

SOLID PHASE CELL LYSIS AND CAPTURE PLATFORM

BACKGROUND OF THE INVENTION

[0001] The present invention relates to the isolation of cellular components, such as polypeptides and nucleic acids, from host cells.

[0002] Recent advances in recombinant DNA technology have made it possible to produce large quantities of peptides in host cells. The extraction and isolation of target peptides, proteins, nucleic acids, or other cellular components from their host cells, however, has thus far been a multiple step process, involving first lysis and then one or more subsequent steps to separate the target product from other cellular components.

[0003] A variety of techniques have been used to lyse cells, each having certain advantages and disadvantages. For example, sonication, French press cell, homogenization, grinding, freeze-thaw lysis, and various other methods of physically or mechanically lysing cells have been used; see, *e.g.*, Bollag & Edelstein, Protein Extraction, in Protein Methods, 27-43 (1993); Schutte & Kula, Biotech. and App. Biochem., 12:559-620 (1990); and Hughes, et al., Methods in Microbiology, 5B:1-54 (1969). Mechanical lysis, however, requires specialized equipment that may not be readily available and, in addition, sonication also generates heat that may be detrimental to some proteins. Enzymes and detergents have also been used to enzymatically or chemically lyse cells; see, *e.g.*, Hughes, et al., Methods in Microbiology, 5B:1-54; Andrews & Asenjo, Trends in Biotech., 5:273-77 (1987); Wiseman, Process Biochem., 63-65 (1969); and Wolska-Mitaszko, et al., Analytical Biochem., 116:241-47 (1981).

[0004] The addition of an enzyme or detergent solution, however, results in a dilution of the solution containing the cells to be lysed and, in addition, the desired product must still be separated from resulting membrane fragments, undesired proteins, and other cellular debris.

[0005] Similarly, a variety of affinity capture methods have been employed to purify peptides, proteins and nucleic acids. U.S. Patent Nos. 4,569,794, 5,310,663, and 5,594,115 describe the use of metal chelating peptides, which include histidine residues, and their use in protein purification. U.S. Patent Nos. 4,703,004, 4,851,341, 5,011,912, and 6,461,154 describe the antigenic FLAG[®] peptide, and the purification of proteins comprising the peptide. U.S. Patent No. 5,654,176 describes the use of glutathione-S-transferase for the purification of proteins. U.S. Patent No. 5,998,155 describes the use of an avidin/biotin capture system. In each of these instances, the interaction between an affinity tag or sequence on the target product and the corresponding ligand results in the "capture" of the target product. Unbound compositions and other cellular debris can then be washed away, leaving the target product bound to the tag- or sequence-specific ligand. A specific eluant is then used to release the bound target product, resulting in a purified target product.

[0006] Disadvantageously, the multiple steps involved in first lysing a host cell and then purifying the target product increases the cost and time required for isolating the product, especially in high throughput applications.

SUMMARY OF THE INVENTION

[0007] Among the various aspects of the present invention, therefore, is the provision of a relatively fast, efficient method for lysing cells and capturing peptides, proteins, nucleic acids, or other cellular components. Advantageously, the process and container of the present invention eliminate the need to centrifuge a cellular solution to remove insoluble material, pipette in additional detergent lysis liquids or enzymatic inhibitors (thereby diluting the original cell-containing solution), or perform subsequent purification steps.

[0008] Briefly, therefore, the present invention is directed to a container for the extraction of a cellular component from a host cell. The container comprises a mouth, an interior surface, and a coating of a lytic reagent on at least a portion of the interior surface wherein the amount of the lytic reagent in the coating is sufficient for the formation of a lysis solution having the capacity to lyse the host cell when a liquid suspension containing the host cell is introduced into the container. In one embodiment, the ratio of the area of the coated interior surface to the volume of the container is less than about 4 mm²/μl.

[0009] In another aspect, the present invention is directed to a container for the extraction and isolation of a cellular component from a host cell. The container comprises a mouth, an interior surface, a volume, a lytic reagent, and a supported capture ligand. The mouth serves as the inlet for the introduction of liquid into and the outlet for the removal of liquid from the container, and the capture ligand is supported at a location in the container which allows the capture ligand to contact intact host cells or solid cellular components derived therefrom when a liquid suspension containing the intact host cells or solid cellular components is introduced into the container through its mouth.

