharmacopeia recommendations. 3.5 mL of sample were filled in 5 mL round-bottom tubes under laminar air flow conditions, and measurement was performed in n=3 mode (0.8 mL per single measurement) after an initial 0.8 mL rinse for each sample. Particle levels provided in the tables refer to the number of particles per mL of protein solution.

Example 3.1.4: Visual Inspection

The protein solution was carefully inspected against both a black and a white background for signs indicating protein physical instability such as haziness, turbidity and particle formation.

Example 3.1.5: Solubility

Protein solubility was assessed in pH 6 (15 mM Histidine) buffer by concentrating the protein to 100 mg/mL.

Example 3.1.6: Protein Viscosity

Protein viscosity was assessed on a Brookfield cone and plate rheometer at a shear rate of 100/s between 8 to 25°C.

Example 3.1.7: DSC

The thermal stability was assessed using a DSC instrument. The DSC instrument used was an automated VP-DSC with Capillary Cell (Microcal). Unfolding of the molecules was studied applying a 1°C/minute scan rate over a 25°C - 95°C temperature range for samples at 1 mg/mL. Additional measurement parameters were: fitting period: 16 seconds, pre-scan wait: 10 minutes, feedback mode: none. Per individual measurement, 420 µL of sample/blank were filled into the DSC measurement sample holder, with a plate fill scheme as provided below. The thermograms hence obtained were fitted to a non two state model such as to obtain the midpoint temperatures and enthalpies of the different transitions.

Example 3.2: Formulation Analysis Development Of D2E7-SL-Hu2B5.7 DVD-Ig

Example 3.2.1: Stability Of D2E7-SL-Hu2B5.7 DVD-Ig Solutions Over Broad Ph Range At Recommended And Accelerated Storage Conditions

Protein stability was assessed at 2mg/ml in10 mM phosphate, 10 mM citrate buffer systems within a pH range of pH 4 to pH 9 values. Stability studies were conducted at recommended storage temperature (2-8°C) and at accelerated conditions (40°C) for up to at least 3 months. Generally, the physico-chemical stability of proteins is highly dependent on the pH and temperatures conditions at which they are stored and/or formulated. Such stress conditions can result in instabilities such as, for example, aggregation, deamidation, etc., during storage and can severly impact safety, efficacy and general product quality. It is generally accepted that providing a stable, safe pharmaceutical product based on large proteins is a challenging task, and very often, protein instability necessitates lyophilization of the product to maintain stability over longer periods of time. It is not uncommon that biological development candidates that are very promising in preclinical models fail clinical development due to inadequate stability.

Table 29: Monomer, Aggregate And Fragment Levels In D2E7-SL-Hu2B5.7 DVD-Ig Samples On Stability As Determined By SEC (3 Months Storage Time)

	Monomer (%)	Aggregates (%)	Fragments (%)
T0			
pH 4	95.9	1.99	2.1
pH 5	95.9	2.12	1.89
pH 6	96	2.06	1.88
pH 7	96	2.04	1.93
pH 8	95.5	2.47	1.94
pH 9	96	2.03	1.92
21d, 40C			
pH 4	29.46	61.4	9.12
pH 5	87.76	8.47	3.75
pH 6	92.54	4.74	2.7
pH 7	91.88	4.91	3.2
pH 8	89.81	5.97	4.02
pH 9	90.42	5.54	4.03
3m, 5C			
pH 4	95.57	2.12	2.3
pH 5	95.05	2.87	2.04

	Monomer (%)	Aggregates (%)	Fragments (%)
pH 6	95.3	2.66	2.03
pH 7	95.44	2.48	2.06
pH 8	95.04	2.82	2.12
pH 9	95.25	2.7	2.04

Table 30: Formation Of Acidic And Basic Isoforms In Samples On Stability As Determined By IEX.(3 Month Storage Time)

	Main Isoform (%)	Acidic Species (%)	Basic Species (%)
T0			
pH 4	48.33	37.78	13.88
pH 5	49.56	37.28	13.15
pH 6	47.87	40.21	11.9
pH 7	47.86	28.75	13.38
pH 8	49.28	37.75	12.95
pH 9	48.18	39.41	12.4
21d, 25C			
pH 4	36.97	37.38	25.64
pH 5	42.65	38.71	18.62
pH 6	46.96	38.74	14.28
pH 7	34.65	53.07	12.26
pH 8	29.49	57.76	12.74
pH 9	26.08	61.12	12.79
3m, 5C			
pH 4	43.2	36.6	20.19
pH 5	46.58	36.26	17.14
pH 6	46.5	39.72	13.69
pH 7	48.74	38.24	13
pH 8	48.7	38.2	13
pH 9	47.13	40.93	11.93

Tables 29 and 30 provide the results of SEC and IEX stability testing for up to 3 months storage of D2E7-SL-Hu2B5.7 DVD-Ig over a very broad pH profile, showing the levels of monomer and main isoform specimen, resp., over time. Table 29 demonstrates that D2E7-SL-Hu2B5.7 DVD-Ig reveals virtually identical monomer levels over a solution pH ranging from pH 4 to pH 9. All formulations reveal monomer levels clearly above a 90% monomer quality level (indicative for very good stability, even over 3 months real-time testing). The formulations actually maintain D2E7-SL-Hu2B5.7 DVD-Ig long-term stability to an extent that even an extremely strict quality specification for real-time stability testing of more than 90% would be met. Similarly, the decrease of the

stability indicating main isoform is less than 5% after 3 months storage over broader pH ranges. Thus, the formulations of the invention are able to provide physical and chemical stability over a very broad D2E7-SL-Hu2B5.7 DVD-Ig concentration range and over a long time with regard to monomer level as well. This is very surprising, since it is well accepted in the scientific community that physical instability (leading to a decrease of monomer) is strongly impacted by solution pH and that proteins usually are stable only within a very narrow pH range.

Example 3.2.2: Stability Of D2E7-SL-Hu2B5.7 DVD-Ig Solutions Over Broad pH Range During Freeze-Thaw Processing

Protein stability was assessed at 1 mg/ml 10 mM phosphate, 10 mM citrate buffer systems at various pH values between pH 4 to pH 9 using freeze thaw studies conducted following a standard procedure. Freezing was performed by keeping the samples at –80°C for at least 6 hours. Thawing was performed at 30°C in a water bath. Generally, proteins are susceptible to physical instabilities such as aggregation and subvisible particle formation due to denaturation that can occur at the ice-water interface which is created during the process of freezing, or due to a variety of other parameters such as cold denaturation and/or cryoconcentration. Since proteins are usually stored in frozen form as bulk drug substance and as lyophilized drug product (which includes freezing as first process unit operations), freeze/thaw processing stability forms an integral part of a protein's stability feature to accommodate compliance with manufacturing operations and general stability requirements.

Table 31: Formation Of Aggregates And Fragments In Samples Following Freeze/Thaw Processing As Determined By SEC (Four Freeze/Thaw Cycles Were Performed)

0 F/T	Monomer (%)	Aggregate (%)	Fragment (%)
pH 4	95.995	1.5	2.49
pH 5	96.13	1.56	2.3
pH 6	95.09	1.69	3.2
pH 7	95.61	1.81	2.56
pH 8	95.84	1.87	2.27
pH 9	95.545	2.125	2.31
1 F/T			

0 F/T	Monomer (%)	Aggregate (%)	Fragment (%)
pH 4	95.88	1.71	2.4
pH 5	96.13	1.71	2.145
pH 6	95.805	1.6	2.58
pH 7	95.58	1.91	2.49
pH 8	95.81536	1.777143	2.3925
pH 9	95.595	2.14	2.255
2 F/T			
pH 4	95.52	1.98	2.48
pH 5	95.995	1.865	2.125
pH 6	95.88	1.6	2.505
pH 7	95.745	1.855	2.38
pH 8	95.75	2.01	2.225
pH 9	95.575	2.11	2.3
3 F/T			
pH 4	95.315	2.185	2.485
pH 5	95.85	1.985	2.15
pH 6	95.55	1.63	2.81
pH 7	95.9	1.85	2.235
pH 8	95.725	2.08	2.18
pH 9	95.31	2.32	2.36
4 F/T			
pH 4	94.23	2.465	3.29
pH 5	95.93	1.92	2.14
pH 6	95.835	1.645	2.505
pH 7	95.95	1.815	2.22
pH 8	95.465	2.11	2.41
pH 9	95.51	2.225	2.25

Table 32: Formation Of Subvisible Particles Greater Than Equal To 10 Microns Per Ml Protein Solution In Samples Following Freeze/Thaw As Determined By KLOTZ Particle Counter (4 Freeze/Thaw Cycles Were Performed)

	0 F/T	1 F/T	2 F/T	3 F/T	4 F/T
pH 4	1.3	15.1	207.5	342	572
pH 5	1.5	3.1	62	136	269
pH 6	2.6	28	142	440	2418
pH 7	2.6	45	165	740	4099
pH 8	5.5	27.1	148	773	4537
pH 9	1.8	16.8	134	375	1425

Table 33: Visual Inspection Of Samples Following Freeze/Thaw (4 Freeze/Thaw Cycles Were Performed). "Clear" Means That No Signs Indicative For Physical Instability Were Observed Such As Particle Formation, Cloudiness, Haziness Or Increased Turbidity And Opalescence

	0 F/T	1 F/T	2 F/T	3 F/T	4 F/T
pH 4	Clear	Clear	Clear	Clear	Clear
pH 5	Clear	Clear	Clear	Clear	Clear
pH 6	Clear	Clear	Clear	Clear	Clear
pH 7	Clear	Clear	Clear	Clear	Clear
pH 8	Clear	Clear	Clear	Clear	Clear
pH 9	Clear	Clear	Clear	Clear	Clear

It is well known that freeze/thaw processing can result in substantial protein denaturation and aggregation, resulting in soluble and insoluble aggregate formation (Parborji et a., Pharm Res 11 (5) 1994). Even minimal amounts of protein (<0.1%) are known to account for precipitation (Hoffman, Analytical methods and stability testing of biopharmaceuticals, in Protein formulation and delivery, ed. by McNally, E. J., 3 (2000) 71-110). All of the formulations presented herein were subjected to repeated freeze thaw processing and the results demonstrated that none of the formulations were sensitive to repeated freeze/thaw cycles (-80°C/30°C). All of the D2E7-SL-Hu2B5.7 DVD-Ig formulations were similarly stable independent of their pH (in all cases there was no significant change as compared to initial values) despite the higher pH of the formulations, which were closer to the pI of D2E7-SL-Hu2B5.7 DVD-Ig. Surprisingly, D2E7-SL-Hu2B5.7 DVD-Ig formulations are physically stable for up to at least four freeze thaw cycles (all samples revealed monomer levels exceeding a 90% quality level), subvisible particle levels (all samples were in compliance with subvisible particle number requirements stated by US and European Pharmacopeias for low-volume parenteral dosage forms), and visual inspection data (all samples were observed free from particles and physical instability).

Example 3.2.3: Stability Of Anti-TNF-Anti-PGE2 DVD-IgG Solutions At Various Selected Formulations During DSC Measurements

The thermal stability of 1mg/ml anti-TNF-anti-PGE2 DVD-IgG between pH 4 to pH 8 was assessed using a DSC instrument. The thermal stability as assessed by DSC is correlated to the kinetic stability of the protein molecules during storage.

Table 34: The Midpoint Temperatures Of The Four Unfolding Transitions In 10 mM Citrate And 10 mM Phosphate Buffer Systems At Different pH Values For The Long And Short Linker Anti-TNF-Anti-PGE2 DVD-IgG Molecule

	Tm1	Tm2	Tm3	Tm4
D2E7-SL-Hu2B5.7				
Universal Buffer pH 4	56.2	60.8	66.9	77.9
Universal Buffer pH 6	58.8	68.5	75	83.3
Universal Buffer pH 8	58.2	65.7	69.7	82.8
D2E7-LL-Hu2B5.7				
Universal Buffer pH 4	55.42	60.95	70.22	79.83
Universal Buffer pH 6	56.49	67.22	75.05	83.39

It could be shown that high temperatures are required (that are well above the recommended storage temperatures of a anti-TNF/anti-PGE_2 DVD-IgG product) to induce forced unfolding of domains of anti-TNF/anti-PGE_2 DVD-IgG, regardless of short linker and long-linker anti-TNF/anti-PGE_2 DVD-IgG molecules.

Example 3.2.4: Stability Of D2E7-SL-Hu2B5.7 DVD-Ig Solutions At 10 mg/ml During Freeze-Thaw Processing

D2E7-SL-Hu2B5.7 DVD-Ig stability during freeze/thaw stress was also assessed at10 mg/ml in pH 6 (15 mM Histidine) buffer. Samples were frozen at –80°C and thawed in a 30°C water bath.

Table 35: Formation Of Aggregates And Fragments In Samples Following Freeze/Thaw As Determined By SEC (3 Freeze/Thaw Cycles Were Performed)

	Monomer (%)	Aggregate (%)	Fragment (%)
0 F/T	99.42	0.57	0
1 F/T	99.39	0.6	0
2 F/T	99.4	0.59	0
3 F/T	99.38	0.61	0

Table 36: Visual Inspection Of Samples Following Freeze/Thaw (3 Freeze/Thaw Cycles Were Performed) Processing Of Anti-TNF-Anti-PGE2 DVD-IgG Solutions

	0 F/T	1 F/T	2 F/T	3 F/T
pH 6	Clear	Clear	Clear	Clear

The formulation presented herein was subjected to repeated freeze thaw processing and the results demonstrated that D2E7-SL-Hu2B5.7 DVD-Ig was not sensitive to instability as a consequence of repeated freeze/thaw cycles (-80°C/30°C) at 10 mg/mL concentrations. It could be demonstrated that anti-TNF-anti-PGE2 DVD-IgG formulations are stable at 10 mg/mL for up to at least three freeze thaw cycles with regard to physical stability (all samples revealed monomer levels exceeding a 90% quality level) and visual inspection data (all samples were observed free from particles and physical instability).

Example 3.2.5: Stability Of D2E7-SL-Hu2B5.7 DVD-Ig In Various Formulations At Recommended And Accelerated Storage Conditions

D2E7-SL-Hu2B5.7 DVD-Ig stability was assessed at 2 mg/ml at pH 6 under various formulation conditions. Stability studies were conducted at 2-8°C and 40°C.

Table 37: Formation Of Aggregates And Fragments In Anti-TNF-Anti-PGE2 DVD-Igg Stability Samples As Determined By SEC (Up To 3 Months Storage)

	Monomer (%)	Aggregates (%)	Fragments (%)
T0			
15 mM citrate	96.51	1.44	2.04
15 mM Histidine	96.42	1.29	2.28
10 mM citrate and 10 mM Phosphate 10 mg/ml sucrose	96.12	1.58	2.28
10 mM citrate and 10 mM Phosphate 0.01 % Tween 80	96.31	1.49	2.18
1m, 40C			
15 mM citrate	90.72	5.3	3.97
15 mM Histidine	91.82	3.85	4.32
10 mM citrate and 10 mM Phosphate 10 mg/ml sucrose	90.03	5.92	4.03
10 mM citrate and 10 mM Phosphate 0.01 % Tween 80	89.54	6.42	4.02
3m, 5C			
15 mM citrate	95.96	1.95	2.07
15 mM Histidine	95.5	1.15	3.33
10 mM citrate and 10 mM Phosphate 10 mg/ml sucrose	95.15	2.38	2.45
10 mM citrate and 10 mM Phosphate 0.01 % Tween 80	94.97	2.59	2.42

Table 37 provides the results of SEC testing for up to 3 months storage of anti-TNF-anti-PGE2 DVD-IgG in selected formulations, showing the levels of monomer over time. Table 37 demonstrates that the anti-TNF-anti-PGE2 DVD-IgG formulations reveal virtually identical monomer levels indicating high stability and formulation robustness, since all formulations reveal monomer levels clearly above a 90% monomer quality level (indicative for very good stability, even over 3 months real-time testing). The formulations actually maintain D2E7-SL-Hu2B5.7 DVD-Ig long-term stability to an extent that even an extremely strict quality specification for real-time stability testing of more than 90% monomer levels would be met.

Table 38: The Midpoint Temperatures Of The Four Unfolding Transitions Under Different Formulation Conditions At pH 6 As Determined By DSC

	Tm1	Tm2	Tm3	Tm4
15 mM citrate	58.9	68.12	74.52	83.75
15 mM Histidine	61.74	67.47	73.87	83.38
10 mM citrate and 10 mM Phosphate 10 mg/ml sucrose	59.22	68.09	74.81	83.65
10 mM citrate and 10 mM Phosphate 0.01 % Tween 80	59.04	67.91	74.84	83.53

High temperatures are required (that are well above the recommended storage temperatures of a anti-TNF-anti-PGE2 DVD-IgG product) to induce forced unfolding of domains of anti-TNF-anti-PGE2 DVD-IgG formulations.

Example 4: Generation and Characterization of Additional Dual Variable Domain Immunoglobulins (DVD-Ig) Specific for PGE2 and Second Target

Dual variable domain immunoglobulins (DVD-Ig) using parent antibodies with known amino acid sequences were generated by synthesizing polynucleotide fragments encoding DVD-Ig variable heavy and DVD-Ig variable light chain sequences and cloning the fragments into a pHybC-D2 vector according to Example 1.4.4.1. The DVD-Ig contructs were cloned into and expressed in 293 cells as described in Example 1,4.4.2. The DVD-Ig protein was purified according to standard methods. Functional characteristics were determined according to the methods described in Example 1.1.1 and 1.1.2 as indicated.

The following examples comprise two tables each. The first table in each example contains the VH and VL sequences of two parent antibodies used in generating

DVD-Igs. The second table in each example contains the sequences of the DVD-Ig VH and VL chains constructed from the sequences of the first table.

Example 4.1: Generation of VEGF and PGE2

Table 39

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345678901234
128	VD162H	AB014VH	AB048VH	EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGM NWVRQAPGKGLEWVGWINTYTGEPTYAADFKRRF TFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHY YGSSHWYFDVWGQGTLVTVSS**ASTKGP**EVQLVQS GAEVKKPGASVKVSCKASGYTFTKYWLGWVRQAP GQGLEWMGDIYPGYDYTHYNEKFKDRVTLTTDTS TSTAYMELRSLRSDDTAVYYCARSDGSSTYWGQG TLVTVSS
129	VD162L	AB014VL	AB048VL	DIQMTQSPSSLSASVGDRVTITCSASQDISNYLN WYQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSG TDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGT KVEIKR**TVAAP**DVLMTQTPLSLPVTPGEPASISC TSSQNIVHSNGNTYLEWYLQKPGQSPQLLIYKVS NRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVY YCFQVSHVPYTFGGGTKVEIKR
130	VD161H	AB048VH	AB014VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTKYWL GWVRQAPGQGLEWMGDIYPGYDYTHYNEKFKDRV TLTTDTSTSTAYMELRSLRSDDTAVYYCARSDGS STYWGQGTLVTVSS**ASTKGP**EVQLVESGGGLVQP GGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWV GWINTYTGEPTYAADFKRRFTFSLDTSKSTAYLQ MNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQG TLVTVSS
131	VD161L	AB048VL	AB014VL	DVLMTQTPLSLPVTPGEPASISCTSSQNIVHSNG NTYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFS GSGSGTDFTLKISRVEAEDVGVYYCFQVSHVPYT FGGGTKVEIKR**TVAAP**DIQMTQSPSSLSASVGDR VTITCSASQDISNYLNWYQQKPGKAPKVLIYFTS SLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQYSTVPWTFGQGTKVEIKR

Example 4.2: Generation of NGF and PGE2 DVD-Igs

Table 40

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
132	VD164H	AB020VH	AB048VH	QVQLQESGPGLVKPSETLSLTCTVSGFSLIGYDLN WIRQPPGKGLEWIGIIWGDGTTDYNSAVKSRVTIS KDTSKNQFSLKLSSVTAADTAVYYCARGGYWYATS YYFDYWGQGTLVTVSS**ASTKGP**EVQLVQSGAEVKK PGASVKVSCKASGYTFTKYWLGWVRQAPGQGLEWM GDIYPGYDYTHYNEKFKDRVTLTTDTSTSTAYMEL RSLRSDDTAVYYCARSDGSSTYWGQGTLVTVSS
133	VD164L	AB020VL	AB048VL	DIQMTQSPSSLSASVGDRVTITCRASQSISNNLNW YQQKPGKAPKLLIYYTSRFHSGVPSRFSGSGSGTD FTFTISSLQPEDIATYYCQQEHTLPYTFGQGTKLE IKR**TVAAP**DVLMTQTPLSLPVTPGEPASISCTSSQ NIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFSG VPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQVS HVPYTFGGGTKVEIKR
134	VD163H	AB048VH	AB020VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTKYWLG WVRQAPGQGLEWMGDIYPGYDYTHYNEKFKDRVTL TTDTSTSTAYMELRSLRSDDTAVYYCARSDGSSTY WGQGTLVTVSS**ASTKGP**QVQLQESGPGLVKPSETL SLTCTVSGFSLIGYDLNWIRQPPGKGLEWIGIIWG DGTTDYNSAVKSRVTISKDTSKNQFSLKLSSVTAA DTAVYYCARGGYWYATSYYFDYWGQGTLVTVSS
135	VD3163L	AB048VL	AB020VL	DVLMTQTPLSLPVTPGEPASISCTSSQNIVHSNGN TYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGS GSGTDFTLKISRVEAEDVGVYYCFQVSHVPYTFGG GTKVEIKR**TVAAP**DIQMTQSPSSLSASVGDRVTIT CRASQSISNNLNWYQQKPGKAPKLLIYYTSRFHSG VPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQEH TLPYTFGQGTKLEIKR

Example 4.3: Generation of IL-17A and PGE2 DVD-Igs

Table 41

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
136	VD156H	AB029VH	AB048VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMN WVRQAPGKGLEWVAAINQDGSEKYYVGSVKGRFTI SRDNAKNSLYLQMNSLRVEDTAVYYCVRDYYDILT DYYIHYWYFDLWGRGTLVTVSS**ASTKGP**EVQLVQS GAEVKKPGASVKVSCKASGYTFTKYWLGWVRQAPG QGLEWMGDIYPGYDYTHYNEKFKDRVTLTTDTSTS TAYMELRSLRSDDTAVYYCARSDGSSTYWGQGTLV TVSS
137	VD156L	AB029VL	AB048VL	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLA WYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGT DFTLTISRLEPEDFAVYYCQQYGSSPCTFGQGTRL EIKR**TVAAP**DVLMTQTPLSLPVTPGEPASISCTSS QNIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFS GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQV SHVPYTFGGGTKVEIKR
138	VD155H	AB048VH	AB029VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTKYWLG WVRQAPGQGLEWMGDIYPGYDYTHYNEKFKDRVTL TTDTSTSTAYMELRSLRSDDTAVYYCARSDGSSTY WGQGTLVTVSS**ASTKGP**EVQLVESGGGLVQPGGSL RLSCAASGFTFSNYWMNWVRQAPGKGLEWVAAINQ DGSEKYYVGSVKGRFTISRDNAKNSLYLQMNSLRV EDTAVYYCVRDYYDILTDYYIHYWYFDLWGRGTLV TVSS
139	VD155L	AB048VL	AB029VL	DVLMTQTPLSLPVTPGEPASISCTSSQNIVHSNGN TYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGS GSGTDFTLKISRVEAEDVGVYYCFQVSHVPYTFGG GTKVEIKR**TVAAP**EIVLTQSPGTLSLSPGERATLS CRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRAT GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSSPCTFGQGTRLEIKR

Example 4.4: Generation of IL-1b and PGE2 (seq. 1) DVD-Igs

Table 42

SEQ ID	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
140	VD158H	AB032VH	AB048VH	QVQLVESGGGVVQPGRSLRLSCAASGFTFSVYGMN WVRQAPGKGLEWVAIIWYDGDNQYYADSVKGRFTI SRDNSKNTLYLQMNGLRAEDTAVYYCARDLRTGPF DYWGQGTLVTVSS**ASTKGP**EVQLVQSGAEVKKPGA SVKVSCKASGYTFTKYWLGWVRQAPGQGLEWMGDI YPGYDYTHYNEKFKDRVTLTTDTSTSTAYMELRSL RSDDTAVYYCARSDGSSTYWGQGTLVTVSS
141	VD158L	AB032VL	AB048VL	EIVLTQSPDFQSVTPKEKVTITCRASQSIGSSLHW YQQKPDQSPKLLIKYASQSFSGVPSRFSGSGSGTD FTLTINSLEAEDAAAYYCHQSSSLPFTFGPGTKVD IKR**TVAAP**DVLMTQTPLSLPVTPGEPASISCTSSQ NIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFSG VPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQVS HVPYTFGGGTKVEIKR
142	VD157H	AB048VH	AB032VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTKYWLG WVRQAPGQGLEWMGDIYPGYDYTHYNEKFKDRVTL TTDTSTSTAYMELRSLRSDDTAVYYCARSDGSSTY WGQGTLVTVSS**ASTKGP**QVQLVESGGGVVQPGRSL RLSCAASGFTFSVYGMNWVRQAPGKGLEWVAIIWY DGDNQYYADSVKGRFTISRDNSKNTLYLQMNGLRA EDTAVYYCARDLRTGPFDYWGQGTLVTVSS
143	VD157L	AB048VL	AB032VL	DVLMTQTPLSLPVTPGEPASISCTSSQNIVHSNGN TYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGS GSGTDFTLKISRVEAEDVGVYYCFQVSHVPYTFGG GTKVEIKR**TVAAP**EIVLTQSPDFQSVTPKEKVTIT CRASQSIGSSLHWYQQKPDQSPKLLIKYASQSFSG VPSRFSGSGSGTDFTLTINSLEAEDAAAYYCHQSS SLPFTFGPGTKVDIKR

Example 4.5: Generation of IL-6 and PGE2 DVD-Igs

Table 43

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
144	VD250H	AB040VH	AB048VH	QVTLKESGPGILQPSQTLSLTCSFSGFSLSTNGMG VSWIRQPSGKGLEWLAHIYWDEDKRYNPSLKSRLT ISKDTSNNQVFLKITNVDTADTATYYCARRRIIYD VEDYFDYWGQGTTLTVSS**ASTKGP**EVQLVQSGAEV KKPGASVKVSCKASGYTFTKYWLGWVRQAPGQGLE WMGDIYPGYDYTHYNEKFKDRVTLTTDTSTSTAYM ELRSLRSDDTAVYYCARSDGSSTYWGQGTLVTVSS
145	VD250L	AB040VL	AB048VL	QIVLIQSPAIMSASPGEKVTMTCSASSSVSYMYWY QQKPGSSPRLLIYDTSNLASGVPVRFSGSGSGTSY SLTISRMEAEDAATYYCQQWSGYPYTFGGGTKLEI KR**TVAAP**DVLMTQTPLSLPVTPGEPASISCTSSQN IVHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFSGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQVSH VPYTFGGGTKVEIKR
146	VD249H	AB048VH	AB040VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTKYWLG WVRQAPGQGLEWMGDIYPGYDYTHYNEKFKDRVTL TTDTSTSTAYMELRSLRSDDTAVYYCARSDGSSTY WGQGTLVTVSS**ASTKGP**QVTLKESGPGILQPSQTL SLTCSFSGFSLSTNGMGVSWIRQPSGKGLEWLAHI YWDEDKRYNPSLKSRLTISKDTSNNQVFLKITNVD TADTATYYCARRRIIYDVEDYFDYWGQGTTLTVSS
147	VD249L	AB048VL	AB040VL	DVLMTQTPLSLPVTPGEPASISCTSSQNIVHSNGN TYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGS GSGTDFTLKISRVEAEDVGVYYCFQVSHVPYTFGG GTKVEIKR**TVAAP**QIVLIQSPAIMSASPGEKVTMT CSASSSVSYMYWYQQKPGSSPRLLIYDTSNLASGV PVRFSGSGSGTSYSLTISRMEAEDAATYYCQQWSG YPYTFGGGTKLEIKR