[0010] In another aspect, the present invention is directed to a multiwell plate for the extraction of a cellular component from a host cell, wherein at least one of the wells of the multiwell plate contains a lytic reagent. The lytic reagent is (i) coated onto at least a portion of the interior surface of the well(s), or (ii) is in the form of a mass of material contained within the well(s).

[0011] In another aspect, the present invention is directed to a process for the extraction of a cellular component from a host cell. The process comprises (a) introducing a liquid suspension containing the host cell into a container, the container having a mouth, an interior surface, a volume, and a coating of a lytic reagent on at least a portion of the interior surface, the ratio of the area of the coated interior surface to the volume of the container being less than about 4 mm²/μl, and (b) lysing the host cell in the container to release the cellular component and form cellular debris.

[0012] In another aspect, the present invention is directed to a process for the extraction and isolation of a cellular component from a host cell. The process comprises (a) introducing a liquid suspension containing the host cell into a container, the container having a mouth, an interior surface, a volume, a lytic reagent, and a supported capture ligand, wherein the mouth serves as the inlet for the introduction of the liquid into and the outlet for the removal of the liquid from the container, (b) lysing the host cell in the container to release the cellular component and form solid cellular debris; and (c) capturing the cellular component with the capture ligand in the presence of the solid cellular debris.

[0013] In another aspect, the present invention is directed to a process for the extraction and isolation of a cellular component from a host cell. The process comprises (a) introducing a liquid suspension containing the host cell into a container, the container having a mouth, an interior surface, a volume, a lytic reagent, and a supported capture ligand, wherein the mouth serves as the inlet for the introduction of the liquid into the container, (b) lysing the host cell in the container to release the cellular component and form solid cellular debris; and (c) capturing the cellular component with the capture ligand in the presence of the solid cellular debris; (d) releasing the cellular component from the capture ligand, and (e) recovering the released cellular component.

[0014] In another aspect, the present invention is directed to a kit for the extraction and isolation of a cellular component from a host cell. The kit comprises a container of the present invention, and instructions for the extraction and isolation of the cellular component from the host cell. In another embodiment, the kit further comprises additional reagents for extracting and/or isolating the cellular component from a host cell, and/or reagents for assaying or detecting a captured cellular component.

[0015] In another aspect, the present invention is directed to a process for the preparation of a container for the extraction of a cellular component from a host cell, the process comprising contacting the interior surfaces of the

container with a liquid containing the lytic reagent and drying the liquid to form an adsorbed layer of lytic reagent on the interior surfaces of the container.

[0016] Other objects and features of the invention will be in part apparent and in part pointed out hereinafter.

BRIEF DESCRIPTION OF THE FIGURES

[0017] FIG. 1 depicts an image of a SDS-PAGE gel of material that was eluted from a HIS-Select™ high capacity plate. Lytic reagents were dried onto the surface of the plate and 0.1 ml of cells were added. The contents of each lane are described in Table 1. This figure illustrates that protein can be bound to the plate in the presence of the crude lysed cells. Increasing amounts of protein can be bound and eluted with varying reagents under these conditions.

[0018] FIG. 2 depicts an image of a SDS-PAGE gel of material that was eluted from a HIS-Select™ high capacity plate. Lytic reagents (0.05 ml) were dried onto the surface of the plate, and 0.1 ml of cells or pure protein was added to each well. The contents of each lane are described in Table 3. This figure illustrates that the protein can be bound in the presence or absence of the crude lysed cells. Increasing amounts of protein can be bound and eluted under these conditions.

[0019] FIG. 3 depicts an image of a SDS-PAGE gel of material that was eluted from a HIS-Select™ high capacity plate. Lytic reagents (0.1 ml) were dried onto the surface of the plate and 0.1 ml of cells or pure protein was added to each well. The contents of each lane are described in Table 3. This figure illustrates that the protein can be bound in the presence or absence of the crude lysed cells. Increasing amounts of protein can be bound and eluted under these conditions.

[0020] Fig. 4 depicts corrected absorbance (A_{450}) readings from an enzyme immunodetection assay using an ANTI-FLAG® M2 high sensitivity plate. The striped bars on the chart represent results for proteins with a DYKDDDDK (SEQ. ID. NO. 1) tag; the bars with horizontal lines represent results for proteins

with a DYKDDDDK (SEQ. ID. NO. 1) /his tag; the white bars represent results for proteins with a his-tag. The lytic reagents used are described in Example 4, and represented on the chart by the letters A-H.