Example 4.6: Generation of Abeta (Seq. 1) and PGE2 DVD-Igs

Table 44

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
148	VD227H	AB043VH	AB048VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYGMS WVRQAPGKGLEWVASIRSGGGRTYYSDNVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCVRYDHYSGS SDYWGQGTLVTVSS**ASTKGP**EVQLVQSGAEVKKPG ASVKVSCKASGYTFTKYWLGWVRQAPGQGLEWMGD IYPGYDYTHYNEKFKDRVTLTTDTSTSTAYMELRS LRSDDTAVYYCARSDGSSTYWGQGTLVTVSS
149	VD227L	AB043VL	AB048VL	DVVMTQSPLSLPVTPGEPASISCKSSQSLLDSDGK TYLNWLLQKPGQSPQRLIYLVSKLDSGVPDRFSGS GSGTDFTLKISRVEAEDVGVYYCWQGTHFPRTFGQ GTKVEIKR**TVAAP**DVLMTQTPLSLPVTPGEPASIS CTSSQNIVHSNGNTYLEWYLQKPGQSPQLLIYKVS NRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYY CFQVSHVPYTFGGGTKVEIKR
150	VD228H	AB048VH	AB043VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTKYWLG WVRQAPGQGLEWMGDIYPGYDYTHYNEKFKDRVTL TTDTSTSTAYMELRSLRSDDTAVYYCARSDGSSTY WGQGTLVTVSS**ASTKGP**EVQLLESGGGLVQPGGSL RLSCAASGFTFSNYGMSWVRQAPGKGLEWVASIRS GGGRTYYSDNVKGRFTISRDNSKNTLYLQMNSLRA EDTAVYYCVRYDHYSGSSDYWGQGTLVTVSS
151	VD228L	AB048VL	AB043VL	DVLMTQTPLSLPVTPGEPASISCTSSQNIVHSNGN TYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGS GSGTDFTLKISRVEAEDVGVYYCFQVSHVPYTFGG GTKVEIKR**TVAAP**DVVMTQSPLSLPVTPGEPASIS CKSSQSLLDSDGKTYLNWLLQKPGQSPQRLIYLVS KLDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYY CWQGTHFPRTFGQGTKVEIKR

Example 4.7: Generation of Abeta (Seq. 2) and PGE2 DVD-Igs

Table 45

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
152	**VD199H**	AB044VH	AB048VH	EVKLVESGGGLVKPGGSLRLSCAASGFTFSSYAMS WVRQAPGKGLEWVASIHNRGTIFYLDSVKGRFTIS RDNVRNTLYLQMNSLRAEDTAVYYCTRGRSNSYAM DYWGQGTSVTVSS**ASTKGP**EVQLVQSGAEVKKPGA SVKVSCKASGYTFTKYWLGWVRQAPGQGLEWMGDI YPGYDYTHYNEKFKDRVTLTTDTSTSTAYMELRSL RSDDTAVYYCARSDGSSTYWGQGTLVTVSS
153	**VD199L**	AB044VL	AB048VL	DVLVTQSPLSLPVTPGEPASISCRSTQTLVHRNGD TYLEWYLQKPGQSPQSLIYKVSNRFSGVPDRFSGS GSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGQ GTKLEIKR**TVAAP**DVLMTQTPLSLPVTPGEPASIS CTSSQNIVHSNGNTYLEWYLQKPGQSPQLLIYKVS NRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYY CFQVSHVPYTFGGGTKVEIKR
154	**VD200H**	AB048VH	AB044VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTKYWLG WVRQAPGQGLEWMGDIYPGYDYTHYNEKFKDRVTL TTDTSTSTAYMELRSLRSDDTAVYYCARSDGSSTY WGQGTLVTVSS**ASTKGP**EVKLVESGGGLVKPGGSL RLSCAASGFTFSSYAMSWVRQAPGKGLEWVASIHN RGTIFYLDSVKGRFTISRDNVRNTLYLQMNSLRAE DTAVYYCTRGRSNSYAMDYWGQGTSVTVSS
155	**VD200L**	AB048VL	AB044VL	DVLMTQTPLSLPVTPGEPASISCTSSQNIVHSNGN TYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGS GSGTDFTLKISRVEAEDVGVYYCFQVSHVPYTFGG GTKVEIKR**TVAAP**DVLVTQSPLSLPVTPGEPASIS CRSTQTLVHRNGDTYLEWYLQKPGQSPQSLIYKVS NRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYY CFQGSHVPYTFGQGTKLEIKR

Example 4.8: Generation of Abeta (Seq. 3) and PGE2 DVD-Igs

Table 46

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
156	**VD213H**	AB045VH	AB048VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMS WVRQAPGKGLELVASINSNGGSTYYPDSVKGRFTI SRDNAKNTLYLQMNSLRAEDTAVYYCASGDYWGQG TLVTVSS**ASTKGP**EVQLVQSGAEVKKPGASVKVSC KASGYTFTKYWLGWVRQAPGQGLEWMGDIYPGYDY THYNEKFKDRVTLTTDTSTSTAYMELRSLRSDDTA VYYCARSDGSSTYWGQGTLVTVSS
157	**VD213L**	AB045VL	AB048VL	DIVMTQSPLSLPVTPGEPASISCRSSQSLVYSNGD TYLHWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGS GSGTDFTLKISRVEAEDVGVYYCSQSTHVPWTFGG GTKVEIKR**TVAAP**DVLMTQTPLSLPVTPGEPASIS CTSSQNIVHSNGNTYLEWYLQKPGQSPQLLIYKVS NRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYY CFQVSHVPYTFGGGTKVEIKR
158	**VD214H**	AB048VH	AB045VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTKYWLG WVRQAPGQGLEWMGDIYPGYDYTHYNEKFKDRVTL TTDTSTSTAYMELRSLRSDDTAVYYCARSDGSSTY WGQGTLVTVSS**ASTKGP**EVQLVESGGGLVQPGGSL RLSCAASGFTFSSYGMSWVRQAPGKGLELVASINS NGGSTYYPDSVKGRFTISRDNAKNTLYLQMNSLRA EDTAVYYCASGDYWGQGTLVTVSS
159	**VD214L**	AB048VL	AB045VL	DVLMTQTPLSLPVTPGEPASISCTSSQNIVHSNGN TYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGS GSGTDFTLKISRVEAEDVGVYYCFQVSHVPYTFGG GTKVEIKR**TVAAP**DIVMTQSPLSLPVTPGEPASIS CRSSQSLVYSNGDTYLHWYLQKPGQSPKLLIYKVS NRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYY CSQSTHVPWTFGGGTKVEIKR

Example 4.9: Generation of IL-18 and PGE2 DVD-Igs

Table 47

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
160	**VD241H**	AB046VH	AB048VH	EVQLVQSGTEVKKPGESLKISCKGSGYTVTSYWIG WVRQMPGKGLEWMGFIYPGDSETRYSPTFQGQVTI SADKSFNTAFLQWSSLKASDTAMYYCARVGSGWYP YTFDIWGQGTMVTVSS**ASTKGP**EVQLVQSGAEVKK PGASVKVSCKASGYTFTKYWLGWVRQAPGQGLEWM GDIYPGYDYTHYNEKFKDRVTLTTDTSTSTAYMEL RSLRSDDTAVYYCARSDGSSTYWGQGTLVTVSS
161	**VD241L**	AB046VL	AB048VL	EIVMTQSPATLSVSPGERATLSCRASESISSNLAW YQQKPGQAPRLFIYTASTRATDIPARFSGSGSGTE FTLTISSLQSEDFAVYYCQQYNNWPSITFGQGTRL EIKR**TVAAP**DVLMTQTPLSLPVTPGEPASISCTSS QNIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFS GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQV SHVPYTFGGGTKVEIKR
162	**VD242H**	AB048VH	AB046VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTKYWLG WVRQAPGQGLEWMGDIYPGYDYTHYNEKFKDRVTL TTDTSTSTAYMELRSLRSDDTAVYYCARSDGSSTY WGQGTLVTVSS**ASTKGP**EVQLVQSGTEVKKPGESL KISCKGSGYTVTSYWIGWVRQMPGKGLEWMGFIYP GDSETRYSPTFQGQVTISADKSFNTAFLQWSSLKA SDTAMYYCARVGSGWYPYTFDIWGQGTMVTVSS
163	**VD242L**	AB048VL	AB046VL	DVLMTQTPLSLPVTPGEPASISCTSSQNIVHSNGN TYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGS GSGTDFTLKISRVEAEDVGVYYCFQVSHVPYTFGG GTKVEIKR**TVAAP**EIVMTQSPATLSVSPGERATLS CRASESISSNLAWYQQKPGQAPRLFIYTASTRATD IPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYN NWPSITFGQGTRLEIKR

Example 4.10: Generation of IL-15 and PGE2 DVD-Igs

Table 48

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
164	**VD154H**	AB049VH	AB048VH	EVQLVQSGAEVKKPGESLKISCKVSGYFFTTYWIG WVRQMPGKGLEYMGIIYPGDSDTRYSPSFQGQVTI SADKSISTAYLQWSSLKASDTAMYYCARGGNWNCF DYWGQGTLVTVSSA**STKGP**EVQLVQSGAEVKKPGA SVKVSCKASGYTFTKYWLGWVRQAPGQGLEWMGDI YPGYDYTHYNEKFKDRVTLTTDTSTSTAYMELRSL RSDDTAVYYCARSDGSSTYWGQGTLVTVSS
165	**VD154L**	AB049VL	AB048VL	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLA WYQQKPGQAPRLLIYGASRRATGIPDRFSGSGSGT DFTLTISRLEPEDFAVYYCQRYGSSHTFGQGTKLE ISR**TVAAP**DVLMTQTPLSLPVTPGEPASISCTSSQ NIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFSG VPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQVS HVPYTFGGGTKVEIKR
166	**VD153H**	AB048VH	AB049VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTKYWLG WVRQAPGQGLEWMGDIYPGYDYTHYNEKFKDRVTL TTDTSTSTAYMELRSLRSDDTAVYYCARSDGSSTY WGQGTLVTVSS**ASTKGP**EVQLVQSGAEVKKPGESL KISCKVSGYFFTTYWIGWVRQMPGKGLEYMGIIYP GDSDTRYSPSFQGQVTISADKSISTAYLQWSSLKA SDTAMYYCARGGNWNCFDYWGQGTLVTVSS
167	**VD153L**	AB048VL	AB049VL	DVLMTQTPLSLPVTPGEPASISCTSSQNIVHSNGN TYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGS GSGTDFTLKISRVEAEDVGVYYCFQVSHVPYTFGG GTKVEIKR**TVAAP**EIVLTQSPGTLSLSPGERATLS CRASQSVSSSYLAWYQQKPGQAPRLLIYGASRRAT GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQRY GSSHTFGQGTKLEISR

Example 4.11: Generation of S1P and PGE2 DVD-Igs

Table 49

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
168	**VD252H**	AB052VH	AB048VH	EVQLVQSGAEVKKPGESLKISCQSFGYIFIDHTIH WMRQMPGQGLEWMGAISPRHDITKYNEMFRGQVTI SADKSSSTAYLQWSSLKASDTAMYFCARGGFYGST IWFDFWGQGTMVTVSS**ASTKGP**EVQLVQSGAEVKK PGASVKVSCKASGYTFTKYWLGWVRQAPGQGLEWM GDIYPGYDYTHYNEKFKDRVTLTTDTSTSTAYMEL RSLRSDDTAVYYCARSDGSSTYWGQGTLVTVSS
169	**VD252L**	AB052VL	AB048VL	ETTVTQSPSFLSASVGDRVTITCITTTDIDDDMNW FQQEPGKAPKLLISEGNILRPGVPSRFSSSGYGTD FTLTISKLQPEDFATYYCLQSDNLPFTFGQGTKLE IKR**TVAAP**DVLMTQTPLSLPVTPGEPASISCTSSQ NIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFSG VPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQVS HVPYTFGGGTKVEIKR
170	**VD251H**	AB048VH	AB052VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTKYWLG WVRQAPGQGLEWMGDIYPGYDYTHYNEKFKDRVTL TTDTSTSTAYMELRSLRSDDTAVYYCARSDGSSTY WGQGTLVTVSS**ASTKGP**EVQLVQSGAEVKKPGESL KISCQSFGYIFIDHTIHWMRQMPGQGLEWMGAISP RHDITKYNEMFRGQVTISADKSSSTAYLQWSSLKA SDTAMYFCARGGFYGSTIWFDFWGQGTMVTVSS
171	**VD251L**	AB048VL	AB052VL	DVLMTQTPLSLPVTPGEPASISCTSSQNIVHSNGN TYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGS GSGTDFTLKISRVEAEDVGVYYCFQVSHVPYTFGG GTKVEIKR**TVAAP**ETTVTQSPSFLSASVGDRVTIT CITTTDIDDDMNWFQQEPGKAPKLLISEGNILRPG VPSRFSSSGYGTDFTLTISKLQPEDFATYYCLQSD NLPFTFGQGTKLEIKR

Example 4.12: Generation of IL-6R and PGE2 DVD-Igs

Table 50

SEQ ID NO	DVD Variable Domain Name	Outer Variable Domain Name	Inner Variable Domain Name	Sequence 12345678901234567890123456789012345
172	VD152H	AB054VH	AB048VH	EVQLQESGPGLVRPSQTLSLTCTVSGYSITSDHAW SWVRQPPGRGLEWIGYISYSGITTYNPSLKSRVTM LRDTSKNQFSLRLSSVTAADTAVYYCARSLARTTA MDYWGQGSLVTVSS**ASTKGP**EVQLVQSGAEVKKPG ASVKVSCKASGYTFTKYWLGWVRQAPGQGLEWMGD IYPGYDYTHYNEKFKDRVTLTTDTSTSTAYMELRS LRSDDTAVYYCARSDGSSTYWGQGTLVTVSS
173	VD152L	AB054VL	AB048VL	DIQMTQSPSSLSASVGDRVTITCRASQDISSYLNW YQQKPGKAPKLLIYYTSRLHSGVPSRFSGSGSGTD FTFTISSLQPEDIATYYCQQGNTLPYTFGQGTKVE IKR**TVAAP**DVLMTQTPLSLPVTPGEPASISCTSSQ NIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFSG VPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQVS HVPYTFGGGTKVEIKR
174	VD151H	AB048VH	AB054VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTKYWLG WVRQAPGQGLEWMGDIYPGYDYTHYNEKFKDRVTL TTDTSTSTAYMELRSLRSDDTAVYYCARSDGSSTY WGQGTLVTVSS**ASTKGP**EVQLQESGPGLVRPSQTL SLTCTVSGYSITSDHAWSWVRQPPGRGLEWIGYIS YSGITTYNPSLKSRVTMLRDTSKNQFSLRLSSVTA ADTAVYYCARSLARTTAMDYWGQGSLVTVSS
175	VD151L	AB048VL	AB054VL	DVLMTQTPLSLPVTPGEPASISCTSSQNIVHSNGN TYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGS GSGTDFTLKISRVEAEDVGVYYCFQVSHVPYTFGG GTKVEIKR**TVAAP**DIQMTQSPSSLSASVGDRVTIT CRASQDISSYLNWYQQKPGKAPKLLIYYTSRLHSG VPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQGN TLPYTFGQGTKVEIKR

Example 4.13: Cloning Vector Sequences Used to Clone Parent Antibody and DVD-Ig Sequences

Table 51

Vector name	SEQ ID NO	Nucleotide sequences 1234567890123456789012345678901234567890123456789012345678901
V1	867	GCGTCGACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGC ACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCC GAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCAC ACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTG GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTG AATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCT TGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGG GGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATC TCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGAC CCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCC AAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGC GTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC AAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAA GCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGC GAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTC TATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTC TACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTC TCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGC CTCTCCCTGTCTCCGGGTAAATGAGCGGCCGCTCGAGGCCGGCAAGGCCGG ATCCCCCGACCTCGACCTCTGGCTAATAAAGGAAATTTATTTTCATTGCAA TAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGG CAAATCATTTGGTCGAGATCCCTCGGAGATCTCTAGCTAGAGGATCGATCC CCGCCCCGGACGAACTAAACCTGACTACGACATCTCTGCCCCTTCTTCGCG GGGCAGTGCATGTAATCCCTTCAGTTGGTTGGTACAACTTGCCAACTGGGC CCTGTTCCACATGTGACACGGGGGGGGACCAAACACAAAGGGGTTCTCTGA CTGTAGTTGACATCCTTATAAATGGATGTGCACATTTGCCAACACTGAGTG GCTTTCATCCTGGAGCAGACTTTGCAGTCTGTGGACTGCAACACAACATTC CCTTTATGTGTAACTCTTGGCTGAAGCTCTTACACCAATGCTGGGGGACAT GTACCTCCCAGGGGCCCAGGAAGACTACGGGAGGCTACACCAACGTCAATC AGAGGGGCCTGTGTAGCTACCGATAAGCGGACCCTCAAGAGGGCATTAGCA ATAGTGTTTATAAGGCCCCCTTGTTAACCCTAAACGGGTAGCATATGCTTC CCGGGTAGTAGTATATACTATCCAGACTAACCCTAATTCAATAGCATATGT TACCCAACGGGAAGCATATGCTATCGAATTAGGGTTAGTAAAAGGGTCCTA AGGAACAGCGATATCTCCCACCCCATGAGCTGTCACGGTTTTATTTACATG GGGTCAGGATTCCACGAGGGTAGTGAACCATTTTAGTCACAAGGGCAGTGG CTGAAGATCAAGGAGCGGGCAGTGAACTCTCCTGAATCTTCGCCTGCTTCT TCATTCTCCTTCGTTTAGCTAATAGAATAACTGCTGAGTTGTGAACAGTAA GGTGTATGTGAGGTGCTCGAAAACAAGGTTTCAGGTGACGCCCCCAGAATA AAATTTGGACGGGGGGTTCAGTGGTGGCATTGTGCTATGACACCAATATAA CCCTCACAAACCCCTTGGGCAATAAATACTAGTGTAGGAATGAAACATTCT GAATATCTTTAACAATAGAAATCCATGGGGTGGGGACAAGCCGTAAAGACT GGATGTCCATCTCACACGAATTTATGGCTATGGGCAACACATAATCCTAGT GCAATATGATACTGGGGTTATTAAGATGTGTCCCAGGCAGGGACCAAGACA GGTGAACCATGTTGTTACACTCTATTTGTAACAAGGGGAAAGAGAGTGGAC GCCGACAGCAGCGGACTCCACTGGTTGTCTCTAACACCCCCGAAAATTAAA CGGGGCTCCACGCCAATGGGGCCCATAAACAAAGACAAGTGGCCACTCTTT TTTTTGAAATTGTGGAGTGGGGGCACGCGTCAGCCCCCACACGCCGCCCTG CGGTTTTGGACTGTAAAATAAGGGTGTAATAACTTGGCTGATTGTAACCCC GCTAACCACTGCGGTCAAACCACTTGCCCACAAAACCACTAATGGCACCCC GGGGAATACCTGCATAAGTAGGTGGGCGGGCCAAGATAGGGGCGCGATTGC TGCGATCTGGAGGACAAATTACACACACTTGCGCCTGAGCGCCAAGCACAG GGTTGTTGGTCCTCATATTCACGAGGTCGCTGAGAGCACGGTGGGCTAATG TTGCCATGGGTAGCATATACTACCCAAATATCTGGATAGCATATGCTATCC TAATCTATATCTGGGTAGCATAGGCTATCCTAATCTATATCTGGGTAGCAT ATGCTATCCTAATCTATATCTGGGTAGTATATGCTATCCTAATTTATATCT

Vector name	SEQ ID NO	Nucleotide sequences 12345678901234567890123456789012345678901234567890
		GGGTAGCATAGGCTATCCTAATCTATATCTGGGTAGCATATGCTATCCTAA TCTATATCTGGGTAGTATATGCTATCCTAATCTGTATCCGGGTAGCATATG CTATCCTAATAGAGATTAGGGTAGTATATGCTATCCTAATTTATATCTGGG TAGCATATACTACCCAAATATCTGGATAGCATATGCTATCCTAATCTATAT CTGGGTAGCATATGCTATCCTAATCTATATCTGGGTAGCATAGGCTATCCT AATCTATATCTGGGTAGCATATGCTATCCTAATCTATATCTGGGTAGTATA TGCTATCCTAATTTATATCTGGGTAGCATAGGCTATCCTAATCTATATCTG GGTAGCATATGCTATCCTAATCTATATCTGGGTAGTATATGCTATCCTAAT CTGTATCCGGGTAGCATATGCTATCCTCATGATAAGCTGTCAAACATGAGA ATTTTCTTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAA TGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAA TGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTA TCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAG GAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGC GGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAA AGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCT CAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAAT GATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTTGA CGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTT GGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGT AAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAA CTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCA CAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAA TGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGC AACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCG GCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCT GCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGG TGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCC CTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGA ACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTA ACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCA TTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGAC CAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGA AAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTG CTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCA AGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGAT ACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAA CTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGC TGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATA GTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACA GCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGA GCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCC GGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGG AAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGA GCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGC CAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCA CATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGC CTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGA GTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCC CGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTG GAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTA GGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAAT TGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGC CAAGCTCTAGCTAGAGGTCGAGTCCCTCCCCAGCAGGCAGAAGTATGCAAA GCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCA TCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGAC TAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTAT TCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGC TTTGCAAAGATGGATAAAGTTTTAAACAGAGAGGAATCTTTGCAGCTAATG GACCTTCTAGGTCTTGAAAGGAGTGGGAATTGGCTCCGGTGCCCGTCAGTG GGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGG CAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGA

Vector name	SEQ ID NO	Nucleotide sequences 12345678901234567890123456789012345678901234567890
		TGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATAT AAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAG AACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGG TTATGGCCCTTGCGTGCCTTGAATTACTTCCACCTGGCTGCAGTACGTGAT TCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTG CGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCG CTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGC TTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCT TTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGG TATTTCGGTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCG CACATGTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACG GGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCC GTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGC GTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAATG GAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAA AAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCG GGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTC TTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTG GGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGA ATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGT GGTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTGAGGAATTCTCTAGAG ATCCCTCGACCTCGAGATCCATTGTGCCCGGGCGCCACCATGGAGTTTGGG CTGAGCTGGCTTTTTCTTGTCGCGATTTTAAAAGGTGTCCAGTGC
V2	868	ACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTG AAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGA GAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCC CAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGC AGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCC TGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAAC AGGGGAGAGTGTTGAGCGGCCGCTCGAGGCCGGCAAGGCCGGATCCCCCGA CCTCGACCTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTT GGAATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATT TGGTCGAGATCCCTCGGAGATCTCTAGCTAGAGGATCGATCCCCGCCCCGG ACGAACTAAACCTGACTACGACATCTCTGCCCCTTCTTCGCGGGGCAGTGC ATGTAATCCCTTCAGTTGGTTGGTACAACTTGCCAACTGGGCCCTGTTCCA CATGTGACACGGGGGGGGACCAAACACAAAGGGGTTCTCTGACTGTAGTTG ACATCCTTATAAATGGATGTGCACATTTGCCAACACTGAGTGGCTTTCATC CTGGAGCAGACTTTGCAGTCTGTGGACTGCAACACAACATTGCCTTTATGT GTAACTCTTGGCTGAAGCTCTTACACCAATGCTGGGGGACATGTACCTCCC AGGGGCCCAGGAAGACTACGGGAGGCTACACCAACGTCAATCAGAGGGGCC TGTGTAGCTACCGATAAGCGGACCCTCAAGAGGGCATTAGCAATAGTGTTT ATAAGGCCCCCTTGTTAACCCTAAACGGGTAGCATATGCTTCCCGGGTAGT AGTATATACTATCCAGACTAACCCTAATTCAATAGCATATGTTACCCAACG GGAAGCATATGCTATCGAATTAGGGTTAGTAAAAGGGTCCTAAGGAACAGC GATATCTCCCACCCCATGAGCTGTCACGGTTTTATTTACATGGGGTCAGGA TTCCACGAGGGTAGTGAACCATTTTAGTCACAAGGGCAGTGGCTGAAGATC AAGGAGCGGGCAGTGAACTCTCCTGAATCTTCGCCTGCTTCTTCATTCTCC TTCGTTTAGCTAATAGAATAACTGCTGAGTTGTGAACAGTAAGGTGTATGT GAGGTGCTCGAAAACAAGGTTTCAGGTGACGCCCCCAGAATAAAATTTGGA CGGGGGGTTCAGTGGTGGCATTGTGCTATGACACCAATATAACCCTCACAA ACCCCTTGGGCAATAAATACTAGTGTAGGAATGAAACATTCTGAATATCTT TAACAATAGAAATCCATGGGGTGGGGACAAGCCGTAAAGACTGGATGTCCA TCTCACACGAATTTATGGCTATGGGCAACACATAATCCTAGTGCAATATGA TACTGGGGTTATTAAGATGTGTCCCAGGCAGGGACCAAGACAGGTGAACCA TGTTGTTACACTCTATTTGTAACAAGGGGAAAGAGAGTGGACGCCGACAGC AGCGGACTCCACTGGTTGTCTCTAACACCCCCGAAAATTAAACGGGGCTCC ACGCCAATGGGGCCCATAAACAAAGACAAGTGGCCACTCTTTTTTTTGAAA TTGTGGAGTGGGGGCACGCGTCAGCCCCCACACGCCGCCCTGCGGTTTTGG ACTGTAAAATAAGGGTGTAATAACTTGGCTGATTGTAACCCCGCTAACCAC TGCGGTCAAACCACTTGCCCACAAAACCACTAATGGCACCCCGGGGAATAC CTGCATAAGTAGGTGGGCGGGCCAAGATAGGGGCGCGATTGCTGCGATCTG GAGGACAAATTACACACACTTGCGCCTGAGCGCCAAGCACAGGGTTGTTGG

Vector name	SEQ ID NO	Nucleotide sequences 12345678901234567890123456789012345678901234567890
		TCCTCATATTCACGAGGTCGCTGAGAGCACGGTGGGCTAATGTTGCCATGG GTAGCATATACTACCCAAATATCTGGATAGCATATGCTATCCTAATCTATA TCTGGGTAGCATAGGCTATCCTAATCTATATCTGGGTAGCATATGCTATCC TAATCTATATCTGGGTAGTATATGCTATCCTAATTTATATCTGGGTAGCAT AGGCTATCCTAATCTATATCTGGGTAGCATATGCTATCCTAATCTATATCT GGGTAGTATATGCTATCCTAATCTGTATCCGGGTAGCATATGCTATCCTAA TAGAGATTAGGGTAGTATATGCTATCCTAATTTATATCTGGGTAGCATATA CTACCCAAATATCTGGATAGCATATGCTATCCTAATCTATATCTGGGTAGC ATATGCTATCCTAATCTATATCTGGGTAGCATAGGCTATCCTAATCTATAT CTGGGTAGCATATGCTATCCTAATCTATATCTGGGTAGTATATGCTATCCT AATTTATATCTGGGTAGCATAGGCTATCCTAATCTATATCTGGGTAGCATA TGCTATCCTAATCTATATCTGGGTAGTATATGCTATCCTAATCTGTATCCG GGTAGCATATGCTATCCTCATGATAAGCTGTCAAACATGAGAATTTTCTTG AAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGAT AATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGG AACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCAT GAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTAT GAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTG CCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGA AGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGG TAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCAC TTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCA AGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTA CTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATT ATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCT GACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGG GGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCAT ACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAACGTT GCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATT AATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGC CCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGG GTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTAT CGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAG ACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGA CCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATT TAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCC TTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAA AGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAAC AAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACC AACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATAC TGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGC ACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAG TGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGA TAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTT GGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGA AAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGG CAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTG GTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATT TTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGC GGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTT TCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTG AGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAG CGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTG GCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGG CAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCA GGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGG ATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTCTA GCTAGAGGTCGAGTCCCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATC TCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCC TAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTT TTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGT AGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTTGCAAAG

Vector name	SEQ ID NO	Nucleotide sequences 12345678901234567890123456789012345678901234567890
		ATGGATAAAGTTTTAAACAGAGAGGAATCTTTGCAGCTAATGGACCTTCTA GGTCTTGAAAGGAGTGGGAATTGGCTCCGGTGCCCGTCAGTGGGCAGAGCG CACATCGCCCACAGTCCCCGAGAAGTTGGGGGGGAGGGGTCGGCAATTGAAC CGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTA CTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGT AGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGT AAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCC TTGCGTGCCTTGAATTACTTCCACCTGGCTGCAGTACGTGATTCTTGATCC CGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGG AGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCG CCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAA GTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTTTTCTG GCAAGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGGTATTTCGGT TTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTC GGCGAGGCGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTC TCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGC CCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGA AAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCG GCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTT TCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTC CAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTG GGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGAC TGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCT TTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAG TTTTTTTCTTCCATTTCAGGTGTCGTGAGGAATTCTCTAGAGATCCCTCGA CCTCGAGATCCATTGTGCCCGGGCGCACCATGGACATGCGCGTGCCCGCCC AGCTGCTGGGCCTGCTGCTGCTGTGGTTCCCCGGCTCGCGATGC
V3	869	CAACCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCCTCCTCTGAGGAG CTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTCTACCCG GGAGCCGTGACAGTGGCCTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGA GTGGAGACCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGC AGCTACCTGAGCCTGACGCCTGAGCAGTGGAAGTCCCACAGAAGCTACAGC TGCCAGGTCACGCATGAAGGGAGCACCGTGGAGAAGACAGTGGCCCCTACA GAATGTTCATGAGCGGCCGCTCGAGGCCGGCAAGGCCGGATCCCCCGACCT CGACCTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGA ATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTGG TCGAGATCCCTCGGAGATCTCTAGCTAGAGGATCGATCCCCGCCCCGGACG AACTAAACCTGACTACGACATCTCTGCCCCTTCTTCGCGGGGCAGTGCATG TAATCCCTTCAGTTGGTTGGTACAACTTGCCAACTGGGCCCTGTTCCACAT GTGACACGGGGGGGGACCAAACACAAAGGGGTTCTCTGACTGTAGTTGACA TCCTTATAAATGGATGTGCACATTTGCCAACACTGAGTGGCTTTCATCCTG GAGCAGACTTTGCAGTCTGTGGACTGCAACACAACATTGCCTTTATGTGTA ACTCTTGGCTGAAGCTCTTACACCAATGCTGGGGGACATGTACCTCCCAGG GGCCCAGGAAGACTACGGGAGGCTACACCAACGTCAATCAGAGGGGCCTGT GTAGCTACCGATAAGCGGACCCTCAAGAGGGCATTAGCAATAGTGTTTATA AGGCCCCCTTGTTAACCCTAAACGGGTAGCATATGCTTCCCGGGTAGTAGT ATATACTATCCAGACTAACCCTAATTCAATAGCATATGTTACCCAACGGGA AGCATATGCTATCGAATTAGGGTTAGTAAAAGGGTCCTAAGGAACAGCGAT ATCTCCCACCCCATGAGCTGTCACGGTTTTATTTACATGGGGTCAGGATTC CACGAGGGTAGTGAACCATTTTAGTCACAAGGGCAGTGGCTGAAGATCAAG GAGCGGGCAGTGAACTCTCCTGAATCTTCGCCTGCTTCTTCATTCTCCTTC GTTTAGCTAATAGAATAACTGCTGAGTTGTGAACAGTAAGGTGTATGTGAG GTGCTCGAAAACAAGGTTTCAGGTGACGCCCCCAGAATAAAATTTGGACGG GGGGTTCAGTGGTGGCATTGTGCTATGACACCAATATAACCCTCACAAACC CCTTGGGCAATAAATACTAGTGTAGGAATGAAACATTCTGAATATCTTTAA CAATAGAAATCCATGGGGTGGGGACAAGCCGTAAAGACTGGATGTCCATCT CACACGAATTTATGGCTATGGGCAACACATAATCCTAGTGCAATATGATAC TGGGGTTATTAAGATGTGTCCCAGGCAGGGACCAAGACAGGTGAACCATGT TGTTACACTCTATTTGTAACAAGGGGAAAGAGAGTGGACGCCGACAGCAGC GGACTCCACTGGTTGTCTCTAACACCCCCGAAAATTAAACGGGGCTCCACG CCAATGGGGCCCATAAACAAAGACAAGTGGCCACTCTTTTTTTTGAAATTG TGGAGTGGGGGCACGCGTCAGCCCCCACACGCCGCCCTGCGGTTTTGGACT

Vector name	SEQ ID NO	Nucleotide sequences 1234567890123456789012345678901234567890123456789012345678901
		GTAAAATAAGGGTGTAATAACTTGGCTGATTGTAACCCCGCTAACCACTGC GGTCAAACCACTTGCCCACAAAACCACTAATGGCACCCCGGGGAATACCTG CATAAGTAGGTGGGCGGGCCAAGATAGGGGCGCGATTGCTGCGATCTGGAG GACAAATTACACACACTTGCGCCTGAGCGCCAAGCACAGGGTTGTTGGTCC TCATATTCACGAGGTCGCTGAGAGCACGGTGGGCTAATGTTGCCATGGGTA GCATATACTACCCAAATATCTGGATAGCATATGCTATCCTAATCTATATCT GGGTAGCATAGGCTATCCTAATCTATATCTGGGTAGCATATGCTATCCTAA TCTATATCTGGGTAGTATATGCTATCCTAATTTATATCTGGGTAGCATAGG CTATCCTAATCTATATCTGGGTAGCATATGCTATCCTAATCTATATCTGGG TAGTATATGCTATCCTAATCTGTATCCGGGTAGCATATGCTATCCTAATAG AGATTAGGGTAGTATATGCTATCCTAATTTATATCTGGGTAGCATATACTA CCCAAATATCTGGATAGCATATGCTATCCTAATCTATATCTGGGTAGCATA TGCTATCCTAATCTATATCTGGGTAGCATAGGCTATCCTAATCTATATCTG GGTAGCATATGCTATCCTAATCTATATCTGGGTAGTATATGCTATCCTAAT TTATATCTGGGTAGCATAGGCTATCCTAATCTATATCTGGGTAGCATATGC TATCCTAATCTATATCTGGGTAGTATATGCTATCCTAATCTGTATCCGGGT AGCATATGCTATCCTCATGATAAGCTGTCAAACATGAGAATTTTCTTGAAG ACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAAT AATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAAC CCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAG ACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAG TATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCT TCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGA TCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAA GATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTT TAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAAGA GCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTC ACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATG CAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGAC AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGA TCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACC AAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAACGTTGCG CAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAAT AGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCT TCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTC TCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGT AGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACA GATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCA AGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAA AAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTA ACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGG ATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAA AAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAAC TCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGT TCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACC GCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGG CGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAA GGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGA GCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAG CGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAG GGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTA TCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTT GTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGC CTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCC TGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGC TGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGA GGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCC GATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAG TGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGC TTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATA ACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTCTAGCT AGAGGTCGAGTCCCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCA

Vector name	SEQ ID NO	Nucleotide sequences 12345678901234567890123456789012345678901234567890
		ATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAA CTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTA TTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGT GAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTTGCAAAGATG GATAAAGTTTTAAACAGAGAGGAATCTTTGCAGCTAATGGACCTTCTAGGT CTTGAAAGGAGTGGGAATTGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCAC ATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGG TGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTG GCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGT CGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAG TGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTG CGTGCCTTGAATTACTTCCACCTGGCTGCAGTACGTGATTCTTGATCCCGA GCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGC CCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCG CGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTC TCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCA AGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTT TGGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGC GAGGCGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCA AGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCC GCCCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAG ATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCG CTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCC GTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAG GCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGG GGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGA AGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTT TGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTT TTTTCTTCCATTTCAGGTGTCGTGAGGAATTCTCTAGAGATCCCTCGACCT CGAGATCCATTGTGCCCGGGCGCCACCATGACTTGGACCCCACTCCTCTTC CTCACCCTCCTCCTCCACTGCACAGGAAGCTTATCG
V4	870	ACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTG AAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGA GAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCC CAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGC AGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCC TGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAAC AGGGGAGAGTGTTGAGCGGCCGCTCGAGGCCGGCAAGGCCGGATCCCCCGA CCTCGACCTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTT GGAATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATT TGGTCGAGATCCCTCGGAGATCTCTAGCTAGAGGATCGATCCCCGCCCCGG ACGAACTAAACCTGACTACGACATCTCTGCCCCTTCTTCGCGGGGCAGTGC ATGTAATCCCTTCAGTTGGTTGGTACAACTTGCCAACTGGGCCCTGTTCCA CATGTGACACGGGGGGGGACCAAACACAAAGGGGTTCTCTGACTGTAGTTG ACATCCTTATAAATGGATGTGCACATTTGCCAACACTGAGTGGCTTTCATC CTGGAGCAGACTTTGCAGTCTGTGGACTGCAACACAACATTGCCTTTATGT GTAACTCTTGGCTGAAGCTCTTACACCAATGCTGGGGGACATGTACCTCCC AGGGGCCCAGGAAGACTACGGGAGGCTACACCAACGTCAATCAGAGGGGCC TGTGTAGCTACCGATAAGCGGACCCTCAAGAGGGCATTAGCAATAGTGTTT ATAAGGCCCCCTTGTTAACCCTAAACGGGTAGCATATGCTTCCCGGGTAGT AGTATATACTATCCAGACTAACCCTAATTCAATAGCATATGTTACCCAACG GGAAGCATATGCTATCGAATTAGGGTTAGTAAAAGGGTCCTAAGGAACAGC GATATCTCCCACCCCATGAGCTGTCACGGTTTTATTTACATGGGGTCAGGA TTCCACGAGGGTAGTGAACCATTTTAGTCACAAGGGCAGTGGCTGAAGATC AAGGAGCGGGCAGTGAACTCTCCTGAATCTTCGCCTGCTTCTTCATTCTCC TTCGTTTAGCTAATAGAATAACTGCTGAGTTGTGAACAGTAAGGTGTATGT GAGGTGCTCGAAAACAAGGTTTCAGGTGACGCCCCCAGAATAAAATTTGGA CGGGGGGTTCAGTGGTGGCATTGTGCTATGACACCAATATAACCCTCACAA ACCCCTTGGGCAATAAATACTAGTGTAGGAATGAAACATTCTGAATATCTT TAACAATAGAAATCCATGGGGTGGGGACAAGCCGTAAAGACTGGATGTCCA TCTCACACGAATTTATGGCTATGGGCAACACATAATCCTAGTGCAATATGA TACTGGGGTTATTAAGATGTGTCCCAGGCAGGGACCAAGACAGGTGAACCA

Vector name	SEQ ID NO	Nucleotide sequences 12345678901234567890123456789012345678901234567890
		TGTTGTTACACTCTATTTGTAACAAGGGGAAAGAGAGTGGACGCCGACAGC AGCGGACTCCACTGGTTGTCTCTAACACCCCCGAAAATTAAACGGGGCTCC ACGCCAATGGGGCCCATAAACAAAGACAAGTGGCCACTCTTTTTTTTGAAA TTGTGGAGTGGGGGCACGCGTCAGCCCCCACACGCCGCCCTGCGGTTTTGG ACTGTAAAATAAGGGTGTAATAACTTGGCTGATTGTAACCCCGCTAACCAC TGCGGTCAAACCACTTGCCCACAAAACCACTAATGGCACCCCGGGGAATAC CTGCATAAGTAGGTGGGCGGGCCAAGATAGGGGCGCGATTGCTGCGATCTG GAGGACAAATTACACACACTTGCGCCTGAGCGCCAAGCACAGGGTTGTTGG TCCTCATATTCACGAGGTCGCTGAGAGCACGGTGGGCTAATGTTGCCATGG GTAGCATATACTACCCAAATATCTGGATAGCATATGCTATCCTAATCTATA TCTGGGTAGCATAGGCTATCCTAATCTATATCTGGGTAGCATATGCTATCC TAATCTATATCTGGGTAGTATATGCTATCCTAATTTATATCTGGGTAGCAT AGGCTATCCTAATCTATATCTGGGTAGCATATGCTATCCTAATCTATATCT GGGTAGTATATGCTATCCTAATCTGTATCCGGGTAGCATATGCTATCCTAA TAGAGATTAGGGTAGTATATGCTATCCTAATTTATATCTGGGTAGCATATA CTACCCAAATATCTGGATAGCATATGCTATCCTAATCTATATCTGGGTAGC ATATGCTATCCTAATCTATATCTGGGTAGCATAGGCTATCCTAATCTATAT CTGGGTAGCATATGCTATCCTAATCTATATCTGGGTAGTATATGCTATCCT AATTTATATCTGGGTAGCATAGGCTATCCTAATCTATATCTGGGTAGCATA TGCTATCCTAATCTATATCTGGGTAGTATATGCTATCCTAATCTGTATCCG GGTAGCATATGCTATCCTCATGATAAGCTGTCAAACATGAGAATTTTCTTG AAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGAT AATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGG AACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCAT GAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTAT GAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTG CCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGA AGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGG TAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCAC TTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCA AGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTA CTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATT ATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCT GACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTTGCACAACATGGG GGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCAT ACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAACGTT GCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATT AATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGC CCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGG GTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTAT CGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAG ACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGA CCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATT TAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCC TTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAA AGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAAC AAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACC AACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATAC TGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGC ACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAG TGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGA TAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTT GGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGA AAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGG CAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTG GTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATT TTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGC GGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTT TCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTG AGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAG CGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTG GCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGG

Vector name	SEQ ID NO	Nucleotide sequences 12345678901234567890123456789012345678901234567890
		CAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCA GGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGG ATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTCTA GCTAGAGGTCGAGTCCCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATC TCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCC TAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTT TTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGT AGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTTGCAAAG ATGGATAAAGTTTTAAACAGAGAGGAATCTTTGCAGCTAATGGACCTTCTA GGTCTTGAAAGGAGTGGGAATTGGCTCCGGTGCCCGTCAGTGGGCAGAGCG CACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAAC CGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTA CTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGT AGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGT AAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCC TTGCGTGCCTTGAATTACTTCCACCTGGCTGCAGTACGTGATTCTTGATCC CGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGG AGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCG CCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAA GTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTTTTCTG GCAAGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGGTATTTCGGT TTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTC GGCGAGGCGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTC TCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGC CCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGA AAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCG GCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTT TCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTC CAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTG GGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGAC TGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCT TTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAG TTTTTTTCTTCCATTTCAGGTGTCGTGAGGAATTCTCTAGAGATCCCTCGA CCTCGAGATCCATTGTGCCCGGGCGCACCATGACTTGGACCCCACTCCTCT TCCTCACCCTCCTCCTCCACTGCACAGGAAGCTTATCG
V5	871	CAACCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCCTCCTCTGAGGAG CTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTCTACCCG GGAGCCGTGACAGTGGCCTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGA GTGGAGACCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGC AGCTACCTGAGCCTGACGCCTGAGCAGTGGAAGTCCCACAGAAGCTACAGC TGCCAGGTCACGCATGAAGGGAGCACCGTGGAGAAGACAGTGGCCCCTACA GAATGTTCATGAGCGGCCGCTCGAGGCCGGCAAGGCCGGATCCCCCGACCT CGACCTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGA ATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTGG TCGAGATCCCTCGGAGATCTCTAGCTAGAGGATCGATCCCCGCCCCGGACG AACTAAACCTGACTACGACATCTCTGCCCCTTCTTCGCGGGGCAGTGCATG TAATCCCTTCAGTTGGTTGGTACAACTTGCCAACTGGGCCCTGTTCCACAT GTGACACGGGGGGGGACCAAACACAAAGGGGTTCTCTGACTGTAGTTGACA TCCTTATAAATGGATGTGCACATTTGCCAACACTGAGTGGCTTTCATCCTG GAGCAGACTTTGCAGTCTGTGGACTGCAACACAACATTGCCTTTATGTGTA ACTCTTGGCTGAAGCTCTTACACCAATGCTGGGGGACATGTACCTCCCAGG GGCCCAGGAAGACTACGGGAGGCTACACCAACGTCAATCAGAGGGGCCTGT GTAGCTACCGATAAGCGGACCCTCAAGAGGGCATTAGCAATAGTGTTTATA AGGCCCCCTTGTTAACCCTAAACGGGTAGCATATGCTTCCCGGGTAGTAGT ATATACTATCCAGACTAACCCTAATTCAATAGCATATGTTACCCAACGGGA AGCATATGCTATCGAATTAGGGTTAGTAAAAGGGTCCTAAGGAACAGCGAT ATCTCCCACCCCATGAGCTGTCACGGTTTTATTTACATGGGGTCAGGATTC CACGAGGGTAGTGAACCATTTTAGTCACAAGGGCAGTGGCTGAAGATCAAG GAGCGGGCAGTGAACTCTCCTGAATCTTCGCCTGCTTCTTCATTCTCCTTC GTTTAGCTAATAGAATAACTGCTGAGTTGTGAACAGTAAGGTGTATGTGAG GTGCTCGAAAACAAGGTTTCAGGTGACGCCCCCAGAATAAAATTTGGACGG GGGGTTCAGTGGTGGCATTGTGCTATGACACCAATATAACCCTCACAAACC

Vector name	SEQ ID NO	Nucleotide sequences 12345678901234567890123456789012345678901234567890
		CCTTGGGCAATAAATACTAGTGTAGGAATGAAACATTCTGAATATCTTTAA CAATAGAAATCCATGGGGTGGGGACAAGCCGTAAAGACTGGATGTCCATCT CACACGAATTTATGGCTATGGGCAACACATAATCCTAGTGCAATATGATAC TGGGGTTATTAAGATGTGTCCCAGGCAGGGACCAAGACAGGTGAACCATGT TGTTACACTCTATTTGTAACAAGGGGAAAGAGAGTGGACGCCGACAGCAGC GGACTCCACTGGTTGTCTCTAACACCCCCGAAAATTAAACGGGGCTCCACG CCAATGGGGCCCATAAACAAAGACAAGTGGCCACTCTTTTTTTTGAAATTG TGGAGTGGGGGCACGCGTCAGCCCCCACACGCCGCCCTGCGGTTTTGGACT GTAAAATAAGGGTGTAATAACTTGGCTGATTGTAACCCCGCTAACCACTGC GGTCAAACCACTTGCCCACAAAACCACTAATGGCACCCCGGGGAATACCTG CATAAGTAGGTGGGCGGGCCAAGATAGGGGCGCGATTGCTGCGATCTGGAG GACAAATTACACACACTTGCGCCTGAGCGCCAAGCACAGGGTTGTTGGTCC TCATATTCACGAGGTCGCTGAGAGCACGGTGGGCTAATGTTGCCATGGGTA GCATATACTACCCAAATATCTGGATAGCATATGCTATCCTAATCTATATCT GGGTAGCATAGGCTATCCTAATCTATATCTGGGTAGCATATGCTATCCTAA TCTATATCTGGGTAGTATATGCTATCCTAATTTATATCTGGGTAGCATAGG CTATCCTAATCTATATCTGGGTAGCATATGCTATCCTAATCTATATCTGGG TAGTATATGCTATCCTAATCTGTATCCGGGTAGCATATGCTATCCTAATAG AGATTAGGGTAGTATATGCTATCCTAATTTATATCTGGGTAGCATATACTA CCCAAATATCTGGATAGCATATGCTATCCTAATCTATATCTGGGTAGCATA TGCTATCCTAATCTATATCTGGGTAGCATAGGCTATCCTAATCTATATCTG GGTAGCATATGCTATCCTAATCTATATCTGGGTAGTATATGCTATCCTAAT TTATATCTGGGTAGCATAGGCTATCCTAATCTATATCTGGGTAGCATATGC TATCCTAATCTATATCTGGGTAGTATATGCTATCCTAATCTGTATCCGGGT AGCATATGCTATCCTCATGATAAGCTGTCAAACATGAGAATTTTCTTGAAG ACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAAT AATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAAC CCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAG ACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAG TATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCT TCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGA TCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAA GATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTT TAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAAGA GCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTC ACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATG CAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGAC AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGA TCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACC AAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAACGTTGCG CAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAAT AGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCT TCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTC TCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGT AGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACA GATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCA AGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAA AAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTA ACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGG ATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAA AAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAAC TCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGT TCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACC GCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGG CGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAA GGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGA GCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAG CGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAG GGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTA TCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTT GTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGC CTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCC

Vector name	SEQ ID NO	Nucleotide sequences 12345678901234567890123456789012345678901234567890123456789012345678901
		TGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGC TGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGA GGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCC GATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAG TGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGC TTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATA ACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTCTAGCT AGAGGTCGAGTCCCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCA ATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAA CTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTA TTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGT GAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTTGCAAAGATG GATAAAGTTTTAAACAGAGAGGAATCTTTGCAGCTAATGGACCTTCTAGGT CTTGAAAGGAGTGGGAATTGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCAC ATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGG TGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTG GCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGT CGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAG TGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTG CGTGCCTTGAATTACTTCCACCTGGCTGCAGTACGTGATTCTTGATCCCGA GCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGC CCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCG CGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTC TCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCA AGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTT TGGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGC GAGGCGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCA AGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCC GCCCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAG ATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCG CTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCC GTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAG GCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGG GGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGA AGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTT TGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTT TTTTCTTCCATTTCAGGTGTCGTGAGGAATTCTCTAGAGATCCCTCGACCT CGAGATCCATTGTGCCCGGGCGCCACCATGGACATGCGCGTGCCCGCCCAG CTGCTGGGCCTGCTGCTGCTGTGGTTCCCCGGCTCGCGATGC
V7	872	GCGTCGACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGC ACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCC GAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCAC ACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTG GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTG AATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCT TGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGCCGCGGGG GGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATC TCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGAC CCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCC AAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGC GTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC AAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAA GCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGC GAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTC TATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTC TACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTC TCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGC CTCTCCCTGTCTCCGGGTAAATGAGCGGCCGCTCGAGGCCGGCAAGGCCGG ATCCCCCGACCTCGACCTCTGGCTAATAAAGGAAATTTATTTTCATTGCAA TAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGG CAAATCATTTGGTCGAGATCCCTCGGAGATCTCTAGCTAGAGGATCGATCC

Vector name	SEQ ID NO	Nucleotide sequences 12345678901234567890123456789012345678901234567890
		CCGCCCCGGACGAACTAAACCTGACTACGACATCTCTGCCCCTTCTTCGCG GGGCAGTGCATGTAATCCCTTCAGTTGGTTGGTACAACTTGCCAACTGGGC CCTGTTCCACATGTGACACGGGGGGGGACCAAACACAAAGGGGTTCTCTGA CTGTAGTTGACATCCTTATAAATGGATGTGCACATTTGCCAACACTGAGTG GCTTTCATCCTGGAGCAGACTTTGCAGTCTGTGGACTGCAACACAACATTG CCTTTATGTGTAACTCTTGGCTGAAGCTCTTACACCAATGCTGGGGGACAT GTACCTCCCAGGGGCCCAGGAAGACTACGGGAGGCTACACCAACGTCAATC AGAGGGGCCTGTGTAGCTACCGATAAGCGGACCCTCAAGAGGGCATTAGCA ATAGTGTTTATAAGGCCCCCTTGTTAACCCTAAACGGGTAGCATATGCTTC CCGGGTAGTAGTATATACTATCCAGACTAACCCTAATTCAATAGCATATGT TACCCAACGGGAAGCATATGCTATCGAATTAGGGTTAGTAAAAGGGTCCTA AGGAACAGCGATATCTCCCACCCCATGAGCTGTCACGGTTTTATTTACATG GGGTCAGGATTCCACGAGGGTAGTGAACCATTTTAGTCACAAGGGCAGTGG CTGAAGATCAAGGAGCGGGCAGTGAACTCTCCTGAATCTTCGCCTGCTTCT TCATTCTCCTTCGTTTAGCTAATAGAATAACTGCTGAGTTGTGAACAGTAA GGTGTATGTGAGGTGCTCGAAAACAAGGTTTCAGGTGACGCCCCCAGAATA AAATTTGGACGGGGGGTTCAGTGGTGGCATTGTGCTATGACACCAATATAA CCCTCACAAACCCCTTGGGCAATAAATACTAGTGTAGGAATGAAACATTCT GAATATCTTTAACAATAGAAATCCATGGGGTGGGGACAAGCCGTAAAGACT GGATGTCCATCTCACACGAATTTATGGCTATGGGCAACACATAATCCTAGT GCAATATGATACTGGGGTTATTAAGATGTGTCCCAGGCAGGGACCAAGACA GGTGAACCATGTTGTTACACTCTATTTGTAACAAGGGGAAAGAGAGTGGAC GCCGACAGCAGCGGACTCCACTGGTTGTCTCTAACACCCCCGAAAATTAAA CGGGGCTCCACGCCAATGGGGCCCATAAACAAAGACAAGTGGCCACTCTTT TTTTTGAAATTGTGGAGTGGGGGCACGCGTCAGCCCCCACACGCCGCCCTG CGGTTTTGGACTGTAAAATAAGGGTGTAATAACTTGGCTGATTGTAACCCC GCTAACCACTGCGGTCAAACCACTTGCCCACAAAACCACTAATGGCACCCC GGGGAATACCTGCATAAGTAGGTGGGCGGGCCAAGATAGGGGCGCGATTGC TGCGATCTGGAGGACAAATTACACACACTTGCGCCTGAGCGCCAAGCACAG GGTTGTTGGTCCTCATATTCACGAGGTCGCTGAGAGCACGGTGGGCTAATG TTGCCATGGGTAGCATATACTACCCAAATATCTGGATAGCATATGCTATCC TAATCTATATCTGGGTAGCATAGGCTATCCTAATCTATATCTGGGTAGCAT ATGCTATCCTAATCTATATCTGGGTAGTATATGCTATCCTAATTTATATCT GGGTAGCATAGGCTATCCTAATCTATATCTGGGTAGCATATGCTATCCTAA TCTATATCTGGGTAGTATATGCTATCCTAATCTGTATCCGGGTAGCATATG CTATCCTAATAGAGATTAGGGTAGTATATGCTATCCTAATTTATATCTGGG TAGCATATACTACCCAAATATCTGGATAGCATATGCTATCCTAATCTATAT CTGGGTAGCATATGCTATCCTAATCTATATCTGGGTAGCATAGGCTATCCT AATCTATATCTGGGTAGCATATGCTATCCTAATCTATATCTGGGTAGTATA TGCTATCCTAATTTATATCTGGGTAGCATAGGCTATCCTAATCTATATCTG GGTAGCATATGCTATCCTAATCTATATCTGGGTAGTATATGCTATCCTAAT CTGTATCCGGGTAGCATATGCTATCCTCATGATAAGCTGTCAAACATGAGA ATTTTCTTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAA TGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAA TGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTA TCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAG GAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGC GGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAA AGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCT CAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAAT GATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTTGA CGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTT GGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGT AAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAA CTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCA CAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAA TGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGC AACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCG GCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCT GCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGG TGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCC CTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGA

Vector name	SEQ ID NO	Nucleotide sequences 12345678901234567890123456789012345678901234567890123456789012345678901
		ACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTA ACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCA TTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGAC CAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGA AAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTG CTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCA AGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGAT ACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAA CTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGC TGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATA GTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACA GCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGA GCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCC GGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGG AAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGA GCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGC CAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCA CATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGC CTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGA GTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCC CGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTG GAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTA GGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAAT TGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGC CAAGCTCTAGCTAGAGGTCGAGTCCCTCCCCAGCAGGCAGAAGTATGCAAA GCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCA TCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGAC TAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTAT TCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGC TTTGCAAAGATGGATAAAGTTTTAAACAGAGAGGAATCTTTGCAGCTAATG GACCTTCTAGGTCTTGAAAGGAGTGGGAATTGGCTCCGGTGCCCGTCAGTG GGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGG CAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGA TGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATAT AAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAG AACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGG TTATGGCCCTTGCGTGCCTTGAATTACTTCCACCTGGCTGCAGTACGTGAT TCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTG CGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCG CTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGC TTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCT TTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGG TATTTCGGTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCG CACATGTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACG GGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCC GTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGC GTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAATG GAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAA AAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCG GGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTC TTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTG GGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGA ATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGT GGTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTGAGGAATTCTCTAGAG ATCCCTCGACCTCGAGATCCATTGTGCCCGGGCGCCACCATGGAGTTTGGG CTGAGCTGGCTTTTTCTTGTCGCGATTTTAAAAGGTGTCCAGTGC

Example 5: Assays Used to Identify and Characterize Parent Antibodies and DVD-Igs

The following assays were used to identify and characterize parent antibodies and DVD-Ig, unless otherwise stated.

Example 5.1: Assays Used To Determine Binding and Affinity of Parent Antibodies and DVD-Ig for Their Target Antigen(s)

Example 5.1.1: Direct Bind ELISA

Enzyme Linked Immunosorbent Assays to screen for antibodies that bind a desired target antigen were performed as follows. High bind ELISA plates (Corning Costar # 3369, Acton, MA) were coated with 100µL/well of 10µg/ml of desired target antigen (R&D Systems, Minneapolis, MN) or desired target antigen extra-cellular domain / FC fusion protein (R&D Systems, Minneapolis, MN) or monoclonal mouse anti-polyHistidine antibody (R&D Systems # MAB050, Minneapolis, MN) in phosphate buffered saline (10X PBS, Abbott Bioresearch Center, Media Prep# MPS-073, Worcester, MA) overnight at 4°C. Plates were washed four times with PBS containing 0.02% Tween 20. Plates were blocked by the addition of 300 µL/well blocking solution (non-fat dry milk powder, various retail suppliers, diluted to 2% in PBS) for 1/2 hour at room temperature. Plates were washed four times after blocking with PBS containing 0.02% Tween 20.

Alternatively, one hundred microliters per well of 10 µg/ml of Histidine (His) tagged desired target antigen (R&D Systems, Minneapolis, MN) was added to ELISA plates coated with monoclonal mouse anti-polyHistidine antibody as described above and incubated for 1 hour at room temperature. Wells were washed four times with PBS containing 0.02% Tween 20.

One hundred microliters of antibody or DVD-Ig preparations diluted in blocking solution as described above was added to the desired target antigen plate or desired target antigen / FC fusion plate or the anti-polyHistidine antibody / His tagged desired target antigen plate prepared as described above and incubated for 1 hour at room temperature. Wells are washed four times with PBS containing 0.02% Tween 20.