[0021] Fig. 5 depicts corrected absorbance (A_{450}) readings from an enzyme immunodetection assay using a HIS-Select™ high sensitivity plate. The striped bars on the chart represent results for proteins with a DYKDDDDK (SEQ. ID. NO. 1) tag; the bars with horizontal lines represent results for proteins with a DYKDDDDK (SEQ. ID. NO. 1) /his tag; the white bars represent results for proteins with a his-tag. The lytic reagents used are described in Example 4, and represented on the chart by the letters A-H.

[0022] Fig. 6 depicts an image of a SDS-PAGE gel of material that was eluted from a HIS-Select™ high capacity plate. Various combinations of lytic reagents, processing reagents, and enzymes were dried onto the surface of a HIS-Select™ high capacity plate, and cells comprising a target protein were added. The contents of each lane are described in Table 6. This figure illustrates that the various lysis reagents were capable of lysing the cells, and that the target protein was successfully captured and eluted from the HIS-Select™ high capacity plate.

[0023] Fig. 7 depicts a container of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

1. Container

[0024] In general, the container of the present invention is suitable for holding a liquid, the container comprising a bottom, a mouth, and a sidewall formation. In one embodiment, the sidewall formation may have any of a variety of geometric shapes; for example, in this embodiment, the sidewall formation may be cylindrical, polygonal, conical, or concave (e.g., hemispherical). Similarly, in one embodiment, the bottom has any of a variety of geometric shapes; for example, in this embodiment, the bottom may be flat, curved or even comprise a single point (e.g., the lower most point of an inverted cone). The

mouth serves as an opening through which a liquid may be introduced to the container; in one embodiment, the mouth and the bottom are at opposite ends of the sidewall formation with the mouth being defined by the opening at the top of the sidewall formation. In its various embodiments, therefore, the container may be a cylinder, flask, jar, beaker, vial, bottle, column, or even a depression in a surface. In addition, the container may be presented as a single, free-standing, receptacle or it may be one of a plurality of physically integrated receptacles. In one embodiment, therefore, the container is an individual well of a unitary multiwell plate such as a 48 well, 96 well, 384 well, 1536 well, etc., multiwell plate. Also, the container may have a permanently closed bottom or the bottom may comprise a valved or capped opening through which liquid in the container may optionally be removed.

[0025] Containers used for the extraction or the extraction and affinity capture of peptides, protein, nucleic acids, or other cellular components may be of a variety of dimensions, and need not contain large volumes of liquids. In general, the container will hold a volume of less than 50 L. In one embodiment, the container will hold a volume of no more than 1 L, but no less than 1.0 μl . In another embodiment, the container will hold a volume from about 10 μl to about 100 ml.

[0026] The interior surface of the container, which comprises the sidewall formation and bottom, defines the liquid volume capacity of the container. In one embodiment, the ratio of the surface area defined by the interior surface to the volume defined by the interior surfaces is less than about 4 $\text{mm}^2/\mu\text{l}$. In another embodiment, the surface area to volume ratio defined by the interior surface of the container does not exceed about 3 $\text{mm}^2/\mu\text{l}$. In another embodiment, the surface area to volume ratio defined by the interior surface of the container does not exceed about 2 $\text{mm}^2/\mu\text{l}$. In another embodiment, the surface area to volume ratio defined by the interior surface of the container does not exceed about 1 $\text{mm}^2/\mu\text{l}$.

[0027] Depending upon the intended use and operator preferences, the containers may optionally be sealed. In one embodiment, therefore, the container comprises a lid or cap which fits over the mouth to isolate the contents of the container from the surrounding ambient. In another embodiment, the top of the container is open to the environment. Thus, for example, when the container is in the format of a multiwell plate, (i) each well may be individually sealed by a separate lid (e.g., a plastic cover wrapping), (ii) a fraction or a plurality of wells may be sealed by a common lid, leaving the remaining fraction of wells open to the surrounding ambient, (iii) all of the wells may be sealed by a common lid, or (iv) all of the wells may be open to the surrounding ambient. In addition, the lid may comprise a single port for the introduction of liquid into the container or it may comprise a plurality of ports for the introduction or introduction and removal of liquid from the container. In another embodiment, when the bottom of the container comprises an opening through which liquid in the container may optionally be removed, the mouth and bottom of the container may both optionally be capped.