One hundred microliters of 10ng/mL goat anti-human IgG –FC specific HRP conjugated antibody (Southern Biotech # 2040-05, Birmingham, AL) was added to each well of the desired target antigen plate or anti-polyHistidine antibody / Histidine tagged desired target antigen plate. Alternatively, one hundred microliters of 10 ng/mL goat anti-human IgG –kappa light chain specific HRP conjugated antibody (Southern Biotech # 2060-05 Birmingham, AL) was added to

each well of the desired target antigen / FC fusion plate and incubated for 1 hour at room temperature. Plates were washed 4 times with PBS containing 0.02% Tween 20.

One hundred microliters of enhanced TMB solution (Neogen Corp. #308177, K Blue, Lexington, KY) was added to each well and incubated for 10 minutes at room temperature. The reaction was stopped by the addition of 50 µL 1N sulphuric acid. Plates were read spectrophotometrically at a wavelength of 450 nm.

Table 52 contains a list of the antigens used in the Direct Bind Assay.

Table 53 contains the binding data expressed as EC50 in nM for those antibodies and DVD-Ig constructs tested in the Direct Bind ELISA assay.

In the Direct Bind ELISA, binding was occasionally not observed, probably because the antibody binding site on the target antigen was either "masked" or the antigen "distorted" when coated to the plastic surface. The inability of a DVD-Ig to bind its target may also be due to steric limitation imposed on DVD-Ig by the Direct Bind ELISA format. The parent antibodies and DVD-Igs that did not bind in the Direct Bind ELISA format bound to target antigen in other ELISA formats, such as FACS, Biacore or bioassay. Non-binding of a DVD-Ig was also restored by adjusting the linker length between the two variable domains of the DVD-Ig, as shown previously.

Table 52: Antigens Used in Direct Bind ELISA

Assay	Antigen	Vendor Designation	Vendor	Catalog #
NGF	NGF	β–NGF	R&D	256-GF
IL-17A	IL-17A	IL-17A	R&D	317-IL
IL-1β	IL-1β	IL-1β	R&D	201-LB
IL-6R	IL-6R	IL-6sR	R&D	227-SR
VEGF	VEGF	VEGF165	R&D	293-VE

/ECD= Extracellular Domain
/FC = Fc region fusion

Table 53: Direct Binding ELISA of Parental Antibodies and DVD Constructs

Parent Antibody or DVD ID	N-terminal Variable Domain	C-terminal Variable Domain	Direct Bind ELISA N-terminal VD EC50 (nM)	Direct Bind ELISA C-terminal VD EC50 (nM)
AB054	IL-6R		1.75	
DVD151	PGE2	IL-6R		26452.34
DVD152	IL-6R	PGE2	1.62	
AB029	IL-17A (seq. 1)		0.89	
DVD155	PGE2	IL-17A (seq. 1)		248.1

Parent Antibody or DVD ID	N-terminal Variable Domain	C-terminal Variable Domain	Direct Bind ELISA N-terminal VD EC50 (nM)	Direct Bind ELISA C-terminal VD EC50 (nM)
DVD156	IL-17A (seq. 1)	PGE2	0.62	
AB032	IL-1b (seq. 1)		5.27	
DVD157	PGE2	IL-1b (seq. 1)		2480.71
DVD158	IL-1b (seq. 1)	PGE2	17.61	
AB014	VEGF		2.93	
DVD161	PGE2	VEGF		391.23
DVD162	VEGF	PGE2	1.79	
AB020	NGF		2.59	
DVD163	PGE2	NGF		>10,000
DVD164	NGF	PGE2	1.21	

Binding of all DVD constructs was maintained and comparable to parent antibodies. All N-terminal variable domains bound with a similar high affinity as the parent.

Example 5.1.2: Affinity Determination using BIACORE technology

Table 54: Reagent Used in Biacore Analyses

Assay	Antigen	Vendor Designation	Vendor	Catalog #
	IL-1β	Recombinant Human IL-1β	R&D systems	201-LB
	IL-17	Recombinant Human IL-17	R&D systems	317-IL
	VEGF	Recombinant Human VEGF	R&D systems	293-VE
	IL-6R	Recombinant Human IL-6 sR	R&D systems	227-SR
	NGF	Recombinant Human β-NGF	R&D systems	256-GF
	IL-15	Recombinant Human IL-15	R&D systems	247-IL

ECD = Extracellular Domain
/FC = antigen/IgG FC domain fusion protein

BIACORE Methods:

The BIACORE assay (Biacore, Inc, Piscataway, NJ) determines the affinity of antibodies or DVD-Ig with kinetic measurements of on-rate and off-rate constants. Binding of antibodies or DVD-Ig to a target antigen (for example, a purified recombinant target antigen) is determined by surface plasmon resonance-based measurements with a Biacore® 1000 or 3000 instrument (Biacore® AB, Uppsala, Sweden) using running HBS-EP (10 mM HEPES [pH 7.4], 150 mM

NaCl, 3 mM EDTA, and 0.005% surfactant P20) at 25° C. All chemicals are obtained from Biacore® AB (Uppsala, Sweden) or otherwise from a different source as described in the text. For example, approximately 5000 RU of goat anti-mouse IgG, (Fcγ), fragment specific polyclonal antibody (Pierce Biotechnology Inc, Rockford, IL) diluted in 10 mM sodium acetate (pH 4.5) is directly immobilized across a CM5 research grade biosensor chip using a standard amine coupling kit according to manufacturer's instructions and procedures at 25 µg/ml. Unreacted moieties on the biosensor surface are blocked with ethanolamine. Modified carboxymethyl dextran surface in flowcell 2 and 4 is used as a reaction surface. Unmodified carboxymethyl dextran without goat anti-mouse IgG in flow cell 1 and 3 is used as the reference surface. For kinetic analysis, rate equations derived from the 1:1 Langmuir binding model are fitted simultaneously to association and dissociation phases of all eight injections (using global fit analysis) with the use of Biaevaluation 4.0.1 software. Purified antibodies or DVD-Ig are diluted in HEPES-buffered saline for capture across goat anti-mouse IgG specific reaction surfaces. Antibodies or DVD-Ig to be captured as a ligand (25 µg/ml) are injected over reaction matrices at a flow rate of 5 µl/min. The association and dissociation rate constants, k_{on} ($M^{-1}s^{-1}$) and k_{off} (s^{-1}) are determined under a continuous flow rate of 25 µl/min. Rate constants are derived by making kinetic binding measurements at different antigen concentrations ranging from 10 – 200 nM. The equilibrium dissociation constant (M) of the reaction between antibodies or DVD-Igs and the target antigen is then calculated from the kinetic rate constants by the following formula: $K_D = k_{off}/k_{on}$. Binding is recorded as a function of time and kinetic rate constants are calculated. In this assay, on-rates as fast as 10^6 $M^{-1}s^{-1}$ and off-rates as slow as 10^{-6} s^{-1} can be measured.

Table 55: BIACORE Analysis of Parental Antibodies and DVD Constructs

Parent Antibody or DVD-Ig ID	N-Terminal Variable Domain (VD)	C-Terminal Variable Domain (VD)	k_{on} (M-1s-1)	k_{off} (s-1)	K_D (M)
AB054	IL-6R		3.57E+05	1.60E-04	4.48E-10
AB048	PGE2		-	-	-
DVD151	PGE2		-	-	-
DVD152		IL-6R	2.53E+04	1.50E-04	5.94E-09
DVD152	IL-6R		4.20E+05	1.15E-04	2.75E-10
DVD152		PGE2	-	-	-
AB049	IL-15		4.05E+05	9.44E-04	2.33E-09
AB048	PGE2		-	-	-
DVD153	PGE2		-	-	-
DVD153		IL-15	-	-	-
DVD154	IL-15		4 04E+05	1.98E-03	4.89E-09
DVD154		PGE2	-	-	-
AB048	PGE2		-	-	-
AB029	IL-17		1.43E+05	1.14E-04	7.98E-10

Parent Antibody or DVD-Ig ID	N-Terminal Variable Domain (VD)	C-Terminal Variable Domain (VD)	k_{on} (M-1s-1)	k_{off} (s-1)	K_D (M)
DVD155	PGE2		-	-	-
DVD155		IL-17	3.70E+04	1.25E-04	3.37E-09
DVD156	IL-17		8.68E+04	<1E-6	1.15E-11
DVD156		PGE2	-	-	-
AB048	PGE2		-	-	-
AB032	IL-1B		1.32E+06	1.73E-05	1.31E-11
DVD157	PGE2		-	-	-
DVD157		IL-1B	-	-	-
DVD158	IL-1B		9.92E+05	3.84E-05	3.87E-11
DVD158		PGE2	-	-	-
AB048	PGE2		-	-	-
AB014	VEGF		3.06E+05	3.99E-06	1.30E-11
DVD161	PGE2		-	-	-
DVD161		VEGF	2.77E+05	2.09E-04	7.55E-10
DVD162	VEGF		4.90E+05	1.31E-04	2.66E-10
DVD162		PGE2	-	-	-
AB048	PGE2		-	-	-
AB020	NGF		2.61E+05	1.12E-04	4.28E-10
DVD163	PGE2	-	-	-	-
DVD163		NGF	8.40E+03	1.03E-06	1.22E-10
DVD164	NGF		4.14E+05	1.03E-04	2.48E-10
DVD164		PGE2	-	-	-

Binding of all DVD-Ig constructs characterized by Biacore technology was maintained and comparable to that of parent antibodies. All N-terminal variable domains bound with a similar high affinity as the parent antibody.

Example 5.1.3: IL-1α/β Bioassay and Neutralization Assay

MRC5 cells were plated at 1.5-2 x 10^4 cells per well in a 100 µL volume and incubated overnight at 37^{0}C, 5% CO_2. A 20 µg/mL working stock of antibody (4x concentrated) was prepared in complete MEM medium. An eight point serial dilution was performed (5 µg/mL-0.0003 µg/mL) in complete MEM in Marsh dilution plates. Sixty-five uL /well of each antibody dilution was added in quadruplicate to a 96 well v-bottom (Costar# 3894) plate and 65 µL of a 200 pg/mL solution of IL-1α or IL-1β or 65 µL of a mixed solution containing a 50 pg/mL solution of both IL-1 α and IL-1β. Control wells received 65 µL 200 pg/ml of IL-1α or IL-1β or 50 pg/mL mixed IL-1α/β (4x concentrated) plus 65 µL MEM media and media control wells received 130 µL of media Following a 1 hour incubation, 100 µL of the Ab/Ag mixture was added to the MRC5 cells. All well volumes were equal to 200 µL. All plate reagents were then 1x concentrated. After a 16-20 hr incubation, the well contents (150 µL) were transferred into a 96-well round bottom plate (Costar# 3799) and placed in a –20^{0}C freezer. The supernatants were tested for hIL-8 levels by using a human IL-8 ELISA kit (R&D Systems, Minneapolis, MN) or

MSD hIL-8 (chemiluminescence kit).Neutralization potency was determined by calculating percent inhibition relative to the IL-1α, IL-1β, or the IL-1α/β alone control value.

Table 56: IL-1βNeutralization Assay With IL-1β Parent Antibody and DVD-Ig Constructs

Parent Antibody or DVD-Ig ID	N-terminal Variable Domain (VD)	C-terminal Variable Domain (VD)	N-terminal VD IL-1β NeutralizationAssay EC50 nM	C-terminal VD IL-1β NeutralizationAssayEC50 nM
AB032	IL-1β		0.05	
DVD157	PGE2	IL-1β		3.22
DVD158	IL-1β	PGE2	0.11	

All DVD-Igs containing VDs from AB032 in either the N-terminal or C-terminal position showed neutralization in the MRC5 IL-1α/β neutralization assay.

Example 5.1.4: IL-17 Bioassay and Neutralization Assay

The human HS27 cell line (ATCC #CRL-1634) secretes IL-6 in response to IL-17. The IL-17-induced IL-6 secretion is inhibited by neutralizing anti-IL-17 antibodies (See, e.g., J. Imm. 155:5483-5486, 1995 or Cytokine 9:794-800, 1997).

HS27 cells were maintained in assay medium: DMEM high glucose medium (Gibco #11965) with 10% fetal bovine serum (Gibco#26140), 4 mM L- glutamine, 1 mM sodium pyruvate, penicillin G (100 U/500 ml) and streptomycin (100 µg/500 ml). Cells were grown in T150 flasks until they were about 80-90% confluent the day of the assay. Human IL-17 (R&D Systems, #317-IL/CF) was reconstituted in sterile PBS without Ca^{2+} and Mg^{2+} stored frozen, freshly thawed for use and diluted to 40 ng/ml (4X) in assay medium. Serial dilutions of antibodies were made in a separate plate (4X concentrations), mixed with equal volume of 40ng/ml (4X) of hu IL-17 and incubated at 37°C for 1 hour. HS27 cells (typically about 20,000 cells in 50 µl assay medium) are added to each well of a 96-well flat-bottom tissue culture plate (Costar #3599), followed by addition of 50 µl of the pre-incubated antibody plus IL-17 mix. The final concentration of IL-17 was 10ng/ml. Cells are incubated for about 24 hours at 37°C. The media supernatants were then collected. The level of IL- 17 neutralization was measured by determination of IL-6 amounts in supernatant using a commercial Meso Scale Discovery kit according to manufacturer's instruction. IC50 values were obtained using logarithm of antibody vs. IL-6 amount variable slope fit (Table 57).

Table 57: IL-17 Neutralization Assay With IL-17 Parent Antibody and DVD-Ig Constructs

Parent Antibody or DVD-Ig ID	N-terminal Variable Domain (VD)	C-terminal Variable Domain (VD)	N-terminal VD IL-17 NeutralizationAssay EC50 nM	C-terminal VD IL-17 NeutralizationAssayEC50 nM
AB029	IL-17A (seq. 1)		0.4	
DVD155	PGE2	IL-17A (seq. 1)		5.7E-5
DVD156	IL-17A (seq. 1)	PGE2	0.2	

All DVD-Igs containing VDs from AB029 in either the N-terminal or C-terminal position showed neutralization in the IL-17 neutralization assay.

Example 5.1.5: Enzyme Linked Immunosorbent Assays To Determine PGE_2 Binding Ability For Anti-PGE_2 Antibodies Or Anti-PGE_2 Containing DVD-Ig Molecules

Enzyme linked immunosorbent assays to screen for anti-PGE_2 antibodies or anti-PGE_2 containing DVD-Ig molecules that bind prostaglandin E_2 were performed as follows.

ELISA plates (Costar 3369, Corning, NY) were coated with 50 µl of anti-host Fc IgG (Sigma, St. Louis, MO) at 2 µg/ml in PBS (Invitrogen, Carlsbad, CA). Following an overnight incubation at 4°C, the plate was blocked with 200 µl Superblock (Pierce #37535, Rockford, IL). The IgG containing samples were diluted to 1 µg/ml in Assay Buffer (10% Superblock in PBS containing 0.05% Surfactamps (Pierce #37535, Rockford, IL) and incubated on the plate at 50 µl/well for 1 hour at room temperature. Following the incubation, plates were washed four times with TTBS (Tween-Tris Buffered Solution). PGE2-biotinamide (Cayman Chemicals, Ann Arbor, MI) was diluted to 30 nM and serially diluted in Assay Buffer. The titration curve was added to each IgG sample at a volume of 50 µl/well and incubated for 1 hour at room temperature. The plates were washed as previously described and 50 µl/well of 1:5000 dilution of streptavidin polyhrp40 (Fitzgerald Industries, Concord, MA) in Assay Buffer was added and incubated for 45 minutes at room temperature. A final wash step was performed and the plates were developed using a single step TMB system (Sigma #T8665, St. Louis, MO) and 2N H_2SO_4. Plates were read at 450 nm on a Molecular Devices Spectramax plate reader (Sunnyvale, CA). EC_{50} was determined using GraphPad Prism 5 (GraphPad Software, La Jolla, CA).

Table 58: PGE2 Binding by ELISA of Parental Antibodies and DVD Constructs

Parent Antibody or DVD ID	N-terminal Variable Domain	C-terminal Variable Domain	Direct Bind ELISA N-terminal VD EC50 (nM)	Direct Bind ELISA C-terminal VD EC50 (nM)
AB048	PGE2		1.49	
DVD151	PGE2	IL-6R	1.52	
DVD152	IL-6R	PGE2		1.65
DVD153	PGE2	IL-15	1.29	
DVD154	IL-15	PGE2		1.54
DVD155	PGE2	IL-17	1.44	
DVD156	IL-17	PGE2		1.55
DVD157	PGE2	IL-1B	1.23	
DVD158	IL-1B	PGE2		1.14
DVD161	PGE2	VEGF	1.02	
DVD162	VEGF	PGE2		1.32
DVD163	PGE2	NGF	0.71	
DVD164	NGF	PGE2		1.3
DVD227	Abeta (seq1)	PGE2		1.21
DVD228	PGE2	Abeta (seq1)	1.25	

Binding of all DVD-Igs was maintained and comparable to the parent antibody.

Example 5.5.6: EP4 Bioassay To Determine PGE_2 Neutralization Ability For Anti-PGE_2 Antibodies Or Anti-PGE_2 Containing DVD-Ig Molecules

The ability of anti-PGE_2 antibodies and anti-PGE_2 containing DVD-Ig molecules to inhibit the cellular response of PGE_2 was determined in a Ca++ flux assay using stably transfected human EP4 in HEK293 Gα16 cells. Cells were plated in black/clear Poly-D-Lysine plates, (Corning #3667) and incubated with Ca++-sensitive dye (Molecular Devices) for 90 minutes. Stock PGE_2 (in 200 proof ethanol) was diluted with FLIPR buffer (containing 1X HBSS, 20 mM HEPES, 0.1% BSA and 2.5 mM Probenecid). Anti-PGE_2 antibodies, DVD-Ig molecules or isotype matched control antibodies were also pre-diluted in FLIPR buffer. 25 µl of PGE_2 or pre-incubated PGE_2/antibody mixture or pre-incubated PGE_2/DVD-Ig molecule mixture was added to the wells pre-plated with cells. A dose response of PGE2 was done by a serial titration of PGE_2 and was determined using FLIPR1 or Tetra (Molecular Devices). EC50 was determined using GraphPad Prism 5. For testing antibodies and DVD-Ig molecules, PGE_2 at EC50 concentration was incubated with varying concentrations of test articles or isotype matched antibody (negative control) for 20 minutes, added to dye-loaded human EP4 in HEK293 Gα16

cells. Ca++ flux was monitored using FLIPR1 and data was analyzed using GraphPad Prism 5.

Table 59: PGE2 Bioassay of Parental Antibodies and DVD Constructs

Parent Antibody or DVD ID	N-terminal Variable Domain	C-terminal Variable Domain	N-terminal VD PGE2 Neutralization EC50 (nM)	C-terminal VD PGE2 Neutralization EC50 (nM)
AB048	PGE2		0.017	
DVD151	PGE2	IL-6R	0.036	
DVD152	IL-6R	PGE2		0.025
DVD153	PGE2	IL-15	0.048	
DVD154	IL-15	PGE2		0.029
DVD155	PGE2	IL-17	0.023	
DVD156	IL-17	PGE2		0.025
DVD157	PGE2	IL-1B	0.217	
DVD158	IL-1B	PGE2		0.21
DVD161	PGE2	VEGF	0.193	
DVD162	VEGF	PGE2		0.067
DVD163	PGE2	NGF	0.245	
DVD164	NGF	PGE2		0.079
DVD227	Abeta (seq1)	PGE2		0.084
DVD228	PGE2	Abeta (seq1)	0.049	

The DVD-Igs maintained their ability to inhibit the cellular response to PGE2 and were comparable to the parent antibodies

Example 5.5.7: Physicochemical And In Vitro Stability Analysis Of Humanized Monoclonal Antibodies

Size Exclusion Chromatography

Antibodies were diluted to 2.5 mg/mL with water and 20 mL is analyzed on a Shimadzu HPLC system using a TSK gel G3000 SWXL column (Tosoh Bioscience, cat# k5539-05k). Samples were eluted from the column with 211 mM sodium sulfate, 92 mM sodium phosphate, pH 7.0, at a flow rate of 0.3 mL/minutes. The HPLC system operating conditions are the following:

Mobile phase: 211 mM Na_2SO_4, 92 mM $Na_2HPO_4*7H_2O$, pH 7.0

Gradient: Isocratic

Flow rate: 0.3 mL/minute

Detector wavelength: 280 nm

Autosampler cooler temp: 4°C

Column oven temperature: Ambient

Run time: 50 minutes

Table 60 contains purity data of parent antibodies and DVD-Ig constructs expressed as percent monomer (unaggregated protein of the expected molecular weight).

Table 60: Purity of Parent Antibodies and DVD-Ig Constructs as Determined by Size Exclusion Chromatography

Parent Antibody or DVD-Ig ID	N-terminal Variable Domain (VD)	C-terminal Variable Domain (VD)	% Monomer (purity)
AB054	IL-6R		97.3
DVD151	PGE2	IL-6R	92.7
DVD152	IL-6R	PGE2	87.6
AB049	IL-15		97.9
DVD153	PGE2	IL-15	95.2
DVD154	IL-15	PGE2	88.7
AB029	IL-17A (seq. 1)		97.9
DVD155	PGE2	IL-17A (seq. 1)	95.8
DVD156	IL-17A (seq. 1)	PGE2	82.3
AB032	IL-1β		100
DVD157	PGE2	IL-1β	88.8
DVD158	IL-1β	PGE2	92.8
AB014	VEGF		98.5
DVD161	PGE2	VEGF	93.1
DVD162	VEGF	PGE2	85.5
AB020	NGF		90
DVD163	PGE2	NGF	83.1
DVD164	NGF	PGE2	96.0

DVD-Igs showed an excellent SEC profile with most DVD-Ig showing >90% monomer. This DVD-Ig profile is similar to that observed for parent antibodies.

Example 5.5.8: Binding of Monoclonal Antibodies to the Surface of Human Tumor Cell Lines as Assessed by Flow Cytometry

Stable cell lines overexpressing a cell-surface antigen of interest or human tumor cell lines were harvested from tissue culture flasks and resuspended in phosphate buffered saline (PBS) containing 5% fetal bovine serum (PBS/FBS). Prior to staining, human tumor cells were incubated on ice with (100µl) human IgG at 5µg/ml in PBS/FCS. 1-5 x10^5 cells were incubated with antibody or DVD-Ig (2 µg/mL) in PBS/FBS for 30-60 minutes on ice. Cells were washed twice and 100µl of F(ab')2 goat anti human IgG, Fcγ- phycoerythrin (1:200 dilution in PBS) (Jackson ImmunoResearch, West Grove, PA, Cat.#109-116-170) was added. After 30 minutes incubation on ice, cells were washed twice and resuspended in PBS/FBS. Fluorescence was measured using a Becton Dickinson FACSCalibur (Becton Dickinson, San Jose, CA).

Table 61 shows the FACS data for the DVD-Ig constructs. The geometric mean is the n root of the multiplication product of n fluorescent signals (a1 x a2 x a3....an). With log-transformed data the geometric mean is used to normalize the weighting of the data distribution. The following table contains the FACS geometric mean of parent antibodies and DVD-Ig constructs.

Table 61: Fluorescent Activated Cell Sorting of DVD-Ig Constructs

Parent Antibody or DVD-Ig ID	N-terminal Variable Domain (VD)	C-terminal Variable Domain (VD)	FACS Geometric Mean N-terminal	FACS Geometric Mean C-terminal
AB054	IL-6R		5.94	
DVD151	PGE2	IL-6R		0.21
DVD152	IL-6R	PGE2	9.13	

All DVDs showed binding to their cell surface targets. The N-terminal domains of DVDs bound their targets on the cell surface as well as or better than the parent antibody. Binding can be restored or improved by adjusting linker length.

Example 5.5.9: Transfection And Expression In 293 Cells

Expression of the reference antibodies and DVD's was accomplished by transiently cotransfecting HEK293(EBNA) cells with plasmids containing the corresponding light-chain (LC) and heavy-chain (HC) genes. HEK293(EBNA) cells were propagated in Freestyle 293 media (Invitrogen, Carlsbad CA) at a 0.5L-scale in flasks (2L Corning Cat# 431198) shaking in a CO_2 incubator (8% CO_2, 125 RPM, 37°C). When the cultures reached a density of 1x10^6 cell/ml, cells were transfected with

transfection complex. Transfection complex was prepared by first mixing 150ug LC-plasmid and 100ug HC-plasmid together in 25ml of Freestyle media, followed by addition of 500ul PEI stock solution [stock solution: 1 mg/ml (pH 7.0) Linear 25kDa PEI, Polysciences Cat# 23966]. The transfection complex was mixed by inversion and allowed to incubate at room temperature for 10 minutes prior to being added to the cell culture. Following transfection, cultures continued to be grown in the CO2 incubator (8% CO_2, 125 RPM, 37°C). Twenty-four hours after transfection, the culture was supplemented with 25ml of a 10% Tryptone N1 solution (Organo Technie, La Courneuve France Cat# 19553). Nine days after transfection, cells were removed from the cultures by centrifugation (16,000 g, 10 min), and the retained supernatant was sterile filtered (Millipore HV Durapore Stericup, 0.45um) and placed at 4°C until initiation of the purification step.

Each antibody or DVD-Ig was individually purified using a disposable 1ml packed column (packed by Orochem Technologies) containing MabSelect SuRe resin (GE Healthcare). Columns were pre-equilibriated in PBS and then loaded with the harvested 0.55L samples overnight (15 hours) at 1 ml/minute with the flow-through being recirculated back into the feed container. Following the loading step, columns were washed with 20ml PBS and protein was eluted by feeding elution buffer [50mM Citric acid pH 3.5] at 4 ml/minute and collecting fractions (1 ml) in tubes already containing 0.2ml of 1.5M Tris pH 8.2 (bringing the final pH to approximately 6.0). Fractions containing antibody were pooled based on the chromatograms and dialyzed into the final storage buffer [10mM citric acid, 10mM Na_2HPO_4, pH 6.0]. Following dialysis, samples were filtered through a 0.22um Steriflip (Millipore) and the protein concentration was determined by absorbance [Hewlett Packard 8453 diode array spectrophotometer].

SDS-PAGE analysis was performed on analytical samples (both reduced and non-reduced) to assess final purity, verify the presence of appropriately sized heavy- and light- chain bands, and confirm the absence of significant amounts of free (i.e. uncomplexed) light chain (in the non-reduced samples).

Table 62 contains the yield data for parent antibodies or DVD-Ig constructs expressed as milligrams per liter in 293 cells.

Table 62: Transient Expression in Yields of Parent Antibodies and DVD-Ig Constructs in 293 Cells

Parent Antibody or DVD-Ig ID	N-terminal Variable Domain (VD)	C-terminal Variable Domain (VD)	Expression Yield (mg/L)
AB014	VEGF		52.4
AB048	PGE2		19.4
DVD161	PGE2	VEGF	0.3
DVD162	VEGF	PGE2	
AB020	NGF		28
AB048	PGE2		19.4
DVD163	PGE2	NGF	0.2
DVD164	NGF	PGE2	1.2
AB029	IL-17A		66.8
AB048	PGE2		19.4
DVD155	PGE2	IL-17A	26.8
DVD156	IL-17A	PGE2	20.8
AB032	IL-1B		27
AB048	PGE2		19.4
DVD157	PGE2	IL-1B	0.6
DVD158	IL-1B	PGE2	1.2
AB040	IL-6		25.8
AB048	PGE2		19.4
DVD249	PGE2	IL-6	
DVD250	IL-6	PGE2	
AB043	Abeta (seq1)		
AB048	PGE2		19.4
DVD227	Abeta (seq1)	PGE2	5.2
DVD228	PGE2	Abeta (seq1)	10.2
AB044	Abeta (seq2)		48.8
AB048	PGE2		19.4
DVD199	Abeta (seq2)	PGE2	
DVD200	PGE2	Abeta (seq2)	
AB045	Abeta (seq3)		
AB048	PGE2		19.4
DVD213	Abeta (seq3)	PGE2	
DVD214	PGE2	Abeta (seq3)	
AB046	IL-18		
AB048	PGE2		19.4
DVD241	IL-18	PGE2	
DVD242	PGE2	IL-18	
AB049	IL-15		
AB048	PGE2		19.4
DVD153	PGE2	IL-15	17.4
DVD154	IL-15	PGE2	14
AB052	S1P		
AB048	PGE2		19.4
DVD251	PGE2	S1P	

Parent Antibody or DVD-Ig ID	N-terminal Variable Domain (VD)	C-terminal Variable Domain (VD)	Expression Yield (mg/L)
DVD252	S1P	PGE2	
AB054	IL-6R		103.6
AB048	PGE2		19.4
DVD151	PGE2	IL-6R	20.2
DVD152	IL-6R	PGE2	12.6
AB033	EGFR (SEQ2)		44.4
AB048	PGE2		19.4
DVD311	PGE2	EGFR (SEQ2)	
DVD312	EGFR (SEQ2)	PGE2	
AB003	EGFR (SEQ1)		
AB048	PGE2		19.4
DVD313	PGE2	EGFR (SEQ1)	
DVD314	EGFR (SEQ1)	PGE2	
AB011	IGF1R		28.5
AB048	PGE2		19.4
DVD315	PGE2	IGF1R	
DVD316	IGF1R	PGE2	
AB010	IGF1,2		38.6
AB048	PGE2		19.4
DVD317	PGE2	IGF1,2	
DVD318	IGF1,2	PGE2	
AB004	HER2		108.2
AB048	PGE2		19.4
DVD319	PGE2	HER2	
DVD320	HER2	PGE2	

All DVDs expressed well in 293 cells. DVDs could be easily purified over a protein A column. In most cases >5 mg/L purified DVD-Ig could be obtained easily from supernatants of 293 cells.

Example 5.5.10: <u>Characterization and lead selection of A/B DVD-Igs</u>

The binding affinities of anti-A/B DVD-Igs are analyzed on Biacore against both protein A and protein B. The tetravalent property of the DVD-Ig is examined by multiple binding studies on Biacore. Meanwhile, the neutralization potency of the DVD-Igs for protein A and protein B are assessed by bioassays, respectively, as described herein. The DVD-Ig molecules that best retain the affinity and potency of the original parent mAbs are selected for in-depth physicochemical and bio-analytical (rat PK) characterizations as described herein for each mAb. Based on the collection of analyses, the final lead DVD-Ig is advanced into CHO stable cell line development, and the CHO-derived material is employed in stability, pharmacokinetic and efficacy studies in cynomolgus monkey, and preformulation activities.

The present invention incorporates by reference in their entirety techniques well known in the field of molecular biology and drug delivery. These techniques include, but are not limited to, techniques described in the following publications:

Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley &Sons, NY (1993);

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Incorporation by Reference

The contents of all cited references (including literature references, patents, patent applications, databases, and websites) that maybe cited throughout this application are hereby expressly incorporated by reference in their entirety for any purpose, as are the references cited therein. The practice of the present invention will employ, unless otherwise indicated, conventional techniques of immunology, molecular biology and cell biology, which are well known in the art.

Equivalents

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting of the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are therefore intended to be embraced herein.

We claim:

1. A binding protein comprising a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein;

VD1 is a first heavy chain variable domain;

VD2 is a second heavy chain variable domain;

C is a heavy chain constant domain;

X1 is a linker with the proviso that it is not CH1;

X2 is an Fc region; and

n is 0 or 1;

wherein the binding protein is capable of binding a pair of antigens selected from the group consisting of TNF and PGE2, NGF and PGE2, IL-17A and PGE2, IL-1b and PGE2, IL-6 and PGE2, IL-6R and PGE2, VEGF and PGE2, Abeta (seq. 1) and PGE2, Abeta (seq. 2) and PGE2, Abeta (seq. 3) and PGE2, IL-18 and PGE2, PGE2 and PGE2, IL-15 and PGE2, S1P and PGE2, EGFR (seq. 1) and PGE2, EGFR (seq. 2) and PGE2, and IGFR and PGE2.

2. The binding protein according to claim 1, wherein VD1 and VD2 comprise an amino acid sequence selected from the group consisting of SEQ ID NOs: 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 87, 90, 110, and 112.

3. A binding protein comprising a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein;

VD1 is a first light heavy chain variable domain;

VD2 is a second light heavy chain variable domain;

C is a light chain constant domain;

X1 is a linker with the proviso that it is not CH1;

X2 does not comprise an Fc region; and

n is 0 or 1;

wherein the binding protein is capable of binding a pair of antigens selected from the group consisting of TNF and PGE2, NGF and PGE2, IL-17A and PGE2, IL-1b and PGE2, IL-6 and PGE2, IL-6R and PGE2, VEGF and PGE2, Abeta (seq. 1) and PGE2, Abeta (seq. 2) and PGE2, Abeta (seq. 3) and PGE2, IL-18 and PGE2, PGE2 and PGE2, IL-15 and PGE2, S1P and PGE2, EGFR (seq. 1) and PGE2, EGFR (seq. 2) and PGE2, and IGFR and PGE2.

4. The binding protein according to claim 3, wherein the VD1 and VD2 light chain variable domains comprise an amino acid sequence selected from the group consisting of SEQ ID NOs: 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 88, 91, 111, and 113.

5. The binding protein according to claim 1 or 3, wherein n is 0.

6. A binding protein comprising first and second polypeptide chains, wherein said first polypeptide chain comprises a first VD1-(X1)n-VD2-C-(X2)n, wherein

VD1 is a first heavy chain variable domain;

VD2 is a second heavy chain variable domain;

C is a heavy chain constant domain;

X1 is a linker with the proviso that it is not CH1; and

X2 is an Fc region; and

wherein said second polypeptide chain comprises a second VD1-(X1)n-VD2-C-(X2)n, wherein

VD1 is a first light chain variable domain;

VD2 is a second light chain variable domain;

C is a light chain constant domain;

X1 is a linker with the proviso that it is not CH1;

X2 does not comprise an Fc region; and

n is 0 or 1, wherein the binding protein is capable of binding a pair of antigens selected from the group consisting of TNF and PGE2, NGF and PGE2, IL-17A and PGE2, IL-1b and PGE2, IL-6 and PGE2, IL-6R and PGE2, VEGF and PGE2, Abeta (seq. 1) and PGE2, Abeta (seq. 2) and PGE2, Abeta (seq. 3) and PGE2, IL-18 and PGE2, PGE2 and PGE2, IL-15 and PGE2, S1P and PGE2, EGFR (seq. 1) and PGE2, EGFR (seq. 2) and PGE2, and IGFR and PGE2.

7. The binding protein according to claim 6, wherein the VD1 and VD2 heavy chain variable domains comprise an amino acid sequence selected from the group consisting of SEQ ID NOs: 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 87, 90, 110, and 112 and wherein the VD1 and VD2 light chain variable domains comprise an amino acid sequence selected from the group consisting of SEQ ID NOs: 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 88, 91, 111, and 113.

8. The binding protein according to claim 1, 3, or 6, wherein X1 or X2 is an amino acid sequence selected from the group consisting of SEQ ID NOs 1-26.

9. The binding protein according to claim 6, wherein the binding protein comprises two first polypeptide chains and two second polypeptide chains.

10. The binding protein according to claim 1, 3, or 6, wherein the Fc region is selected from the group consisting of native sequence Fc region and a variant sequence Fc region.

11. The binding protein according to claim 10, wherein the Fc region is selected from the group consisting of an Fc region from an IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgE, and IgD.

12. The binding protein according to claim 1, 3, or 6, wherein said VD1 of the first polypeptide chain and said VD1 of the second polypeptide chain are obtained from the same first and second parent antibody, respectively, or antigen binding portion thereof.

13. The binding protein according to claim 1, 3, or 6, wherein said VD1 of the first polypeptide chain and said VD1 of the second polypeptide chain are obtained from a different first and second parent antibody, respectively, or antigen binding portion thereof.

14. The binding protein according to claim 1, 3, or 6, wherein said VD2 of the first polypeptide chain and said VD2 of the second polypeptide chain are obtained from the same first and second parent antibody, respectively, or antigen binding portion thereof.

15. The binding protein according to claim 1, 3, or 6, wherein said VD2 of the first polypeptide chain and said VD2 of the second polypeptide chain are obtained from different first and second parent antibody, respectively, or antigen binding portion thereof.

16. The binding protein according to any one of claims 13-15, wherein said first and said second parent antibodies bind different epitopes on said antigen.

17. The binding protein according to any one of claims 13-15, wherein said first parent antibody or antigen binding portion thereof, binds said first antigen with a potency different from the potency with which said second parent antibody or antigen binding portion thereof, binds said second antigen.

18. The binding protein according to any one of claims 13-15, wherein said first parent antibody or antigen binding portion thereof, binds said first antigen with an affinity different from the affinity with which said second parent antibody or antigen binding portion thereof, binds said second antigen.

19. The binding protein according to any one of claims 13-15, wherein said first parent antibody or antigen binding portion thereof, and said second parent antibody or antigen binding portion thereof, are selected from the group consisting of a human antibody, a CDR grafted antibody, and a humanized antibody.

20. The binding protein according to any one of claims 13-15, wherein said first parent antibody or antigen binding portion thereof, and said second parent antibody or antigen binding portion thereof, are selected from the group consisting of a Fab fragment; a $F(ab')_2$ fragment; a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the VH and CH1 domains; a Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a dAb fragment; an isolated complementarity determining region (CDR); a single chain antibody; and a diabody.

21. The binding protein according to claim 1, 3, or 6, wherein said binding protein possesses at least one desired property exhibited by said first parent antibody or antigen binding portion thereof, or said second parent antibody or antigen binding portion thereof.

22. The binding protein according to claim 2, wherein said desired property is selected from one or more antibody parameters.

23. The binding protein according to claim 21, wherein said antibody parameters are selected from the group consisting of antigen specificity, affinity to antigen, potency, biological function, epitope recognition, stability, solubility, production efficiency, immunogenicity, pharmacokinetics, bioavailability, tissue cross reactivity, and orthologous antigen binding.

24. A binding protein capable of binding two antigens comprising four polypeptide chains, wherein two polypeptide chains comprise VD1-(X1)n-VD2-C-(X2)n, wherein

VD1 is a first heavy chain variable domain;
VD2 is a second heavy chain variable domain;
C is a heavy chain constant domain;
X1 is a linker with the proviso that it is not CH1; and
X2 is an Fc region; and
wherein two polypeptide chains comprise VD1-(X1)n-VD2-C-(X2)n, wherein
VD1 is a first light chain variable domain;
VD2 is a second light chain variable domain;

C is a light chain constant domain;

X1 is a linker with the proviso that it is not CH1;

X2 does not comprise an Fc region; and

n is 0 or 1; wherein the VD1 and VD2 heavy chain variable domains comprise an amino acid sequence selected from the group consisting of SEQ ID NOs: 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 87, 90, 110, and 112 and wherein the VD1 and VD2 light chain variable domains comprise an amino acid sequence selected from the group consisting of SEQ ID NOs: 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 88, 91, 111, and 113.

25. A binding protein capable of binding two antigens comprising four polypeptide chains, wherein two polypeptide chains comprise VD1-(X1)n-VD2-C-(X2)n, wherein

VD1 is a first heavy chain variable domain;

VD2 is a second heavy chain variable domain;

C is a heavy chain constant domain;

X1 is a linker with the proviso that it is not CH1;

X2 is an Fc region; and

n is 0 or 1; and

wherein two polypeptide chains comprise VD1-(X1)n-VD2-C-(X2)n, wherein

VD1 is a first light chain variable domain;

VD2 is a second light chain variable domain;

C is a light chain constant domain;

X1 is a linker with the proviso that it is not CH1;

X2 does not comprise an Fc region; and

n is 0 or 1;

wherein the DVD-Ig binds at least one antigen selected from the group consisting of Prostaglandin E2 (PGE2), Amyloid beta (Abeta) (seq. 1), Abeta (seq. 2), Abeta (seq. 3), Tumor Necrosis Factor alpha (TNF-α), interleukin 1β (IL-1β), Insulin-like Growth Factor Receptor (IGFR), Interleukin 17A (IL-17A), Interleukin 6 (IL-6), Interleukin 6 receptor (IL-6R), Interleukin 15 (IL-15), Interleukin 18 (IL-18), Nerve

Growth Factor (NGF), Epidermal Growth Factor Receptor (EGFR) (seq. 1), EGFR (seq. 2), vascular endothelial growth factor (VEGF), and S1P.

26. The binding protein according to claim 1, 3, 6, 24, or 25, wherein said binding protein has an on rate constant (Kon) to said one or more targets selected from the group consisting of: at least about $10^2 M^{-1}s^{-1}$; at least about $10^3 M^{-1}s^{-1}$; at least about $10^4 M^{-1}s^{-1}$; at least about $10^5 M^{-1}s^{-1}$; and at least about $10^6 M^{-1}s^{-1}$, as measured by surface plasmon resonance.

27. The binding protein according to claim 1, 3, 6, 24, or 25, wherein said binding protein has an off rate constant (Koff) to said one or more targets selected from the group consisting of: at most about $10^{-3}s^{-1}$; at most about $10^{-4}s^{-1}$; at most about $10^{-5}s^{-1}$; and at most about $10^{-6}s^{-1}$, as measured by surface plasmon resonance.

28. The binding protein according to claim 1, 3, 6, 24, or 25, wherein said binding protein has a dissociation constant (K_D) to said one or more targets selected from the group consisting of: at most about 10^{-7} M; at most about 10^{-8} M; at most about 10^{-9} M; at most about 10^{-10} M; at most about 10^{-11} M; at most about 10^{-12} M; and at most 10^{-13} M.

29. A binding protein conjugate comprising a binding protein according to any one of claims 1, 3, 6, 24, or 25, said binding protein conjugate further comprising an agent selected from the group consisting of; an immunoadhesion molecule, an imaging agent, a therapeutic agent, and a cytotoxic agent.

30. The binding protein conjugate according to claim 29, wherein said agent is an imaging agent selected from the group consisting of a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, and biotin.

31. The binding protein conjugate according to claim 30, wherein said imaging agent is a radiolabel selected from the group consisting of: ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, and ^{153}Sm.

32. The binding protein conjugate according to claim 30, wherein said agent is a therapeutic or cytotoxic agent selected from the group consisting of; an anti-metabolite, an alkylating agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, toxin, and an apoptotic agent.

33. The binding protein according to claim 1, 3, 6, 24, or 25, wherein said binding protein is a crystallized binding protein.

34. The binding protein according to claim 33, wherein said crystal is a carrier-free pharmaceutical controlled release crystal.

35. The binding protein according to claim 33, wherein said binding protein has a greater half life in vivo than the soluble counterpart of said binding protein.

36. The binding protein according to claim 33, wherein said binding protein retains biological activity.

37. An isolated nucleic acid encoding a binding protein amino acid sequence according to any one of claims 1, 3, 6, 24, or 25.

38. A vector comprising an isolated nucleic acid according to claim 37.

39. The vector according to claim 38, wherein said vector is selected from the group consisting of pcDNA, pTT, pTT3, pEFBOS, pBV, pJV, pcDNA3.1 TOPO, pEF6 TOPO, and pBJ.

40. A host cell comprising a vector according to claim 38.

41. The host cell according to claim 40, wherein said host cell is a prokaryotic cell.

42. The host cell according to claim 41, wherein said host cell is E.Coli.

43. The host cell according to claim 40, wherein said host cell is a eukaryotic cell.

44. The host cell according to claim 43, wherein said eukaryotic cell is selected from the group consisting of protist cell, animal cell, plant cell and fungal cell.

45. The host cell according to claim 43, wherein said eukaryotic cell is an animal cell selected from the group consisting of; a mammalian cell, an avian cell, and an insect cell.

46. The host cell according to claim 45, wherein said host cell is a CHO cell.

47. The host cell according to claim 45, wherein said host cell is COS.

48. The host cell according to claim 43, wherein said host cell is a yeast cell.

49. The host cell according to claim 48, wherein said yeast cell is *Saccharomyces cerevisiae*.

50. The host cell according to claim 45, wherein said host cell is an insect Sf9 cell.

51. A method of producing a binding protein, comprising culturing a host cell described in any one of claims 40-50 in culture medium under conditions sufficient to produce the binding protein

52. The method according to claim 51, wherein 50%-75% of the binding protein produced is a dual specific tetravalent binding protein.

53. The method according to claim 51, wherein 75%-90% of the binding protein produced is a dual specific tetravalent binding protein.

54. The method according to claim 51, wherein 90%-95% of the binding protein produced is a dual specific tetravalent binding protein.

55. A protein produced according to the method of claim 51.

56. A pharmaceutical composition comprising the binding protein of any one of claims 1-36 and 55, and a pharmaceutically acceptable carrier.

57. The pharmaceutical composition of claim 56 further comprising at least one additional therapeutic agent.

58. The pharmaceutical composition of claim 57, wherein said additional therapeutic agent is selected from the group consisting of: Therapeutic agent, imaging agent, cytotoxic agent, angiogenesis inhibitors; kinase inhibitors; co-stimulation molecule blockers; adhesion molecule blockers; anti-cytokine antibody or functional fragment thereof; methotrexate; cyclosporin; rapamycin; FK506; detectable label or reporter; a TNF antagonist; an antirheumatic; a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteriod, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, and a cytokine antagonist.

59. A method for treating a subject for a disease or a disorder by administering to the subject the binding protein of any one of claims 1-36 and 55 such that treatment is achieved.

60. The method of claim 59, wherein said disorder is selected from the group comprising rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, septic arthritis, Lyme arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, inflammatory bowel disease, insulin dependent diabetes mellitus, thyroiditis, asthma, allergic diseases, psoriasis, dermatitis scleroderma, graft versus host disease, organ transplant rejection, acute or

chronic immune disease associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, Wegener's granulomatosis, Henoch-Schoenlein purpurea, microscopic vasculitis of the kidneys, chronic active hepatitis, uveitis, septic shock, toxic shock syndrome, sepsis syndrome, cachexia, infectious diseases, parasitic diseases, acquired immunodeficiency syndrome, acute transverse myelitis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary cirrhosis, hemolytic anemia, malignancies, heart failure, myocardial infarction, Addison's disease, sporadic, polyglandular deficiency type I and polyglandular deficiency type II, Schmidt's syndrome, adult (acute) respiratory distress syndrome, alopecia, alopecia areata, seronegative arthopathy, arthropathy, Reiter's disease, psoriatic arthropathy, ulcerative colitic arthropathy, enteropathic synovitis, chlamydia, yersinia and salmonella associated arthropathy, spondyloarthopathy, atheromatous disease/arteriosclerosis, atopic allergy, autoimmune bullous disease, pemphigus vulgaris, pemphigus foliaceus, pemphigoid, linear IgA disease, autoimmune haemolytic anaemia, Coombs positive haemolytic anaemia, acquired pernicious anaemia, juvenile pernicious anaemia, myalgic encephalitis/Royal Free Disease, chronic mucocutaneous candidiasis, giant cell arteritis, primary sclerosing hepatitis, cryptogenic autoimmune hepatitis, Acquired Immunodeficiency Disease Syndrome, Acquired Immunodeficiency Related Diseases, Hepatitis B, Hepatitis C, common varied immunodeficiency (common variable hypogammaglobulinaemia), dilated cardiomyopathy, female infertility, ovarian failure, premature ovarian failure, fibrotic lung disease, cryptogenic fibrosing alveolitis, post-inflammatory interstitial lung disease, interstitial pneumonitis, connective tissue disease associated interstitial lung disease, mixed connective tissue disease associated lung disease, systemic sclerosis associated interstitial lung disease, rheumatoid arthritis associated interstitial lung disease, systemic lupus erythematosus associated lung disease, dermatomyositis/polymyositis associated lung disease, Sjögren's disease associated lung disease, ankylosing spondylitis associated lung disease, vasculitic diffuse lung disease, haemosiderosis associated lung disease, drug-induced interstitial lung disease, fibrosis, radiation fibrosis, bronchiolitis obliterans, chronic eosinophilic pneumonia, lymphocytic infiltrative lung disease, postinfectious interstitial lung disease, gouty arthritis, autoimmune hepatitis, type-1 autoimmune

hepatitis (classical autoimmune or lupoid hepatitis), type-2 autoimmune hepatitis (anti-LKM antibody hepatitis), autoimmune mediated hypoglycaemia, type B insulin resistance with acanthosis nigricans, hypoparathyroidism, acute immune disease associated with organ transplantation, chronic immune disease associated with organ transplantation, osteoarthrosis, primary sclerosing cholangitis, psoriasis type 1, psoriasis type 2, idiopathic leucopaenia, autoimmune neutropaenia, renal disease NOS, glomerulonephritides, microscopic vasulitis of the kidneys, lyme disease, discoid lupus erythematosus, male infertility idiopathic or NOS, sperm autoimmunity, multiple sclerosis (all subtypes), sympathetic ophthalmia, pulmonary hypertension secondary to connective tissue disease, Goodpasture's syndrome, pulmonary manifestation of polyarteritis nodosa, acute rheumatic fever, rheumatoid spondylitis, Still's disease, systemic sclerosis, Sjörgren's syndrome, Takayasu's disease/arteritis, autoimmune thrombocytopaenia, idiopathic thrombocytopaenia, autoimmune thyroid disease, hyperthyroidism, goitrous autoimmune hypothyroidism (Hashimoto's disease), atrophic autoimmune hypothyroidism, primary myxoedema, phacogenic uveitis, primary vasculitis, vitiligo acute liver disease, chronic liver diseases, alcoholic cirrhosis, alcohol-induced liver injury, choleosatatis, idiosyncratic liver disease, Drug-Induced hepatitis, Non-alcoholic Steatohepatitis, allergy and asthma, group B streptococci (GBS) infection, mental disorders (*e.g.*, depression and schizophrenia), Th2 Type and Th1 Type mediated diseases, acute and chronic pain (different forms of pain), and cancers such as lung, breast, stomach, bladder, colon, pancreas, ovarian, prostate and rectal cancer and hematopoietic malignancies (leukemia and lymphoma) Abetalipoprotemia, Acrocyanosis, acute and chronic parasitic or infectious processes, acute leukemia, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), acute or chronic bacterial infection, acute pancreatitis, acute renal failure, adenocarcinomas, aerial ectopic beats, AIDS dementia complex, alcohol-induced hepatitis, allergic conjunctivitis, allergic contact dermatitis, allergic rhinitis, allograft rejection, alpha-l- antitrypsin deficiency, amyotrophic lateral sclerosis, anemia, angina pectoris, anterior horn cell degeneration, anti cd3 therapy, antiphospholipid syndrome, anti-receptor hypersensitivity reactions, aordic and peripheral aneuryisms, aortic dissection, arterial hypertension, arteriosclerosis, arteriovenous fistula, ataxia, atrial fibrillation (sustained or paroxysmal), atrial flutter, atrioventricular block, B cell lymphoma, bone graft

rejection, bone marrow transplant (BMT) rejection, bundle branch block, Burkitt's lymphoma, Burns, cardiac arrhythmias, cardiac stun syndrome, cardiac tumors, cardiomyopathy, cardiopulmonary bypass inflammation response, cartilage transplant rejection, cerebellar cortical degenerations, cerebellar disorders, chaotic or multifocal atrial tachycardia, chemotherapy associated disorders, chromic myelocytic leukemia (CML), chronic alcoholism, chronic inflammatory pathologies, chronic lymphocytic leukemia (CLL), chronic obstructive pulmonary disease (COPD), chronic salicylate intoxication, colorectal carcinoma, congestive heart failure, conjunctivitis, contact dermatitis, cor pulmonale, coronary artery disease, Creutzfeldt-Jakob disease, culture negative sepsis, cystic fibrosis, cytokine therapy associated disorders, Dementia pugilistica, demyelinating diseases, dengue hemorrhagic fever, dermatitis, dermatologic conditions, diabetes, diabetes mellitus, diabetic ateriosclerotic disease, Diffuse Lewy body disease, dilated congestive cardiomyopathy, disorders of the basal ganglia, Down's Syndrome in middle age, drug- induced movement disorders induced by drugs which block CNS dopamine receptors, drug sensitivity, eczema, encephalomyelitis, endocarditis, endocrinopathy, epiglottitis, epstein-barr virus infection, erythromelalgia, extrapyramidal and cerebellar disorders, familial hematophagocytic lymphohistiocytosis, fetal thymus implant rejection, Friedreich's ataxia, functional peripheral arterial disorders, fungal sepsis, gas gangrene, gastric ulcer, glomerular nephritis, graft rejection of any organ or tissue, gram negative sepsis, gram positive sepsis, granulomas due to intracellular organisms, hairy cell leukemia, Hallervorden-Spatz disease, hashimoto's thyroiditis, hay fever, heart transplant rejection, hemachromatosis, hemodialysis, hemolytic uremic syndrome/thrombolytic thrombocytopenic purpura, hemorrhage, hepatitis (A), His bundle arrythmias, HIV infection/HIV neuropathy, Hodgkin's disease, hyperkinetic movement disorders, hypersensitity reactions, hypersensitivity pneumonitis, hypertension, hypokinetic movement disorders, hypothalamic-pituitary-adrenal axis evaluation, idiopathic Addison's disease, idiopathic pulmonary fibrosis, antibody mediated cytotoxicity, Asthenia, infantile spinal muscular atrophy, inflammation of the aorta, influenza a, ionizing radiation exposure, iridocyclitis/uveitis/optic neuritis, ischemia- reperfusion injury, ischemic stroke, juvenile rheumatoid arthritis, juvenile spinal muscular atrophy, Kaposi's sarcoma, kidney transplant rejection, legionella, leishmaniasis, leprosy, lesions of the corticospinal system, lipedema, liver transplant

rejection, lymphederma, malaria, malignamt Lymphoma, malignant histiocytosis, malignant melanoma, meningitis, meningococcemia, metabolic/idiopathic, migraine headache, mitochondrial multi.system disorder, mixed connective tissue disease, monoclonal gammopathy, multiple myeloma, multiple systems degenerations (Mencel Dejerine- Thomas Shi-Drager and Machado-Joseph), myasthenia gravis, mycobacterium avium intracellulare, mycobacterium tuberculosis, myelodyplastic syndrome, myocardial infarction, myocardial ischemic disorders, nasopharyngeal carcinoma, neonatal chronic lung disease, nephritis, nephrosis, neurodegenerative diseases, neurogenic I muscular atrophies , neutropenic fever, non- hodgkins lymphoma, occlusion of the abdominal aorta and its branches, occulsive arterial disorders, okt3 therapy, orchitis/epidydimitis, orchitis/vasectomy reversal procedures, organomegaly, osteoporosis, pancreas transplant rejection, pancreatic carcinoma, paraneoplastic syndrome/hypercalcemia of malignancy, parathyroid transplant rejection, pelvic inflammatory disease, perennial rhinitis, pericardial disease, peripheral atherlosclerotic disease, peripheral vascular disorders, peritonitis, pernicious anemia, pneumocystis carinii pneumonia, pneumonia, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes syndrome), post perfusion syndrome, post pump syndrome, post-MI cardiotomy syndrome, preeclampsia, Progressive supranucleo Palsy, primary pulmonary hypertension, radiation therapy, Raynaud's phenomenon and disease, Raynoud's disease, Refsum's disease, regular narrow QRS tachycardia, renovascular hypertension, reperfusion injury, restrictive cardiomyopathy, sarcomas, scleroderma, senile chorea, Senile Dementia of Lewy body type, seronegative arthropathies, shock, sickle cell anemia, skin allograft rejection, skin changes syndrome, small bowel transplant rejection, solid tumors, specific arrythmias, spinal ataxia, spinocerebellar degenerations, streptococcal myositis, structural lesions of the cerebellum, Subacute sclerosing panencephalitis, Syncope, syphilis of the cardiovascular system, systemic anaphalaxis, systemic inflammatory response syndrome, systemic onset juvenile rheumatoid arthritis, T-cell or FAB ALL, Telangiectasia, thromboangitis obliterans, thrombocytopenia, toxicity, transplants, trauma/hemorrhage, type III hypersensitivity reactions, type IV hypersensitivity, unstable angina, uremia, urosepsis, urticaria, valvular heart diseases, varicose veins, ,vasculitis, venous diseases, venous thrombosis, ventricular fibrillation, viral and fungal infections, vital

encephalitis/aseptic meningitis, vital-associated hemaphagocytic syndrome, Wernicke- Korsakoff syndrome, Wilson's disease, xenograft rejection of any organ or tissue, acute coronary syndromes, acute idiopathic polyneuritis, acute inflammatory demyelinating polyradiculoneuropathy, acute ischemia, adult Still's disease, alopecia areata, anaphylaxis, anti-phospholipid antibody syndrome, aplastic anemia, arteriosclerosis, atopic eczema, atopic dermatitis, autoimmune dermatitis, autoimmune disorder associated with streptococcus infection, autoimmune enteropathy, autoimmune hearing loss, autoimmune lymphoproliferative syndrome (ALPS), autoimmune myocarditis, autoimmune premature ovarian failure, blepharitis, bronchiectasis, bullous pemphigoid, cardiovascular disease, catastrophic antiphospholipid syndrome, celiac disease, cervical spondylosis, chronic ischemia, cicatricial pemphigoid, clinically isolated syndrome (cis) with risk for multiple sclerosis, conjunctivitis, childhood onset psychiatric disorder, chronic obstructive pulmonary disease (COPD), dacryocystitis, dermatomyositis, diabetic retinopathy, diabetes mellitus, disk herniation, disk prolaps, drug induced immune hemolytic anemia, endocarditis, endometriosis, endophthalmitis, episcleritis, erythema multiforme, erythema multiforme major, gestational pemphigoid, Guillain-Barré syndrome (GBS), hay fever, Hughes syndrome, idiopathic Parkinson's disease, idiopathic interstitial pneumonia, IgE-mediated allergy, immune hemolytic anemia, inclusion body myositis, infectious ocular inflammatory disease, inflammatory demyelinating disease, inflammatory heart disease, inflammatory kidney disease, IPF/UIP, iritis, keratitis, keratojuntivitis sicca, Kussmaul disease or Kussmaul-Meier disease, Landry's paralysis, Langerhan's cell histiocytosis, livedo reticularis, macular degeneration, microscopic polyangiitis, morbus bechterev, motor neuron disorders, mucous membrane pemphigoid, multiple organ failure, myasthenia gravis, myelodysplastic syndrome, myocarditis, nerve root disorders, neuropathy, non-A non-B hepatitis, optic neuritis, osteolysis, ovarian cancer, pauciarticular JRA, peripheral artery occlusive disease (PAOD), peripheral vascular disease (PVD), peripheral artery, disease (PAD), phlebitis, polyarteritis nodosa (or periarteritis nodosa), polychondritis, polymyalgia rheumatica, poliosis, polyarticular JRA, polyendocrine deficiency syndrome, polymyositis, polymyalgia rheumatica (PMR), post-pump syndrome, primary Parkinsonism, prostate and rectal cancer and hematopoietic malignancies (leukemia and lymphoma), prostatitis, pure red cell aplasia, primary

adrenal insufficiency, recurrent neuromyelitis optica, restenosis, rheumatic heart disease, sapho (synovitis, acne, pustulosis, hyperostosis, and osteitis), scleroderma, secondary amyloidosis, shock lung, scleritis, sciatica, secondary adrenal insufficiency, silicone associated connective tissue disease, sneddon-wilkinson dermatosis, spondilitis ankylosans, Stevens-Johnson syndrome (SJS), systemic inflammatory response syndrome, temporal arteritis, toxoplasmic retinitis, toxic epidermal necrolysis, transverse myelitis, TRAPS (tumor necrosis factor receptor, type I allergic reaction, type II diabetes, urticaria, usual interstitial pneumonia (UIP), vasculitis, vernal conjunctivitis, viral retinitis, Vogt-Koyanagi-Harada syndrome (VKH syndrome), wet macular degeneration, wound healing, yersinia and salmonella associated arthropathy.

61. The method according to claim 60, wherein said administering to the subject is by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, and transdermal.

62. A method for generating a Dual Variable Domain Immunoglobulin capable of binding two antigens comprising the steps of

a) obtaining a first parent antibody or antigen binding portion thereof, capable of binding a first antigen;

b) obtaining a second parent antibody or antigen binding portion thereof, capable of binding a second antigen;

c) constructing first and third polypeptide chains comprising VD1-(X1)n-VD2-C-(X2)n, wherein

VD1 is a first heavy chain variable domain obtained from said first parent antibody or antigen binding portion thereof;

VD2 is a second heavy chain variable domain obtained from said second parent antibody or antigen binding portion thereof;
C is a heavy chain constant domain;
X1 is a linker with the proviso that it is not CH1;
X2 is an Fc region; and
n is 0 or 1; and
d) constructing second and fourth polypeptide chains comprising VD1-(X1)n-VD2-C-(X2)n, wherein
VD1 is a first light chain variable domain obtained from said first parent antibody or antigen binding portion thereof;
VD2 is a second light chain variable domain obtained from said second parent antibody or antigen binding thereof;
C is a light chain constant domain;
X1 is a linker with the proviso that it is not CH1;
X2 does not comprise an Fc region; and
n is 0 or 1; and
e) expressing said first, second, third and fourth polypeptide chains;

such that a Dual Variable Domain Immunoglobulin capable of binding said first and said second antigen is generated, wherein the binding protein is capable of binding a pair of antigens selected from the group consisting of TNF and PGE2, NGF and PGE2, IL-17A and PGE2, IL-1b and PGE2, IL-6 and PGE2, IL-6R and PGE2, VEGF and PGE2, Abeta (seq. 1) and PGE2, Abeta (seq. 2) and PGE2, Abeta (seq. 3) and PGE2, IL-18 and PGE2, PGE2 and PGE2, IL-15 and PGE2, S1P and PGE2, EGFR (seq. 1) and PGE2, EGFR (seq. 2) and PGE2, and IGFR and PGE2.

63. The method of claim 62, wherein the VD1 and VD2 heavy chain variable domains comprise an amino acid sequence selected from the group consisting of SEQ ID NOs: 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 87, 90, 110, and 112 and wherein the VD1 and VD2 light chain variable domains comprise an amino acid sequence selected from the group consisting of SEQ ID NOs: 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 88, 91, 111, and 113.

64. The method of claim 62, wherein said first parent antibody or antigen binding portion thereof, and said second parent antibody or antigen binding portion thereof, are selected from the group consisting of a human antibody, a CDR grafted antibody, and a humanized antibody.

65. The method of claim 62, wherein said first parent antibody or antigen binding portion thereof, and said second parent antibody or antigen binding portion thereof, are selected from the group consisting of a Fab fragment, a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the VH and CH1 domains; a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, a dAb fragment, an isolated complementarity determining region (CDR), a single chain antibody, and diabodies.

66. The method of claim 62, wherein said first parent antibody or antigen binding portion thereof possesses at least one desired property exhibited by the Dual Variable Domain Immunoglobulin.

67. The method of claim 62, wherein said second parent antibody or antigen binding portion thereof possesses at least one desired property exhibited by the Dual Variable Domain Immunoglobulin.

68. The method of claim 62, wherein the Fc region is selected from the group consisting of a native sequence Fc region and a variant sequence Fc region.

69. The method of claim 62, wherein the Fc region is selected from the group consisting of an Fc region from an IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgE, and IgD.

70. The method of claim 66, wherein said desired property is selected from one or more antibody parameters.

71. The method of claim 67, wherein said desired property is selected from one or more antibody parameters.

72. The method of claim 70, wherein said antibody parameters are selected from the group consisting of antigen specificity, affinity to antigen, potency, biological

function, epitope recognition, stability, solubility, production efficiency, immunogenicity, pharmacokinetics, bioavailability, tissue cross reactivity, and orthologous antigen binding.

73. The method of claim 71, wherein said antibody parameters are selected from the group consisting of antigen specificity, affinity to antigen, potency, biological function, epitope recognition, stability, solubility, production efficiency, immunogenicity, pharmacokinetics, bioavailability, tissue cross reactivity, and orthologous antigen binding.

74. The method of claim 62, wherein said first parent antibody or antigen binding portion thereof, binds said first antigen with a different affinity than the affinity with which said second parent antibody or antigen binding portion thereof, binds said second antigen.

75. The method of claim 62, wherein said first parent antibody or antigen binding portion thereof, binds said first antigen with a different potency than the potency with which said second parent antibody or antigen binding portion thereof, binds said second antigen.

76. A method for generating a Dual Variable Domain Immunoglobulin capable of binding two antigens with desired properties comprising the steps of

a) obtaining a first parent antibody or antigen binding portion thereof, capable of binding a first antigen and possessing at least one desired property exhibited by the Dual Variable Domain Immunoglobulin;

b) obtaining a second parent antibody or antigen binding portion thereof, capable of binding a second antigen and possessing at least one desired property exhibited by the Dual Variable Domain Immunoglobulin;

c) constructing first and third polypeptide chains comprising VD1-(X1)n-VD2-C-(X2)n, wherein;

VD1 is a first heavy chain variable domain obtained from said first parent antibody or antigen binding portion thereof;

VD2 is a second heavy chain variable domain obtained from said second parent antibody or antigen binding portion thereof;

C is a heavy chain constant domain;

X1 is a linker with the proviso that it is not CH1;

X2 is an Fc region; and

n is 0 or 1;

d) constructing second and fourth polypeptide chains comprising VD1-(X1)n-VD2-C-(X2)n, wherein;

VD1 is a first light chain variable domain obtained from said first parent antibody or antigen binding portion thereof;

VD2 is a second light chain variable domain obtained from said second parent antibody or antigen binding portion thereof;

C is a light chain constant domain;

X1 is a linker with the proviso that it is not CH1;

X2 does not comprise an Fc region; and

n is 0 or 1;

e) expressing said first, second, third and fourth polypeptide chains;

such that a Dual Variable Domain Immunoglobulin capable of binding said first and said second antigen with desired properties is generated, wherein the binding protein is capable of binding a pair of antigens selected from the group consisting of TNF and PGE2, NGF and PGE2, IL-17A and PGE2, IL-1b and PGE2, IL-6 and PGE2, IL-6R and PGE2, VEGF and PGE2, Abeta (seq. 1) and PGE2, Abeta (seq. 2) and PGE2, Abeta (seq. 3) and PGE2, IL-18 and PGE2, PGE2 and PGE2, IL-15 and PGE2, S1P and PGE2, EGFR (seq. 1) and PGE2, EGFR (seq. 2) and PGE2, and IGFR and PGE2.

SEQUENCE LISTING

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<141> 2009-07-08

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<151> 2008-09-11

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20 25 30

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35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
50 55 60

Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

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35 40 45

Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

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Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
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Tyr Ala Ala Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
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Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

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Asp Leu Asn Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Ile Ile Trp Gly Asp Gly Thr Thr Asp Tyr Asn Ser Ala Val Lys
50 55 60

Ser Arg Val Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Gly Gly Tyr Trp Tyr Ala Thr Ser Tyr Tyr Phe Asp Tyr Trp Gly
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Gln Gly Thr Leu Val Thr Val Ser Ser
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35 40 45

Tyr Tyr Thr Ser Arg Phe His Ser Gly Val Pro Ser Arg Phe Ser Gly
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85 90 95

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20 25 30

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35 40 45

Ala Ala Ile Asn Gln Asp Gly Ser Glu Lys Tyr Tyr Val Gly Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Val Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Val Arg Asp Tyr Tyr Asp Ile Leu Thr Asp Tyr Tyr Ile His Tyr Trp
100 105 110

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20 25 30

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35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
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20 25 30

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35 40 45

Ala Ile Ile Trp Tyr Asp Gly Asp Asn Gln Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Gly Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
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35 40 45

Lys Tyr Ala Ser Gln Ser Phe Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asn Ser Leu Glu Ala
65 70 75 80

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35 40 45

Trp Leu Ala His Ile Tyr Trp Asp Glu Asp Lys Arg Tyr Asn Pro Ser
50 55 60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Asn Asn Gln Val
65 70 75 80

Phe Leu Lys Ile Thr Asn Val Asp Thr Ala Asp Thr Ala Thr Tyr Tyr
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Cys Ala Arg Arg Arg Ile Ile Tyr Asp Val Glu Asp Tyr Phe Asp Tyr
100 105 110

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35 40 45

Asp Thr Ser Asn Leu Ala Ser Gly Val Pro Val Arg Phe Ser Gly Ser
50 55 60

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20 25 30

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35 40 45

Ala Ser Ile Arg Ser Gly Gly Gly Arg Thr Tyr Tyr Ser Asp Asn Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Val Arg Tyr Asp His Tyr Ser Gly Ser Ser Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 41
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 41
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
20 25 30

Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Trp Gln Gly
85 90 95

Thr His Phe Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg

<210> 42
<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 42
Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Ser Ile His Asn Arg Gly Thr Ile Phe Tyr Leu Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Val Arg Asn Thr Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr
85 90 95

Arg Gly Arg Ser Asn Ser Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Ser Val Thr Val Ser Ser
115

<210> 43
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 43
Asp Val Leu Val Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Thr Gln Thr Leu Val His Arg
20 25 30

Asn Gly Asp Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Ser Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

Arg

<210> 44
<211> 112
<212> PRT

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 44

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Leu Val
        35                  40                  45

Ala Ser Ile Asn Ser Asn Gly Gly Ser Thr Tyr Tyr Pro Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
            100                 105                 110
```

<210> 45
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 45

```
Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1               5                   10                  15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val Tyr Ser
            20                  25                  30

Asn Gly Asp Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Ser
                85                  90                  95

Thr His Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
```

100 105 110

Arg

<210> 46
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 46
Glu Val Gln Leu Val Gln Ser Gly Thr Glu Val Lys Lys Pro Gly Glu
1 5 10 15

Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Thr Val Thr Ser Tyr
20 25 30

Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Phe Ile Tyr Pro Gly Asp Ser Glu Thr Arg Tyr Ser Pro Thr Phe
50 55 60

Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Phe Asn Thr Ala Phe
65 70 75 80

Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Arg Val Gly Ser Gly Trp Tyr Pro Tyr Thr Phe Asp Ile Trp Gly
100 105 110

Gln Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 47
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 47
Glu Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Glu Ser Ile Ser Ser Asn
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Phe Ile
35 40 45

Tyr Thr Ala Ser Thr Arg Ala Thr Asp Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Ser
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Asn Asn Trp Pro Ser
85 90 95

Ile Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg
100 105

<210> 48
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 48
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser
115

<210> 49
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 49
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly

1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg

<210> 50
<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 50
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1 5 10 15

Ser Leu Lys Ile Ser Cys Lys Val Ser Gly Tyr Phe Phe Thr Thr Tyr
20 25 30

Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Tyr Met
35 40 45

Gly Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe
50 55 60

Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr
65 70 75 80

Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Arg Gly Gly Asn Trp Asn Cys Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> 51
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 51
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Tyr Gly Ala Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Arg Tyr Gly Ser Ser His
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Ser Arg
100 105

<210> 52
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 52
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1 5 10 15

Ser Leu Lys Ile Ser Cys Gln Ser Phe Gly Tyr Ile Phe Ile Asp His
20 25 30

Thr Ile His Trp Met Arg Gln Met Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Ala Ile Ser Pro Arg His Asp Ile Thr Lys Tyr Asn Glu Met Phe
50 55 60

Arg Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Phe Cys
85 90 95

Ala Arg Gly Gly Phe Tyr Gly Ser Thr Ile Trp Phe Asp Phe Trp Gly
100 105 110

Gln Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 53
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 53
Glu Thr Thr Val Thr Gln Ser Pro Ser Phe Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ile Thr Thr Thr Asp Ile Asp Asp Asp
20 25 30

Met Asn Trp Phe Gln Gln Glu Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Ser Glu Gly Asn Ile Leu Arg Pro Gly Val Pro Ser Arg Phe Ser Ser
50 55 60

Ser Gly Tyr Gly Thr Asp Phe Thr Leu Thr Ile Ser Lys Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Ser Asp Asn Leu Pro Phe
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 54
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 54
Glu Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp
20 25 30

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His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
        35                  40                  45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
    50                  55                  60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65                  70                  75                  80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Ser Leu Val Thr Val Ser Ser
        115


<210> 55
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 55
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Ser Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Gly Asn Thr Leu Pro Tyr
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
            100                 105


<210> 56
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide
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<400> 56
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Val Ser Ser Gly
20 25 30

Asp Tyr Tyr Trp Thr Trp Ile Arg Gln Ser Pro Gly Lys Gly Leu Glu
35 40 45

Trp Ile Gly His Ile Tyr Tyr Ser Gly Asn Thr Asn Tyr Asn Pro Ser
50 55 60

Leu Lys Ser Arg Leu Thr Ile Ser Ile Asp Thr Ser Lys Thr Gln Phe
65 70 75 80

Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Ile Tyr Tyr
85 90 95

Cys Val Arg Asp Arg Val Thr Gly Ala Phe Asp Ile Trp Gly Gln Gly
100 105 110

Thr Met Val Thr Val Ser Ser
115

<210> 57
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 57
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Ile Ala Thr Tyr Phe Cys Gln His Phe Asp His Leu Pro Leu
85 90 95

Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg

100 105

<210> 58
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 58
Gln Val Gln Leu Lys Gln Ser Gly Pro Gly Leu Val Gln Pro Ser Gln
1 5 10 15

Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Asn Tyr
20 25 30

Gly Val His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Gly Val Ile Trp Ser Gly Gly Asn Thr Asp Tyr Asn Thr Pro Phe Thr
50 55 60

Ser Arg Leu Ser Ile Asn Lys Asp Asn Ser Lys Ser Gln Val Phe Phe
65 70 75 80

Lys Met Asn Ser Leu Gln Ser Asn Asp Thr Ala Ile Tyr Tyr Cys Ala
85 90 95

Arg Ala Leu Thr Tyr Tyr Asp Tyr Glu Phe Ala Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ala
115

<210> 59
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 59
Asp Ile Leu Leu Thr Gln Ser Pro Val Ile Leu Ser Val Ser Pro Gly
1 5 10 15

Glu Arg Val Ser Phe Ser Cys Arg Ala Ser Gln Ser Ile Gly Thr Asn
20 25 30

Ile His Trp Tyr Gln Gln Arg Thr Asn Gly Ser Pro Arg Leu Leu Ile
35 40 45

Lys Tyr Ala Ser Glu Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Ser
65 70 75 80

Glu Asp Ile Ala Asp Tyr Tyr Cys Gln Gln Asn Asn Asn Trp Pro Thr
85 90 95

Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
100 105

<210> 60
<211> 125
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 60
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Thr Thr Phe Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Arg Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Asp Leu Gly Trp Ser Asp Ser Tyr Tyr Tyr Tyr Tyr Gly Met
100 105 110

Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120 125

<210> 61
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 61
Asp Ile Gln Met Thr Gln Phe Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp

20 25 30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
35 40 45

Tyr Ala Ala Ser Arg Leu His Arg Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln His Asn Ser Tyr Pro Cys
85 90 95

Ser Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 62
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 62
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Arg Ala Thr Leu Thr Val Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser
115

<210> 63
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 63
Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg

<210> 64
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 64
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Arg Ala Thr Leu Thr Val Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser
115

<210> 65
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 65
Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg

<210> 66
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 66
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Arg Ala Thr Leu Thr Val Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser
115

<210> 67
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 67
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg

<210> 68
<211> 116
<212> PRT

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 68
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Arg Ala Thr Leu Thr Val Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser
115

<210> 69
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 69
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val

85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg

<210> 70
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 70
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser
115

<210> 71
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 71
Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
20 25 30

```
Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
                85                  90                  95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105                 110

Arg
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<210> 72
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 72

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Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
            20                  25                  30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
            100                 105                 110

Thr Val Ser Ser
        115
```

<210> 73
<211> 113

<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 73
Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg

<210> 74
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 74
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser
115

<210> 75
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 75
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg

<210> 76
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 76
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser
115

<210> 77
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 77
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg

<210> 78

<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 78
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Arg Val Thr Met Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser
115

<210> 79
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 79
Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg

<210> 80
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 80
Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Arg Pro Gly Ser
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ala
115

<210> 81
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 81
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15

Asp Gln Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser

20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Val Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Val
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

Arg

<210> 82
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 82
Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Arg Pro Gly Ser
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Ile Tyr Tyr Cys
85 90 95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ala
115

<210> 83
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 83
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15

Asp Gln Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Val Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Val
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

Arg

<210> 84
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 84
Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Arg Pro Gly Ser
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile
35 40 45

Gly Asp Ile Tyr Pro Tyr Gly Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ala
115

<210> 85
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 85
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15

Asp Gln Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Val Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Val
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

Arg

<210> 86
<211> 237
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 86
Glu Phe Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

```
Ser Val Arg Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Asp Tyr
            20                  25                  30

Asn Met Asn Trp Val Lys Gln Ser Asn Gly Lys Ser Leu Glu Trp Val
        35                  40                  45

Gly Val Ile Asn Pro Asn Tyr Gly Ser Ser Thr Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Gly Lys Ala Thr Leu Thr Val Asp Gln Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Lys Trp Gly Gln Leu Gly Arg Gly Phe Phe Asp Val Trp Gly
            100                 105                 110

Thr Gly Thr Thr Val Thr Val Ser Ser Gln Val Gln Leu Gln Gln Ser
        115                 120                 125

Gly Pro Glu Leu Val Arg Pro Gly Ser Ser Val Lys Ile Ser Cys Lys
    130                 135                 140

Ala Ser Gly Tyr Thr Phe Thr Lys Tyr Trp Leu Gly Trp Val Lys Gln
145                 150                 155                 160

Arg Pro Gly His Gly Leu Glu Trp Ile Gly Asp Ile Tyr Pro Gly Tyr
                165                 170                 175

Asp Tyr Thr His Tyr Asn Glu Lys Phe Lys Asp Lys Ala Thr Leu Thr
            180                 185                 190

Val Asp Thr Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr
        195                 200                 205

Ser Glu Asp Ser Ala Ile Tyr Tyr Cys Ala Arg Ser Asp Gly Ser Ser
    210                 215                 220

Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
225                 230                 235


<210> 87
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 87
Glu Phe Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1               5                   10                  15
```

```
Ser Val Arg Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Asp Tyr
            20                  25                  30

Asn Met Asn Trp Val Lys Gln Ser Asn Gly Lys Ser Leu Glu Trp Val
        35                  40                  45

Gly Val Ile Asn Pro Asn Tyr Gly Ser Ser Thr Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Gly Lys Ala Thr Leu Thr Val Asp Gln Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Lys Trp Gly Gln Leu Gly Arg Gly Phe Phe Asp Val Trp Gly
            100                 105                 110

Thr Gly Thr Thr Val Thr Val Ser Ser
        115                 120


<210> 88
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 88
Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Arg Pro Gly Ser
1               5                   10                  15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
            20                  25                  30

Trp Leu Gly Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile
        35                  40                  45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Asp Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Ile Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
            100                 105                 110

Thr Val Ser Ala
        115
```

<210> 89
<211> 220
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 89
Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

His Trp Phe Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr
35 40 45

Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Ser Pro Leu Thr
85 90 95

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Asp Val Leu Met Thr
100 105 110

Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile
115 120 125

Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser Asn Gly Asn Thr Tyr
130 135 140

Leu Glu Trp Tyr Leu Gln Arg Pro Gly Gln Ser Pro Lys Leu Leu Ile
145 150 155 160

Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly
165 170 175

Ser Gly Ser Gly Thr Val Phe Thr Leu Lys Ile Ser Arg Val Glu Ala
180 185 190

Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Val Ser His Val Pro Tyr
195 200 205

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
210 215 220

<210> 90

<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 90
Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

His Trp Phe Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr
35 40 45

Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Ser Pro Leu Thr
85 90 95

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
100 105

<210> 91
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 91
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15

Asp Gln Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Val Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Val
85 90 95

```
Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110

Arg



<210> 92
<211> 243
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 92
Glu Phe Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1               5                   10                  15

Ser Val Arg Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Asp Tyr
            20                  25                  30

Asn Met Asn Trp Val Lys Gln Ser Asn Gly Lys Ser Leu Glu Trp Val
        35                  40                  45

Gly Val Ile Asn Pro Asn Tyr Gly Ser Ser Thr Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Gly Lys Ala Thr Leu Thr Val Asp Gln Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Lys Trp Gly Gln Leu Gly Arg Gly Phe Phe Asp Val Trp Gly
            100                 105                 110

Thr Gly Thr Thr Val Thr Val Ser Ser Ala Lys Thr Thr Ala Pro Gln
        115                 120                 125

Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Arg Pro Gly Ser Ser
    130                 135                 140

Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr Trp
145                 150                 155                 160

Leu Gly Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile Gly
                165                 170                 175

Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe Lys
            180                 185                 190

Asp Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr Met
        195                 200                 205
```

Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Ile Tyr Tyr Cys Ala
210 215 220

Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr
225 230 235 240

Val Ser Ala

<210> 93
<211> 225
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 93
Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

His Trp Phe Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr
35 40 45

Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Ser Pro Leu Thr
85 90 95

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp Ala Ala Pro
100 105 110

Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
115 120 125

Asp Gln Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
130 135 140

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Arg Pro Gly Gln Ser
145 150 155 160

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
165 170 175

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Val Phe Thr Leu Lys Ile

180 185 190

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Val
195 200 205

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
210 215 220

Arg
225

<210> 94
<211> 250
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 94
Glu Phe Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Arg Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Asp Tyr
20 25 30

Asn Met Asn Trp Val Lys Gln Ser Asn Gly Lys Ser Leu Glu Trp Val
35 40 45

Gly Val Ile Asn Pro Asn Tyr Gly Ser Ser Thr Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Val Asp Gln Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Lys Trp Gly Gln Leu Gly Arg Gly Phe Phe Asp Val Trp Gly
100 105 110

Thr Gly Thr Thr Val Thr Val Ser Ser Ala Lys Thr Thr Ala Pro Ser
115 120 125

Val Tyr Pro Leu Ala Pro Gln Val Gln Leu Gln Gln Ser Gly Pro Glu
130 135 140

Leu Val Arg Pro Gly Ser Ser Val Lys Ile Ser Cys Lys Ala Ser Gly
145 150 155 160

Tyr Thr Phe Thr Lys Tyr Trp Leu Gly Trp Val Lys Gln Arg Pro Gly
165 170 175

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His Gly Leu Glu Trp Ile Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr
            180                 185                 190

His Tyr Asn Glu Lys Phe Lys Asp Lys Ala Thr Leu Thr Val Asp Thr
        195                 200                 205

Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp
    210                 215                 220

Ser Ala Ile Tyr Tyr Cys Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp
225                 230                 235                 240

Gly Gln Gly Thr Leu Val Thr Val Ser Ala
                245                 250


<210> 95
<211> 232
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 95
Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly
1               5                   10                  15

Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

His Trp Phe Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr
        35                  40                  45

Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu
65                  70                  75                  80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Ser Pro Leu Thr
                85                  90                  95

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp Ala Ala Pro
            100                 105                 110

Thr Val Ser Ile Phe Pro Pro Asp Val Leu Met Thr Gln Thr Pro Leu
        115                 120                 125

Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Thr Ser
    130                 135                 140

Ser Gln Asn Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr
145                 150                 155                 160
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```
Leu Gln Arg Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser
                165                 170                 175

Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly
            180                 185                 190

Thr Val Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly
        195                 200                 205

Val Tyr Tyr Cys Phe Gln Val Ser His Val Pro Tyr Thr Phe Gly Gly
    210                 215                 220

Gly Thr Lys Leu Glu Ile Lys Arg
225                 230
```

<210> 96
<211> 237
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 96

```
Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Arg Pro Gly Ser
1               5                   10                  15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
            20                  25                  30

Trp Leu Gly Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile
        35                  40                  45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Asp Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Ile Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
            100                 105                 110

Thr Val Ser Ala Glu Phe Gln Leu Gln Gln Ser Gly Pro Glu Leu Val
        115                 120                 125

Lys Pro Gly Ala Ser Val Arg Ile Ser Cys Lys Ala Ser Gly Tyr Ser
    130                 135                 140

Phe Thr Asp Tyr Asn Met Asn Trp Val Lys Gln Ser Asn Gly Lys Ser
145                 150                 155                 160
```

Leu Glu Trp Val Gly Val Ile Asn Pro Asn Tyr Gly Ser Ser Thr Tyr
165 170 175

Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Gln Ser Ser
180 185 190

Ser Thr Ala Tyr Met Gln Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala
195 200 205

Val Tyr Tyr Cys Ala Arg Lys Trp Gly Gln Leu Gly Arg Gly Phe Phe
210 215 220

Asp Val Trp Gly Thr Gly Thr Thr Val Thr Val Ser Ser
225 230 235

<210> 97
<211> 220
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 97
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15

Asp Gln Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Val Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Val
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

Arg Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro
115 120 125

Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr
130 135 140

Met His Trp Phe Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile

145 150 155 160

Tyr Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly
165 170 175

Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala
180 185 190

Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Ser Pro Leu
195 200 205

Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
210 215 220

<210> 98
<211> 243
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 98
Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Arg Pro Gly Ser
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Ile Tyr Tyr Cys
85 90 95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ala Ala Lys Thr Thr Ala Pro Glu Phe Gln Leu Gln Gln
115 120 125

Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Val Arg Ile Ser Cys
130 135 140

Lys Ala Ser Gly Tyr Ser Phe Thr Asp Tyr Asn Met Asn Trp Val Lys
145 150 155 160

Gln Ser Asn Gly Lys Ser Leu Glu Trp Val Gly Val Ile Asn Pro Asn
165 170 175

Tyr Gly Ser Ser Thr Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu
180 185 190

Thr Val Asp Gln Ser Ser Ser Thr Ala Tyr Met Gln Leu Asn Ser Leu
195 200 205

Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Lys Trp Gly Gln
210 215 220

Leu Gly Arg Gly Phe Phe Asp Val Trp Gly Thr Gly Thr Thr Val Thr
225 230 235 240

Val Ser Ser

<210> 99
<211> 225
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 99
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15

Asp Gln Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Val Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Val
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

Arg Ala Asp Ala Ala Pro Gln Ile Val Leu Ser Gln Ser Pro Ala Ile
115 120 125

Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser
130 135 140

```
Ser Ser Val Ser Tyr Met His Trp Phe Gln Gln Lys Pro Gly Ser Ser
145                 150                 155                 160

Pro Lys Pro Trp Ile Tyr Ala Thr Ser Asn Leu Ala Ser Gly Val Pro
                165                 170                 175

Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
            180                 185                 190

Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
        195                 200                 205

Ser Ser Ser Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
    210                 215                 220

Arg
225
```

<210> 100
<211> 250
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 100

```
Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Arg Pro Gly Ser
1               5                   10                  15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
            20                  25                  30

Trp Leu Gly Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile
        35                  40                  45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Asp Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Ile Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
            100                 105                 110

Thr Val Ser Ala Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro Leu Ala
        115                 120                 125

Pro Glu Phe Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
    130                 135                 140
```

```
Ala Ser Val Arg Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Asp
145                 150                 155                 160

Tyr Asn Met Asn Trp Val Lys Gln Ser Asn Gly Lys Ser Leu Glu Trp
                165                 170                 175

Val Gly Val Ile Asn Pro Asn Tyr Gly Ser Ser Thr Tyr Asn Gln Lys
            180                 185                 190

Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Gln Ser Ser Ser Thr Ala
        195                 200                 205

Tyr Met Gln Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr
    210                 215                 220

Cys Ala Arg Lys Trp Gly Gln Leu Gly Arg Gly Phe Phe Asp Val Trp
225                 230                 235                 240

Gly Thr Gly Thr Thr Val Thr Val Ser Ser
                245                 250


<210> 101
<211> 232
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 101
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1               5                   10                  15

Asp Gln Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
            20                  25                  30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Arg Pro Gly Gln Ser
        35                  40                  45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Val Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Val
                85                  90                  95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110

Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Gln Ile Val
```

115 120 125

Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly Glu Lys Val
130 135 140

Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Met His Trp Phe
145 150 155 160

Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr Ala Thr Ser
165 170 175

Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly
180 185 190

Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu Asp Ala Ala
195 200 205

Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Ser Pro Leu Thr Phe Gly Ala
210 215 220

Gly Thr Lys Leu Glu Leu Lys Arg
225 230

<210> 102
<211> 243
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 102
Glu Phe Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Arg Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Asp Tyr
20 25 30

Asn Met Asn Trp Val Lys Gln Ser Asn Gly Lys Ser Leu Glu Trp Val
35 40 45

Gly Val Ile Asn Pro Asn Tyr Gly Ser Ser Thr Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Val Asp Gln Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Lys Trp Gly Gln Leu Gly Arg Gly Phe Phe Asp Val Trp Gly
100 105 110

```
Thr Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Gln
        115                 120                 125

Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Arg Pro Gly Ser Ser
    130                 135                 140

Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr Trp
145                 150                 155                 160

Leu Gly Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile Gly
                165                 170                 175

Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe Lys
            180                 185                 190

Asp Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr Met
        195                 200                 205

Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Ile Tyr Tyr Cys Ala
    210                 215                 220

Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr
225                 230                 235                 240

Val Ser Ala
```

<210> 103
<211> 225
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 103

```
Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly
1               5                   10                  15

Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

His Trp Phe Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr
        35                  40                  45

Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu
65                  70                  75                  80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Ser Pro Leu Thr
                85                  90                  95
```

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Thr Val Ala Ala Pro
100 105 110

Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
115 120 125

Asp Gln Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
130 135 140

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Arg Pro Gly Gln Ser
145 150 155 160

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
165 170 175

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Val Phe Thr Leu Lys Ile
180 185 190

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Val
195 200 205

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
210 215 220

Arg
225

<210> 104
<211> 250
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 104
Glu Phe Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Arg Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Asp Tyr
20 25 30

Asn Met Asn Trp Val Lys Gln Ser Asn Gly Lys Ser Leu Glu Trp Val
35 40 45

Gly Val Ile Asn Pro Asn Tyr Gly Ser Ser Thr Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Val Asp Gln Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

```
Ala Arg Lys Trp Gly Gln Leu Gly Arg Gly Phe Phe Asp Val Trp Gly
            100                 105                 110

Thr Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
        115                 120                 125

Val Phe Pro Leu Ala Pro Gln Val Gln Leu Gln Gln Ser Gly Pro Glu
    130                 135                 140

Leu Val Arg Pro Gly Ser Ser Val Lys Ile Ser Cys Lys Ala Ser Gly
145                 150                 155                 160

Tyr Thr Phe Thr Lys Tyr Trp Leu Gly Trp Val Lys Gln Arg Pro Gly
                165                 170                 175

His Gly Leu Glu Trp Ile Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr
            180                 185                 190

His Tyr Asn Glu Lys Phe Lys Asp Lys Ala Thr Leu Thr Val Asp Thr
        195                 200                 205

Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp
    210                 215                 220

Ser Ala Ile Tyr Tyr Cys Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp
225                 230                 235                 240

Gly Gln Gly Thr Leu Val Thr Val Ser Ala
                245                 250


<210> 105
<211> 232
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 105
Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly
1               5                   10                  15

Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

His Trp Phe Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr
        35                  40                  45

Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu
```

65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Ser Pro Leu Thr
85 90 95

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Asp Val Leu Met Thr Gln Thr Pro Leu
115 120 125

Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Thr Ser
130 135 140

Ser Gln Asn Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr
145 150 155 160

Leu Gln Arg Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser
165 170 175

Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly
180 185 190

Thr Val Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly
195 200 205

Val Tyr Tyr Cys Phe Gln Val Ser His Val Pro Tyr Thr Phe Gly Gly
210 215 220

Gly Thr Lys Leu Glu Ile Lys Arg
225 230

<210> 106
<211> 243
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 106
Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Arg Pro Gly Ser
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

```
Lys Asp Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Ile Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
            100                 105                 110

Thr Val Ser Ala Ala Ser Thr Lys Gly Pro Glu Phe Gln Leu Gln Gln
        115                 120                 125

Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Val Arg Ile Ser Cys
    130                 135                 140

Lys Ala Ser Gly Tyr Ser Phe Thr Asp Tyr Asn Met Asn Trp Val Lys
145                 150                 155                 160

Gln Ser Asn Gly Lys Ser Leu Glu Trp Val Gly Val Ile Asn Pro Asn
                165                 170                 175

Tyr Gly Ser Ser Thr Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu
            180                 185                 190

Thr Val Asp Gln Ser Ser Ser Thr Ala Tyr Met Gln Leu Asn Ser Leu
        195                 200                 205

Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Lys Trp Gly Gln
    210                 215                 220

Leu Gly Arg Gly Phe Phe Asp Val Trp Gly Thr Gly Thr Thr Val Thr
225                 230                 235                 240

Val Ser Ser


<210> 107
<211> 225
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 107
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1               5                   10                  15

Asp Gln Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
            20                  25                  30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Arg Pro Gly Gln Ser
        35                  40                  45
```

```
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Val Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Val
                85                  90                  95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110

Arg Thr Val Ala Ala Pro Gln Ile Val Leu Ser Gln Ser Pro Ala Ile
        115                 120                 125

Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser
    130                 135                 140

Ser Ser Val Ser Tyr Met His Trp Phe Gln Gln Lys Pro Gly Ser Ser
145                 150                 155                 160

Pro Lys Pro Trp Ile Tyr Ala Thr Ser Asn Leu Ala Ser Gly Val Pro
                165                 170                 175

Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
            180                 185                 190

Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
        195                 200                 205

Ser Ser Ser Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
    210                 215                 220

Arg
225


<210> 108
<211> 250
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 108
Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Arg Pro Gly Ser
1               5                   10                  15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
            20                  25                  30

Trp Leu Gly Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile
        35                  40                  45
```

```
Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
     50                  55                  60

Lys Asp Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Ile Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
            100                 105                 110

Thr Val Ser Ala Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
        115                 120                 125

Pro Glu Phe Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
    130                 135                 140

Ala Ser Val Arg Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Asp
145                 150                 155                 160

Tyr Asn Met Asn Trp Val Lys Gln Ser Asn Gly Lys Ser Leu Glu Trp
                165                 170                 175

Val Gly Val Ile Asn Pro Asn Tyr Gly Ser Ser Thr Tyr Asn Gln Lys
            180                 185                 190

Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Gln Ser Ser Ser Thr Ala
        195                 200                 205

Tyr Met Gln Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr
    210                 215                 220

Cys Ala Arg Lys Trp Gly Gln Leu Gly Arg Gly Phe Phe Asp Val Trp
225                 230                 235                 240

Gly Thr Gly Thr Thr Val Thr Val Ser Ser
                245                 250


<210> 109
<211> 232
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 109
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1               5                   10                  15

Asp Gln Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
```

20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Val Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Val
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Gln Ile Val
115 120 125

Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly Glu Lys Val
130 135 140

Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Met His Trp Phe
145 150 155 160

Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr Ala Thr Ser
165 170 175

Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly
180 185 190

Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu Asp Ala Ala
195 200 205

Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Ser Pro Leu Thr Phe Gly Ala
210 215 220

Gly Thr Lys Leu Glu Leu Lys Arg
225 230

<210> 110
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 110
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser
115

<210> 111
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 111
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg

<210> 112
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 112
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Thr Trp Asn Ser Gly His Ile Asp Tyr Ala Asp Ser Val
50 55 60

Glu Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Val Ser Tyr Leu Ser Thr Ala Ser Ser Leu Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 113
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 113
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro

65 70 75 80

Glu Asp Val Ala Thr Tyr Tyr Cys Gln Arg Tyr Asn Arg Ala Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> 114
<211> 243
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 114
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Glu Val Gln Leu Val Glu
115 120 125

Ser Gly Gly Gly Leu Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys
130 135 140

Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr Ala Met His Trp Val Arg
145 150 155 160

Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ala Ile Thr Trp Asn
165 170 175

Ser Gly His Ile Asp Tyr Ala Asp Ser Val Glu Gly Arg Phe Thr Ile
180 185 190

```
Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu
        195                 200                 205

Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys Val Ser Tyr Leu
    210                 215                 220

Ser Thr Ala Ser Ser Leu Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
225                 230                 235                 240

Val Ser Ser
```

<210> 115
<211> 226
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 115

```
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1               5                   10                  15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
            20                  25                  30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
                85                  90                  95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105                 110

Arg Thr Val Ala Ala Pro Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
        115                 120                 125

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
    130                 135                 140

Gln Gly Ile Arg Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys
145                 150                 155                 160

Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Thr Leu Gln Ser Gly Val
                165                 170                 175
```

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
180 185 190

Ile Ser Ser Leu Gln Pro Glu Asp Val Ala Thr Tyr Tyr Cys Gln Arg
195 200 205

Tyr Asn Arg Ala Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
210 215 220

Lys Arg
225

<210> 116
<211> 250
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 116
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
115 120 125

Pro Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
130 135 140

Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp
145 150 155 160

Tyr Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
165 170 175

Val Ser Ala Ile Thr Trp Asn Ser Gly His Ile Asp Tyr Ala Asp Ser
180 185 190

Val Glu Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu
195 200 205

Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
210 215 220

Cys Ala Lys Val Ser Tyr Leu Ser Thr Ala Ser Ser Leu Asp Tyr Trp
225 230 235 240

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
245 250

<210> 117
<211> 233
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 117
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Asp Ile Gln
115 120 125

Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val
130 135 140

Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Tyr Leu Ala Trp

145 150 155 160

Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala
165 170 175

Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser
180 185 190

Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Val
195 200 205

Ala Thr Tyr Tyr Cys Gln Arg Tyr Asn Arg Ala Pro Tyr Thr Phe Gly
210 215 220

Gln Gly Thr Lys Val Glu Ile Lys Arg
225 230

<210> 118
<211> 243
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 118
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Thr Trp Asn Ser Gly His Ile Asp Tyr Ala Asp Ser Val
50 55 60

Glu Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Val Ser Tyr Leu Ser Thr Ala Ser Ser Leu Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Glu
115 120 125

Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser
130 135 140

```
Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr Trp
145                 150                 155                 160

Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly
                165                 170                 175

Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe Lys
            180                 185                 190

Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr Met
        195                 200                 205

Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala
    210                 215                 220

Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr
225                 230                 235                 240

Val Ser Ser
```

<210> 119
<211> 226
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 119

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Tyr
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ala Ala Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Val Ala Thr Tyr Tyr Cys Gln Arg Tyr Asn Arg Ala Pro Tyr
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro
        115                 120                 125
```

Gly Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His
130 135 140

Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln
145 150 155 160

Ser Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val
165 170 175

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys
180 185 190

Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln
195 200 205

Val Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
210 215 220

Lys Arg
225

<210> 120
<211> 250
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 120
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Thr Trp Asn Ser Gly His Ile Asp Tyr Ala Asp Ser Val
50 55 60

Glu Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Val Ser Tyr Leu Ser Thr Ala Ser Ser Leu Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115 120 125

Val Phe Pro Leu Ala Pro Glu Val Gln Leu Val Gln Ser Gly Ala Glu
130 135 140

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
145 150 155 160

Tyr Thr Phe Thr Lys Tyr Trp Leu Gly Trp Val Arg Gln Ala Pro Gly
165 170 175

Gln Gly Leu Glu Trp Met Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr
180 185 190

His Tyr Asn Glu Lys Phe Lys Asp Arg Val Thr Leu Thr Thr Asp Thr
195 200 205

Ser Thr Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu Arg Ser Asp Asp
210 215 220

Thr Ala Val Tyr Tyr Cys Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp
225 230 235 240

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
245 250

<210> 121
<211> 233
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 121
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Val Ala Thr Tyr Tyr Cys Gln Arg Tyr Asn Arg Ala Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala

100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Asp Val Leu Met Thr Gln Thr Pro
115 120 125

Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Thr
130 135 140

Ser Ser Gln Asn Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu Trp
145 150 155 160

Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Lys Val
165 170 175

Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser
180 185 190

Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val
195 200 205

Gly Val Tyr Tyr Cys Phe Gln Val Ser His Val Pro Tyr Thr Phe Gly
210 215 220

Gly Gly Thr Lys Val Glu Ile Lys Arg
225 230

<210> 122
<211> 330
<212> PRT
<213> Homo sapiens

<400> 122
Ala Ser Thr Lys Gly Pro Ser Val Phe Phe Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
100 105 110

```
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                165                 170                 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            180                 185                 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        195                 200                 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    210                 215                 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
225                 230                 235                 240

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                245                 250                 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            260                 265                 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        275                 280                 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
    290                 295                 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305                 310                 315                 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                325                 330


<210> 123
<211> 330
<212> PRT
<213> Homo sapiens

<400> 123
Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1               5                   10                  15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
```

```
            20                  25                  30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65                  70                  75                  80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            100                 105                 110

Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                165                 170                 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            180                 185                 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        195                 200                 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    210                 215                 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
225                 230                 235                 240

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                245                 250                 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            260                 265                 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        275                 280                 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
```

290 295 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305 310 315 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
325 330

<210> 124
<211> 106
<212> PRT
<213> Homo sapiens

<400> 124
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
1 5 10 15

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
20 25 30

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
35 40 45

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
50 55 60

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
65 70 75 80

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
85 90 95

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
100 105

<210> 125
<211> 105
<212> PRT
<213> Homo sapiens

<400> 125
Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
1 5 10 15

Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe
20 25 30

Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val
35 40 45

Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
50 55 60

Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser

65 70 75 80

His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu
85 90 95

Lys Thr Val Ala Pro Thr Glu Cys Ser
100 105

<210> 126
<211> 332
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 126
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Val Ala Thr Tyr Tyr Cys Gln Arg Tyr Asn Arg Ala Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro
115 120 125

Gly Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His
130 135 140

Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln
145 150 155 160

Ser Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val
165 170 175

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys
180 185 190

```
Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln
        195                 200                 205

Val Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
    210                 215                 220

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
225                 230                 235                 240

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
                245                 250                 255

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
            260                 265                 270

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
        275                 280                 285

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
    290                 295                 300

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
305                 310                 315                 320

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
                325                 330
```

<210> 127
<211> 573
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 127

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
            20                  25                  30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ser Ala Ile Thr Trp Asn Ser Gly His Ile Asp Tyr Ala Asp Ser Val
    50                  55                  60

Glu Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
```

```
Ala Lys Val Ser Tyr Leu Ser Thr Ala Ser Ser Leu Asp Tyr Trp Gly
            100                 105                 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Glu
        115                 120                 125

Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser
    130                 135                 140

Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr Trp
145                 150                 155                 160

Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly
                165                 170                 175

Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe Lys
            180                 185                 190

Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr Met
        195                 200                 205

Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala
    210                 215                 220

Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr
225                 230                 235                 240

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
                245                 250                 255

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
            260                 265                 270

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
        275                 280                 285

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
    290                 295                 300

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
305                 310                 315                 320

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
                325                 330                 335

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys
            340                 345                 350

Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
        355                 360                 365
```

```
Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
    370                 375                 380

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
385                 390                 395                 400

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
                405                 410                 415

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
            420                 425                 430

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
        435                 440                 445

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
    450                 455                 460

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
465                 470                 475                 480

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
                485                 490                 495

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
            500                 505                 510

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
        515                 520                 525

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
    530                 535                 540

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
545                 550                 555                 560

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                565                 570


<210> 128
<211> 245
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 128
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30
```

```
Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
    50                  55                  60

Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Lys Tyr Pro His Tyr Tyr Gly Ser Ser His Trp Tyr Phe Asp Val
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125

Pro Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
    130                 135                 140

Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys
145                 150                 155                 160

Tyr Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp
                165                 170                 175

Met Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys
            180                 185                 190

Phe Lys Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala
        195                 200                 205

Tyr Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr
    210                 215                 220

Cys Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu
225                 230                 235                 240

Val Thr Val Ser Ser
                245


<210> 129
<211> 226
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 129
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
```

1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
35 40 45

Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro
115 120 125

Gly Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His
130 135 140

Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln
145 150 155 160

Ser Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val
165 170 175

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys
180 185 190

Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln
195 200 205

Val Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
210 215 220

Lys Arg
225

<210> 130
<211> 245
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 130

```
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                  10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
            20                  25                  30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
            100                 105                 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Glu Val Gln Leu Val Glu
        115                 120                 125

Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys
    130                 135                 140

Ala Ala Ser Gly Tyr Thr Phe Thr Asn Tyr Gly Met Asn Trp Val Arg
145                 150                 155                 160

Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Gly Trp Ile Asn Thr Tyr
                165                 170                 175

Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe Lys Arg Arg Phe Thr Phe
            180                 185                 190

Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr Leu Gln Met Asn Ser Leu
        195                 200                 205

Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys Tyr Pro His Tyr
    210                 215                 220

Tyr Gly Ser Ser His Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu
225                 230                 235                 240

Val Thr Val Ser Ser
                245
```

<210> 131
<211> 226
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 131
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr Val Ala Ala Pro Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
115 120 125

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Ser Ala Ser
130 135 140

Gln Asp Ile Ser Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys
145 150 155 160

Ala Pro Lys Val Leu Ile Tyr Phe Thr Ser Ser Leu His Ser Gly Val
165 170 175

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
180 185 190

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
195 200 205

Tyr Ser Thr Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
210 215 220

Lys Arg
225

<210> 132
<211> 243
<212> PRT

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 132

```
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1               5                  10                  15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ile Gly Tyr
            20                  25                  30

Asp Leu Asn Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Ile Ile Trp Gly Asp Gly Thr Thr Asp Tyr Asn Ser Ala Val Lys
    50                  55                  60

Ser Arg Val Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Phe Ser Leu
65                  70                  75                  80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
                85                  90                  95

Arg Gly Gly Tyr Trp Tyr Ala Thr Ser Tyr Tyr Phe Asp Tyr Trp Gly
            100                 105                 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Glu
        115                 120                 125

Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser
    130                 135                 140

Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr Trp
145                 150                 155                 160

Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly
                165                 170                 175

Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe Lys
            180                 185                 190

Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr Met
        195                 200                 205

Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala
    210                 215                 220

Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr
225                 230                 235                 240

Val Ser Ser
```

<210> 133
<211> 226
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 133
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Asn Asn
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Tyr Thr Ser Arg Phe His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Glu His Thr Leu Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro
115 120 125

Gly Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His
130 135 140

Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln
145 150 155 160

Ser Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val
165 170 175

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys
180 185 190

Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln
195 200 205

Val Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
210 215 220

Lys Arg

225

<210> 134
<211> 243
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 134
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Gln Val Gln Leu Gln Glu
115 120 125

Ser Gly Pro Gly Leu Val Lys Pro Ser Glu Thr Leu Ser Leu Thr Cys
130 135 140

Thr Val Ser Gly Phe Ser Leu Ile Gly Tyr Asp Leu Asn Trp Ile Arg
145 150 155 160

Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile Gly Ile Ile Trp Gly Asp
165 170 175

Gly Thr Thr Asp Tyr Asn Ser Ala Val Lys Ser Arg Val Thr Ile Ser
180 185 190

Lys Asp Thr Ser Lys Asn Gln Phe Ser Leu Lys Leu Ser Ser Val Thr
195 200 205

Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly Gly Tyr Trp Tyr
210 215 220

```
Ala Thr Ser Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
225                 230                 235                 240

Val Ser Ser
```

<210> 135
<211> 226
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 135

```
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1               5                   10                  15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
            20                  25                  30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
                85                  90                  95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105                 110

Arg Thr Val Ala Ala Pro Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
        115                 120                 125

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
    130                 135                 140

Gln Ser Ile Ser Asn Asn Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys
145                 150                 155                 160

Ala Pro Lys Leu Leu Ile Tyr Tyr Thr Ser Arg Phe His Ser Gly Val
                165                 170                 175

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr
            180                 185                 190

Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln
        195                 200                 205
```

Glu His Thr Leu Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile
210 215 220

Lys Arg
225

<210> 136
<211> 249
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 136
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30

Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Ala Ile Asn Gln Asp Gly Ser Glu Lys Tyr Tyr Val Gly Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Val Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Val Arg Asp Tyr Tyr Asp Ile Leu Thr Asp Tyr Tyr Ile His Tyr Trp
100 105 110

Tyr Phe Asp Leu Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser Ala
115 120 125

Ser Thr Lys Gly Pro Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val
130 135 140

Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr
145 150 155 160

Thr Phe Thr Lys Tyr Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln
165 170 175

Gly Leu Glu Trp Met Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His
180 185 190

Tyr Asn Glu Lys Phe Lys Asp Arg Val Thr Leu Thr Thr Asp Thr Ser
195 200 205

```
Thr Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr
    210                 215                 220

Ala Val Tyr Tyr Cys Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly
225                 230                 235                 240

Gln Gly Thr Leu Val Thr Val Ser Ser
                245
```

<210> 137
<211> 227
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 137

```
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1               5                   10                  15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
            20                  25                  30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
        35                  40                  45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
    50                  55                  60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65                  70                  75                  80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro
                85                  90                  95

Cys Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg Thr Val Ala
            100                 105                 110

Ala Pro Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr
        115                 120                 125

Pro Gly Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val
    130                 135                 140

His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly
145                 150                 155                 160

Gln Ser Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly
                165                 170                 175

Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu
```

180 185 190

Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe
195 200 205

Gln Val Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu
210 215 220

Ile Lys Arg
225

<210> 138
<211> 249
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 138
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Glu Val Gln Leu Val Glu
115 120 125

Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys
130 135 140

Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr Trp Met Asn Trp Val Arg
145 150 155 160

Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Ala Ile Asn Gln Asp
165 170 175

Gly Ser Glu Lys Tyr Tyr Val Gly Ser Val Lys Gly Arg Phe Thr Ile
180 185 190

Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu
195 200 205

Arg Val Glu Asp Thr Ala Val Tyr Tyr Cys Val Arg Asp Tyr Tyr Asp
210 215 220

Ile Leu Thr Asp Tyr Tyr Ile His Tyr Trp Tyr Phe Asp Leu Trp Gly
225 230 235 240

Arg Gly Thr Leu Val Thr Val Ser Ser
245

<210> 139
<211> 227
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 139
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr Val Ala Ala Pro Glu Ile Val Leu Thr Gln Ser Pro Gly Thr
115 120 125

Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser
130 135 140

Gln Ser Val Ser Ser Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly
145 150 155 160

```
Gln Ala Pro Arg Leu Leu Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly
                165                 170                 175

Ile Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu
            180                 185                 190

Thr Ile Ser Arg Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln
        195                 200                 205

Gln Tyr Gly Ser Ser Pro Cys Thr Phe Gly Gln Gly Thr Arg Leu Glu
    210                 215                 220

Ile Lys Arg
225


<210> 140
<211> 240
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 140
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Val Tyr
            20                  25                  30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Ile Ile Trp Tyr Asp Gly Asp Asn Gln Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Gly Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Asp Leu Arg Thr Gly Pro Phe Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Glu Val Gln Leu
        115                 120                 125

Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val
    130                 135                 140

Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr Trp Leu Gly Trp
145                 150                 155                 160
```

Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Asp Ile Tyr
165 170 175

Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe Lys Asp Arg Val
180 185 190

Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr Met Glu Leu Arg
195 200 205

Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Asp
210 215 220

Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
225 230 235 240

<210> 141
<211> 226
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 141
Glu Ile Val Leu Thr Gln Ser Pro Asp Phe Gln Ser Val Thr Pro Lys
1 5 10 15

Glu Lys Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Gly Ser Ser
20 25 30

Leu His Trp Tyr Gln Gln Lys Pro Asp Gln Ser Pro Lys Leu Leu Ile
35 40 45

Lys Tyr Ala Ser Gln Ser Phe Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asn Ser Leu Glu Ala
65 70 75 80

Glu Asp Ala Ala Ala Tyr Tyr Cys His Gln Ser Ser Ser Leu Pro Phe
85 90 95

Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro
115 120 125

Gly Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His
130 135 140

Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln

145 150 155 160

Ser Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val
165 170 175

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys
180 185 190

Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln
195 200 205

Val Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
210 215 220

Lys Arg
225

<210> 142
<211> 240
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 142
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Gln Val Gln Leu Val Glu
115 120 125

Ser Gly Gly Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys
130 135 140

Ala Ala Ser Gly Phe Thr Phe Ser Val Tyr Gly Met Asn Trp Val Arg
145 150 155 160

Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Ile Ile Trp Tyr Asp
165 170 175

Gly Asp Asn Gln Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile
180 185 190

Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Gly Leu
195 200 205

Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp Leu Arg Thr
210 215 220

Gly Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
225 230 235 240

<210> 143
<211> 226
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 143
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr Val Ala Ala Pro Glu Ile Val Leu Thr Gln Ser Pro Asp Phe
115 120 125

Gln Ser Val Thr Pro Lys Glu Lys Val Thr Ile Thr Cys Arg Ala Ser
130 135 140

```
Gln Ser Ile Gly Ser Ser Leu His Trp Tyr Gln Gln Lys Pro Asp Gln
145                 150                 155                 160

Ser Pro Lys Leu Leu Ile Lys Tyr Ala Ser Gln Ser Phe Ser Gly Val
                165                 170                 175

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
            180                 185                 190

Ile Asn Ser Leu Glu Ala Glu Asp Ala Ala Ala Tyr Tyr Cys His Gln
        195                 200                 205

Ser Ser Ser Leu Pro Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Ile
    210                 215                 220

Lys Arg
225
```

<210> 144
<211> 245
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 144

```
Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Gln Pro Ser Gln
1               5                   10                  15

Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Asn
            20                  25                  30

Gly Met Gly Val Ser Trp Ile Arg Gln Pro Ser Gly Lys Gly Leu Glu
        35                  40                  45

Trp Leu Ala His Ile Tyr Trp Asp Glu Asp Lys Arg Tyr Asn Pro Ser
    50                  55                  60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Asn Asn Gln Val
65                  70                  75                  80

Phe Leu Lys Ile Thr Asn Val Asp Thr Ala Asp Thr Ala Thr Tyr Tyr
                85                  90                  95

Cys Ala Arg Arg Arg Ile Ile Tyr Asp Val Glu Asp Tyr Phe Asp Tyr
            100                 105                 110

Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125

Pro Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
    130                 135                 140
```

```
Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys
145                 150                 155                 160

Tyr Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp
                165                 170                 175

Met Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys
            180                 185                 190

Phe Lys Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala
        195                 200                 205

Tyr Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr
    210                 215                 220

Cys Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu
225                 230                 235                 240

Val Thr Val Ser Ser
                245


<210> 145
<211> 225
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 145
Gln Ile Val Leu Ile Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1               5                   10                  15

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

Tyr Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Arg Leu Leu Ile Tyr
        35                  40                  45

Asp Thr Ser Asn Leu Ala Ser Gly Val Pro Val Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Met Glu Ala Glu
65                  70                  75                  80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Gly Tyr Pro Tyr Thr
                85                  90                  95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
            100                 105                 110

Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
```

115 120 125

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
130 135 140

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
145 150 155 160

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
165 170 175

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
180 185 190

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
195 200 205

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
210 215 220

Arg
225

<210> 146
<211> 245
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 146
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

```
Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Gln Val Thr Leu Lys Glu
        115                 120                 125

Ser Gly Pro Gly Ile Leu Gln Pro Ser Gln Thr Leu Ser Leu Thr Cys
    130                 135                 140

Ser Phe Ser Gly Phe Ser Leu Ser Thr Asn Gly Met Gly Val Ser Trp
145                 150                 155                 160

Ile Arg Gln Pro Ser Gly Lys Gly Leu Glu Trp Leu Ala His Ile Tyr
                165                 170                 175

Trp Asp Glu Asp Lys Arg Tyr Asn Pro Ser Leu Lys Ser Arg Leu Thr
            180                 185                 190

Ile Ser Lys Asp Thr Ser Asn Asn Gln Val Phe Leu Lys Ile Thr Asn
        195                 200                 205

Val Asp Thr Ala Asp Thr Ala Thr Tyr Tyr Cys Ala Arg Arg Arg Ile
    210                 215                 220

Ile Tyr Asp Val Glu Asp Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Thr
225                 230                 235                 240

Leu Thr Val Ser Ser
                245
```

<210> 147
<211> 225
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 147

```
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1               5                   10                  15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
            20                  25                  30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
                85                  90                  95
```

```
Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105                 110

Arg Thr Val Ala Ala Pro Gln Ile Val Leu Ile Gln Ser Pro Ala Ile
        115                 120                 125

Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser
    130                 135                 140

Ser Ser Val Ser Tyr Met Tyr Trp Tyr Gln Gln Lys Pro Gly Ser Ser
145                 150                 155                 160

Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly Val Pro
                165                 170                 175

Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
            180                 185                 190

Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
        195                 200                 205

Ser Gly Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
    210                 215                 220

Arg
225
```

<210> 148
<211> 241
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 148

```
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
            20                  25                  30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Ser Ile Arg Ser Gly Gly Gly Arg Thr Tyr Tyr Ser Asp Asn Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
```

Val Arg Tyr Asp His Tyr Ser Gly Ser Ser Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Glu Val Gln
115 120 125

Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys
130 135 140

Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr Trp Leu Gly
145 150 155 160

Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Asp Ile
165 170 175

Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe Lys Asp Arg
180 185 190

Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr Met Glu Leu
195 200 205

Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser
210 215 220

Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
225 230 235 240

Ser

<210> 149
<211> 231
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 149
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
20 25 30

Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile

65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Trp Gln Gly
85 90 95

Thr His Phe Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr Val Ala Ala Pro Asp Val Leu Met Thr Gln Thr Pro Leu Ser
115 120 125

Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser
130 135 140

Gln Asn Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu
145 150 155 160

Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn
165 170 175

Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr
180 185 190

Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val
195 200 205

Tyr Tyr Cys Phe Gln Val Ser His Val Pro Tyr Thr Phe Gly Gly Gly
210 215 220

Thr Lys Val Glu Ile Lys Arg
225 230

<210> 150
<211> 241
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 150
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

```
Lys Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
            100                 105                 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Glu Val Gln Leu Leu Glu
        115                 120                 125

Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys
    130                 135                 140

Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr Gly Met Ser Trp Val Arg
145                 150                 155                 160

Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Ser Ile Arg Ser Gly
                165                 170                 175

Gly Gly Arg Thr Tyr Tyr Ser Asp Asn Val Lys Gly Arg Phe Thr Ile
            180                 185                 190

Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu
        195                 200                 205

Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Val Arg Tyr Asp His Tyr
    210                 215                 220

Ser Gly Ser Ser Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
225                 230                 235                 240

Ser
```

<210> 151
<211> 231
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 151

```
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1               5                   10                  15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
            20                  25                  30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45
```

```
Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
                85                  90                  95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105                 110

Arg Thr Val Ala Ala Pro Asp Val Val Met Thr Gln Ser Pro Leu Ser
        115                 120                 125

Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Lys Ser Ser
    130                 135                 140

Gln Ser Leu Leu Asp Ser Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu
145                 150                 155                 160

Gln Lys Pro Gly Gln Ser Pro Gln Arg Leu Ile Tyr Leu Val Ser Lys
                165                 170                 175

Leu Asp Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr
            180                 185                 190

Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val
        195                 200                 205

Tyr Tyr Cys Trp Gln Gly Thr His Phe Pro Arg Thr Phe Gly Gln Gly
    210                 215                 220

Thr Lys Val Glu Ile Lys Arg
225                 230


<210> 152
<211> 240
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 152
Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
```

```
Ala Ser Ile His Asn Arg Gly Thr Ile Phe Tyr Leu Asp Ser Val Lys
    50                  55                  60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Val Arg Asn Thr Leu Tyr Leu
65                  70                  75                  80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr
                85                  90                  95

Arg Gly Arg Ser Asn Ser Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110

Ser Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Glu Val Gln Leu
        115                 120                 125

Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val
    130                 135                 140

Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr Trp Leu Gly Trp
145                 150                 155                 160

Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Asp Ile Tyr
                165                 170                 175

Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe Lys Asp Arg Val
            180                 185                 190

Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr Met Glu Leu Arg
        195                 200                 205

Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Asp
    210                 215                 220

Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
225                 230                 235                 240


<210> 153
<211> 231
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 153
Asp Val Leu Val Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1               5                   10                  15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Thr Gln Thr Leu Val His Arg
            20                  25                  30

Asn Gly Asp Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
```

35 40 45

Pro Gln Ser Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

Arg Thr Val Ala Ala Pro Asp Val Leu Met Thr Gln Thr Pro Leu Ser
115 120 125

Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser
130 135 140

Gln Asn Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu
145 150 155 160

Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn
165 170 175

Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr
180 185 190

Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val
195 200 205

Tyr Tyr Cys Phe Gln Val Ser His Val Pro Tyr Thr Phe Gly Gly Gly
210 215 220

Thr Lys Val Glu Ile Lys Arg
225 230

<210> 154
<211> 240
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 154
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

```
Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
            100                 105                 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Glu Val Lys Leu Val Glu
        115                 120                 125

Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Arg Leu Ser Cys
    130                 135                 140

Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met Ser Trp Val Arg
145                 150                 155                 160

Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Ser Ile His Asn Arg
                165                 170                 175

Gly Thr Ile Phe Tyr Leu Asp Ser Val Lys Gly Arg Phe Thr Ile Ser
            180                 185                 190

Arg Asp Asn Val Arg Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg
        195                 200                 205

Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr Arg Gly Arg Ser Asn Ser
    210                 215                 220

Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
225                 230                 235                 240


<210> 155
<211> 231
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 155
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1               5                   10                  15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
            20                  25                  30
```

```
Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
                85                  90                  95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105                 110

Arg Thr Val Ala Ala Pro Asp Val Leu Val Thr Gln Ser Pro Leu Ser
        115                 120                 125

Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Thr
    130                 135                 140

Gln Thr Leu Val His Arg Asn Gly Asp Thr Tyr Leu Glu Trp Tyr Leu
145                 150                 155                 160

Gln Lys Pro Gly Gln Ser Pro Gln Ser Leu Ile Tyr Lys Val Ser Asn
                165                 170                 175

Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr
            180                 185                 190

Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val
        195                 200                 205

Tyr Tyr Cys Phe Gln Gly Ser His Val Pro Tyr Thr Phe Gly Gln Gly
    210                 215                 220

Thr Lys Leu Glu Ile Lys Arg
225                 230


<210> 156
<211> 234
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 156
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30
```

```
Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Leu Val
        35                  40                  45

Ala Ser Ile Asn Ser Asn Gly Gly Ser Thr Tyr Tyr Pro Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
            100                 105                 110

Ala Ser Thr Lys Gly Pro Glu Val Gln Leu Val Gln Ser Gly Ala Glu
        115                 120                 125

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
    130                 135                 140

Tyr Thr Phe Thr Lys Tyr Trp Leu Gly Trp Val Arg Gln Ala Pro Gly
145                 150                 155                 160

Gln Gly Leu Glu Trp Met Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr
                165                 170                 175

His Tyr Asn Glu Lys Phe Lys Asp Arg Val Thr Leu Thr Thr Asp Thr
            180                 185                 190

Ser Thr Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu Arg Ser Asp Asp
        195                 200                 205

Thr Ala Val Tyr Tyr Cys Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp
    210                 215                 220

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
225                 230


<210> 157
<211> 231
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 157
Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1               5                   10                  15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val Tyr Ser
```

20 25 30

Asn Gly Asp Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Ser
85 90 95

Thr His Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr Val Ala Ala Pro Asp Val Leu Met Thr Gln Thr Pro Leu Ser
115 120 125

Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser
130 135 140

Gln Asn Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu
145 150 155 160

Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn
165 170 175

Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr
180 185 190

Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val
195 200 205

Tyr Tyr Cys Phe Gln Val Ser His Val Pro Tyr Thr Phe Gly Gly Gly
210 215 220

Thr Lys Val Glu Ile Lys Arg
225 230

<210> 158
<211> 234
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 158
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

```
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
            20                  25                  30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
            100                 105                 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Glu Val Gln Leu Val Glu
        115                 120                 125

Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys
    130                 135                 140

Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Gly Met Ser Trp Val Arg
145                 150                 155                 160

Gln Ala Pro Gly Lys Gly Leu Glu Leu Val Ala Ser Ile Asn Ser Asn
                165                 170                 175

Gly Gly Ser Thr Tyr Tyr Pro Asp Ser Val Lys Gly Arg Phe Thr Ile
            180                 185                 190

Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu
        195                 200                 205

Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ser Gly Asp Tyr Trp
    210                 215                 220

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
225                 230
```

<210> 159
<211> 231
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 159

```
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1               5                   10                  15
```

```
Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
            20                  25                  30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
                85                  90                  95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105                 110

Arg Thr Val Ala Ala Pro Asp Ile Val Met Thr Gln Ser Pro Leu Ser
        115                 120                 125

Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser
    130                 135                 140

Gln Ser Leu Val Tyr Ser Asn Gly Asp Thr Tyr Leu His Trp Tyr Leu
145                 150                 155                 160

Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn
                165                 170                 175

Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr
            180                 185                 190

Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val
        195                 200                 205

Tyr Tyr Cys Ser Gln Ser Thr His Val Pro Trp Thr Phe Gly Gly Gly
    210                 215                 220

Thr Lys Val Glu Ile Lys Arg
225                 230


<210> 160
<211> 243
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 160
Glu Val Gln Leu Val Gln Ser Gly Thr Glu Val Lys Lys Pro Gly Glu
1               5                   10                  15
```

```
Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Thr Val Thr Ser Tyr
            20                  25                  30

Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
        35                  40                  45

Gly Phe Ile Tyr Pro Gly Asp Ser Glu Thr Arg Tyr Ser Pro Thr Phe
    50                  55                  60

Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Phe Asn Thr Ala Phe
65                  70                  75                  80

Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
                85                  90                  95

Ala Arg Val Gly Ser Gly Trp Tyr Pro Tyr Thr Phe Asp Ile Trp Gly
            100                 105                 110

Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Glu
        115                 120                 125

Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser
    130                 135                 140

Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr Trp
145                 150                 155                 160

Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly
                165                 170                 175

Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe Lys
            180                 185                 190

Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr Met
        195                 200                 205

Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala
    210                 215                 220

Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr
225                 230                 235                 240

Val Ser Ser
```

<210> 161
<211> 227
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 161
Glu Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Glu Ser Ile Ser Ser Asn
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Phe Ile
35 40 45

Tyr Thr Ala Ser Thr Arg Ala Thr Asp Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Ser
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Asn Asn Trp Pro Ser
85 90 95

Ile Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg Thr Val Ala
100 105 110

Ala Pro Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr
115 120 125

Pro Gly Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val
130 135 140

His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly
145 150 155 160

Gln Ser Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly
165 170 175

Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu
180 185 190

Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe
195 200 205

Gln Val Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu
210 215 220

Ile Lys Arg
225

<210> 162
<211> 243
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 162

```
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                  10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
            20                  25                  30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
            100                 105                 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Glu Val Gln Leu Val Gln
        115                 120                 125

Ser Gly Thr Glu Val Lys Lys Pro Gly Glu Ser Leu Lys Ile Ser Cys
    130                 135                 140

Lys Gly Ser Gly Tyr Thr Val Thr Ser Tyr Trp Ile Gly Trp Val Arg
145                 150                 155                 160

Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly Phe Ile Tyr Pro Gly
                165                 170                 175

Asp Ser Glu Thr Arg Tyr Ser Pro Thr Phe Gln Gly Gln Val Thr Ile
            180                 185                 190

Ser Ala Asp Lys Ser Phe Asn Thr Ala Phe Leu Gln Trp Ser Ser Leu
        195                 200                 205

Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala Arg Val Gly Ser Gly
    210                 215                 220

Trp Tyr Pro Tyr Thr Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr
225                 230                 235                 240

Val Ser Ser
```

<210> 163
<211> 227
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 163

```
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1               5                  10                  15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
            20                  25                  30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
                85                  90                  95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105                 110

Arg Thr Val Ala Ala Pro Glu Ile Val Met Thr Gln Ser Pro Ala Thr
        115                 120                 125

Leu Ser Val Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser
    130                 135                 140

Glu Ser Ile Ser Ser Asn Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
145                 150                 155                 160

Ala Pro Arg Leu Phe Ile Tyr Thr Ala Ser Thr Arg Ala Thr Asp Ile
                165                 170                 175

Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr
            180                 185                 190

Ile Ser Ser Leu Gln Ser Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln
        195                 200                 205

Tyr Asn Asn Trp Pro Ser Ile Thr Phe Gly Gln Gly Thr Arg Leu Glu
    210                 215                 220

Ile Lys Arg
225
```

<210> 164
<211> 240
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 164

```
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1               5                   10                  15

Ser Leu Lys Ile Ser Cys Lys Val Ser Gly Tyr Phe Phe Thr Thr Tyr
            20                  25                  30

Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Tyr Met
        35                  40                  45

Gly Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe
    50                  55                  60

Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
                85                  90                  95

Ala Arg Gly Gly Asn Trp Asn Cys Phe Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Glu Val Gln Leu
        115                 120                 125

Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val
    130                 135                 140

Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr Trp Leu Gly Trp
145                 150                 155                 160

Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Asp Ile Tyr
                165                 170                 175

Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe Lys Asp Arg Val
            180                 185                 190

Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr Met Glu Leu Arg
        195                 200                 205

Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Asp
    210                 215                 220

Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
225                 230                 235                 240
```

<210> 165
<211> 226
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 165
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Tyr Gly Ala Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Arg Tyr Gly Ser Ser His
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Ser Arg Thr Val Ala Ala
100 105 110

Pro Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro
115 120 125

Gly Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His
130 135 140

Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln
145 150 155 160

Ser Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val
165 170 175

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys
180 185 190

Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln
195 200 205

Val Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
210 215 220

Lys Arg

225

<210> 166
<211> 240
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 166
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Glu Val Gln Leu Val Gln
115 120 125

Ser Gly Ala Glu Val Lys Lys Pro Gly Glu Ser Leu Lys Ile Ser Cys
130 135 140

Lys Val Ser Gly Tyr Phe Phe Thr Thr Tyr Trp Ile Gly Trp Val Arg
145 150 155 160

Gln Met Pro Gly Lys Gly Leu Glu Tyr Met Gly Ile Ile Tyr Pro Gly
165 170 175

Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe Gln Gly Gln Val Thr Ile
180 185 190

Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu Gln Trp Ser Ser Leu
195 200 205

Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala Arg Gly Gly Asn Trp
210 215 220

Asn Cys Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
225 230 235 240

<210> 167
<211> 226
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 167
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr Val Ala Ala Pro Glu Ile Val Leu Thr Gln Ser Pro Gly Thr
115 120 125

Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser
130 135 140

Gln Ser Val Ser Ser Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly
145 150 155 160

Gln Ala Pro Arg Leu Leu Ile Tyr Gly Ala Ser Arg Arg Ala Thr Gly
165 170 175

Ile Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu
180 185 190

Thr Ile Ser Arg Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln
195 200 205

Arg Tyr Gly Ser Ser His Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile
210 215 220

Ser Arg
225

<210> 168
<211> 243
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 168
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1 5 10 15

Ser Leu Lys Ile Ser Cys Gln Ser Phe Gly Tyr Ile Phe Ile Asp His
20 25 30

Thr Ile His Trp Met Arg Gln Met Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Ala Ile Ser Pro Arg His Asp Ile Thr Lys Tyr Asn Glu Met Phe
50 55 60

Arg Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Phe Cys
85 90 95

Ala Arg Gly Gly Phe Tyr Gly Ser Thr Ile Trp Phe Asp Phe Trp Gly
100 105 110

Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Glu
115 120 125

Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser
130 135 140

Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr Trp
145 150 155 160

Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly
165 170 175

Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe Lys
180 185 190

Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr Met
195 200 205

Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala
210 215 220

Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr
225 230 235 240

Val Ser Ser

<210> 169
<211> 226
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 169
Glu Thr Thr Val Thr Gln Ser Pro Ser Phe Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ile Thr Thr Thr Asp Ile Asp Asp Asp
20 25 30

Met Asn Trp Phe Gln Gln Glu Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Ser Glu Gly Asn Ile Leu Arg Pro Gly Val Pro Ser Arg Phe Ser Ser
50 55 60

Ser Gly Tyr Gly Thr Asp Phe Thr Leu Thr Ile Ser Lys Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Ser Asp Asn Leu Pro Phe
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro
115 120 125

Gly Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His
130 135 140

Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln
145 150 155 160

Ser Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val
165 170 175

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys
180 185 190

Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln

195 200 205

Val Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
210 215 220

Lys Arg
225

<210> 170
<211> 243
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 170
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Glu Val Gln Leu Val Gln
115 120 125

Ser Gly Ala Glu Val Lys Lys Pro Gly Glu Ser Leu Lys Ile Ser Cys
130 135 140

Gln Ser Phe Gly Tyr Ile Phe Ile Asp His Thr Ile His Trp Met Arg
145 150 155 160

Gln Met Pro Gly Gln Gly Leu Glu Trp Met Gly Ala Ile Ser Pro Arg
165 170 175

His Asp Ile Thr Lys Tyr Asn Glu Met Phe Arg Gly Gln Val Thr Ile
180 185 190

```
Ser Ala Asp Lys Ser Ser Ser Thr Ala Tyr Leu Gln Trp Ser Ser Leu
        195                 200                 205

Lys Ala Ser Asp Thr Ala Met Tyr Phe Cys Ala Arg Gly Gly Phe Tyr
    210                 215                 220

Gly Ser Thr Ile Trp Phe Asp Phe Trp Gly Gln Gly Thr Met Val Thr
225                 230                 235                 240

Val Ser Ser
```

<210> 171
<211> 226
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 171

```
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1               5                   10                  15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
            20                  25                  30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
                85                  90                  95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105                 110

Arg Thr Val Ala Ala Pro Glu Thr Thr Val Thr Gln Ser Pro Ser Phe
        115                 120                 125

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Ile Thr Thr
    130                 135                 140

Thr Asp Ile Asp Asp Asp Met Asn Trp Phe Gln Gln Glu Pro Gly Lys
145                 150                 155                 160

Ala Pro Lys Leu Leu Ile Ser Glu Gly Asn Ile Leu Arg Pro Gly Val
                165                 170                 175
```

```
Pro Ser Arg Phe Ser Ser Ser Gly Tyr Gly Thr Asp Phe Thr Leu Thr
            180                 185                 190

Ile Ser Lys Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln
        195                 200                 205

Ser Asp Asn Leu Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile
    210                 215                 220

Lys Arg
225
```

<210> 172
<211> 241
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 172

```
Glu Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1               5                   10                  15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp
            20                  25                  30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
        35                  40                  45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
    50                  55                  60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65                  70                  75                  80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Glu Val Gln
        115                 120                 125

Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys
    130                 135                 140

Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr Trp Leu Gly
145                 150                 155                 160

Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Asp Ile
                165                 170                 175
```

```
Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe Lys Asp Arg
            180                 185                 190

Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr Met Glu Leu
        195                 200                 205

Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser
    210                 215                 220

Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
225                 230                 235                 240

Ser
```

<210> 173
<211> 226
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 173

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Ser Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Gly Asn Thr Leu Pro Tyr
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro
        115                 120                 125

Gly Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His
    130                 135                 140

Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln
```

145 150 155 160

Ser Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val
165 170 175

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys
180 185 190

Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln
195 200 205

Val Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
210 215 220

Lys Arg
225

<210> 174
<211> 241
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 174
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr
20 25 30

Trp Leu Gly Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Asp Ile Tyr Pro Gly Tyr Asp Tyr Thr His Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Arg Val Thr Leu Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Asp Gly Ser Ser Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Glu Val Gln Leu Gln Glu
115 120 125

Ser Gly Pro Gly Leu Val Arg Pro Ser Gln Thr Leu Ser Leu Thr Cys
130 135 140

```
Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp His Ala Trp Ser Trp Val
145                 150                 155                 160

Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile Gly Tyr Ile Ser Tyr
                165                 170                 175

Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu Lys Ser Arg Val Thr Met
            180                 185                 190

Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser Leu Arg Leu Ser Ser Val
        195                 200                 205

Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Leu Ala Arg
    210                 215                 220

Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly Ser Leu Val Thr Val Ser
225                 230                 235                 240

Ser
```

<210> 175
<211> 226
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 175

```
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1               5                   10                  15

Glu Pro Ala Ser Ile Ser Cys Thr Ser Ser Gln Asn Ile Val His Ser
            20                  25                  30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Val
                85                  90                  95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105                 110

Arg Thr Val Ala Ala Pro Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
        115                 120                 125
```

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
130 135 140

Gln Asp Ile Ser Ser Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys
145 150 155 160

Ala Pro Lys Leu Leu Ile Tyr Tyr Thr Ser Arg Leu His Ser Gly Val
165 170 175

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr
180 185 190

Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln
195 200 205

Gly Asn Thr Leu Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
210 215 220

Lys Arg
225

<210> 176
<211> 7185
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 176
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ggcacagcgg ccctgggctg cctggtcaag gactacttcc ccgaaccggt gacggtgtcg 120

tggaactcag gcgccctgac cagcggcgtg cacaccttcc cggctgtcct acagtcctca 180

ggactctact ccctcagcag cgtggtgacc gtgccctcca gcagcttggg cacccagacc 240

tacatctgca acgtgaatca caagcccagc aacaccaagg tggacaagaa agttgagccc 300

aaatcttgtg acaaaactca cacatgccca ccgtgcccag cacctgaact cctgggggga 360

ccgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggaccccct 420

gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg 480

tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac 540

agcacgtacc gtgtggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag 600

gagtacaagt gcaaggtctc caacaaagcc ctcccagccc ccatcgagaa aaccatctcc 660

aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgccccatc ccgcgaggag 720

atgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc 780

gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg 840

ctggactccg acggctcctt cttcctctac agcaagctca ccgtggacaa gagcaggtgg 900

cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg 960

cagaagagcc tctccctgtc tccgggtaaa tgagcggccg ctcgaggccg gcaaggccgg 1020

atcccccgac ctcgacctct ggctaataaa ggaaatttat tttcattgca atagtgtgtt 1080

ggaatttttt gtgtctctca ctcggaagga catatgggag ggcaaatcat ttggtcgaga 1140

tccctcggag atctctagct agaggatcga tccccgcccc ggacgaacta aacctgacta 1200

cgacatctct gccccttctt cgcggggcag tgcatgtaat cccttcagtt ggttggtaca 1260

acttgccaac tgggccctgt tccacatgtg acacgggggg ggaccaaaca caaaggggtt 1320

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ttcatcctgg agcagacttt gcagtctgtg gactgcaaca caacattgcc tttatgtgta 1440

actcttggct gaagctctta caccaatgct gggggacatg tacctcccag gggcccagga 1500

agactacggg aggctacacc aacgtcaatc agaggggcct gtgtagctac cgataagcgg 1560

accctcaaga gggcattagc aatagtgttt ataaggcccc cttgttaacc ctaaacgggt 1620

agcatatgct tcccgggtag tagtatatac tatccagact aaccctaatt caatagcata 1680

tgttacccaa cgggaagcat atgctatcga attagggtta gtaaaagggt cctaaggaac 1740

agcgatatct cccaccccat gagctgtcac ggttttattt acatggggtc aggattccac 1800

gagggtagtg aaccatttta gtcacaaggg cagtggctga agatcaagga gcgggcagtg 1860

aactctcctg aatcttcgcc tgcttcttca ttctccttcg tttagctaat agaataactg 1920

ctgagttgtg aacagtaagg tgtatgtgag gtgctcgaaa acaaggtttc aggtgacgcc 1980

cccagaataa aatttggacg gggggttcag tggtggcatt gtgctatgac accaatataa 2040

ccctcacaaa ccccttgggc aataaatact agtgtaggaa tgaaacattc tgaatatctt 2100

taacaataga aatccatggg gtggggacaa gccgtaaaga ctggatgtcc atctcacacg 2160

aatttatggc tatgggcaac acataatcct agtgcaatat gatactgggg ttattaagat 2220

gtgtcccagg cagggaccaa gacaggtgaa ccatgttgtt acactctatt tgtaacaagg 2280

ggaaagagag tggacgccga cagcagcgga ctccactggt tgtctctaac acccccgaaa 2340

attaaacggg gctccacgcc aatggggccc ataaacaaag acaagtggcc actcttttt 2400

ttgaaattgt ggagtggggg cacgcgtcag cccccacacg ccgccctgcg gttttggact 2460

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acttgcccac aaaaccacta atggcacccc ggggaatacc tgcataagta ggtgggcggg 2580

ccaagatagg ggcgcgattg ctgcgatctg gaggacaaat tacacacact tgcgcctgag 2640

cgccaagcac agggttgttg gtcctcatat tcacgaggtc gctgagagca cggtgggcta 2700

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ggttaatgtc atgataataa tggtttctta gacgtcaggt ggcacttttc ggggaaatgt 3420

gcgcggaacc cctatttgtt tatttttcta aatacattca aatatgtatc cgctcatgag 3480

acaataaccc tgataaatgc ttcaataata ttgaaaaagg aagagtatga gtattcaaca 3540

tttccgtgtc gcccttattc cctttttttgc ggcattttgc cttcctgttt ttgctcaccc 3600

agaaacgctg gtgaaagtaa aagatgctga agatcagttg ggtgcacgag tgggttacat 3660

cgaactggat ctcaacagcg gtaagatcct tgagagtttt cgccccgaag aacgttttcc 3720

aatgatgagc acttttaaag ttctgctatg tggcgcggta ttatcccgtg ttgacgccgg 3780

gcaagagcaa ctcggtcgcc gcatacacta ttctcagaat gacttggttg agtactcacc 3840

agtcacagaa aagcatctta cggatggcat gacagtaaga gaattatgca gtgctgccat 3900

aaccatgagt gataacactg cggccaactt acttctgaca acgatcggag gaccgaagga 3960

gctaaccgct tttttgcaca acatggggga tcatgtaact cgccttgatc gttgggaacc 4020

ggagctgaat gaagccatac caaacgacga gcgtgacacc acgatgcctg cagcaatggc 4080

aacaacgttg cgcaaactat taactggcga actacttact ctagcttccc ggcaacaatt 4140

aatagactgg atggaggcgg ataaagttgc aggaccactt ctgcgctcgg cccttccggc 4200

tggctggttt attgctgata aatctggagc cggtgagcgt gggtctcgcg gtatcattgc 4260

agcactgggg ccagatggta agccctcccg tatcgtagtt atctacacga cggggagtca 4320

ggcaactatg gatgaacgaa atagacagat cgctgagata ggtgcctcac tgattaagca 4380

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acgtgagttt tcgttccact gagcgtcaga ccccgtagaa aagatcaaag gatcttcttg 4560

agatcctttt tttctgcgcg taatctgctg cttgcaaaca aaaaaaccac cgctaccagc 4620

ggtggtttgt ttgccggatc aagagctacc aactcttttt ccgaaggtaa ctggcttcag 4680

cagagcgcag ataccaaata ctgttcttct agtgtagccg tagttaggcc accacttcaa 4740

gaactctgta gcaccgccta catacctcgc tctgctaatc ctgttaccag tggctgctgc 4800

cagtggcgat aagtcgtgtc ttaccgggtt ggactcaaga cgatagttac cggataaggc 4860

gcagcggtcg ggctgaacgg ggggttcgtg cacacagccc agcttggagc gaacgaccta 4920

caccgaactg agatacctac agcgtgagct atgagaaagc gccacgcttc ccgaagggag 4980

aaaggcggac aggtatccgg taagcggcag ggtcggaaca ggagagcgca cgagggagct 5040

tccaggggga aacgcctggt atctttatag tcctgtcggg tttcgccacc tctgacttga 5100

gcgtcgattt ttgtgatgct cgtcaggggg gcggagccta tggaaaaacg ccagcaacgc 5160

ggccttttta cggttcctgg ccttttgctg gccttttgct cacatgttct ttcctgcgtt 5220

atcccctgat tctgtggata accgtattac cgcctttgag tgagctgata ccgctcgccg 5280

cagccgaacg accgagcgca gcgagtcagt gagcgaggaa gcggaagagc gcccaatacg 5340

caaaccgcct ctccccgcgc gttggccgat tcattaatgc agctggcacg acaggtttcc 5400

cgactggaaa gcgggcagtg agcgcaacgc aattaatgtg agttagctca ctcattaggc 5460

accccaggct ttacacttta tgcttccggc tcgtatgttg tgtggaattg tgagcggata 5520

acaatttcac acaggaaaca gctatgacca tgattacgcc aagctctagc tagaggtcga 5580

gtccctcccc agcaggcaga agtatgcaaa gcatgcatct caattagtca gcaaccatag 5640

tcccgcccct aactccgccc atcccgcccc taactccgcc cagttccgcc cattctccgc 5700

cccatggctg actaattttt tttatttatg cagaggccga ggccgcctcg gcctctgagc 5760

tattccagaa gtagtgagga ggcttttttg gaggcctagg cttttgcaaa aagctttgca 5820

aagatggata aagttttaaa cagagaggaa tctttgcagc taatggacct tctaggtctt 5880

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