[0028] In general, the container may be formed from a variety of natural or synthetic materials. For example, the container may be plastic, silica, glass, metal, ceramic, magnetite, polyesters, polystyrene, polypropylene, polyethylene, nylon, polyacrylamide, cellulose, nitrocellulose, latex, etc.

2.Capture Ligands and Product Purification

[0029] Once the host cell has been lysed, the cellular components may be isolated and separated from other cellular debris through the use of a capture ligand immobilized on a support material in the container. The capture ligand may be supported directly or indirectly by the interior surface of the container or by a bead or other support which is placed in, affixed to, or otherwise held in the container. In one embodiment, the capture ligand is positioned on the bottom of the container. In another embodiment, the capture ligand is positioned on a sidewall formation. In another embodiment, the capture ligand is positioned on

both the bottom and the sidewall formation of a container. In another embodiment, the supported capture ligand is positioned in the container at a location which allows the capture ligand to be exposed to intact host cells or solid cellular components derived therefrom which may be present in the container.

[0030] Advantageously, the reagents, components and methods of the present invention permit a range of capture ligands to be used. In one preferred embodiment, the capture ligands are capable of isolating the cellular component in a liquid suspension comprising cellular debris.

[0031] A variety of techniques for purifying proteins, peptides, DNA, RNA, or other cellular components are well known in the art, and can be used in conjunction with the containers and processes described herein. See, e.g., Becker, et al., *Biotech. Advs.*, 1:247-61 (1983). In one embodiment, any capture method may be used, so long as the presence of the lytic reagent does not interfere with binding. For example, a common method of protein purification involves the production of a fusion protein comprising the target protein and an affinity tag capable of binding with high specificity to an affinity matrix. Thus, in one aspect, the containers of the present invention comprise a supported capture ligand capable of binding with high specificity the affinity tag of the target protein or peptide, thus resulting in isolation of the target protein or peptide from other proteins and cellular debris. In some instances, the target protein or peptide naturally contains a sequence capable of binding to a corresponding capture ligand. In this instance, the protein need not be recombinant, so long as there is a capture ligand capable of binding the target protein or peptide. Some specific examples of well known affinity capture systems that can be used to capture proteins or peptides include (i) metal chelate chromatography (e.g., nickel or cobalt interactions with histidine tags), (ii) immunogenic capture systems, such as those using antigen-antibody interactions (e.g., the FLAG[®] peptide, c-myc tags, HA tags, etc.), (iii) a glutathione-S-transferase (GST) capture system, and (iv) the biotin-

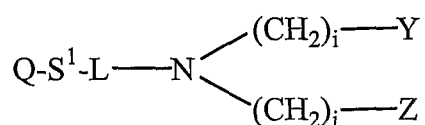
avidin/streptavidin capture system. Other techniques include ion exchange chromatography, including both anion and cation exchange, as well as hydrophobic chromatography, and thiophilic chromatography. Combinations of these various capture methods may also be used, such as with mixed mode chromatography. These techniques are a few of the techniques commonly used to purify proteins. Hydrophobic chromatography, ion exchange chromatography, and various hybridization techniques, for example, utilizing nucleotide sequences with specificity for the target DNA or RNA, are also commonly used to purify DNA and RNA. Another common RNA capture method is poly (dT). Since these and other capture systems are well known in the art, they will only be described briefly herein.

[0032] Immobilized metal affinity chromatography ("IMAC") uses the affinity of certain residues within proteins for metal ions, to purify proteins. In IMAC, metal ions are immobilized onto a solid support, and used to capture proteins comprising a metal chelating peptide. The metal chelating peptide may occur naturally in the protein, or the protein may be a recombinant protein with an affinity tag comprising a metal chelating peptide. Some of the most commonly used metal ions include nickel (Ni^{2+}), zinc (Zn^{2+}), copper (Cu^{2+}), iron (Fe^{3+}), cobalt (Co^{2+}), calcium (Ca^{2+}), aluminum (Al^{3+}), magnesium (Mg^{2+}), manganese (Mn^{2+}), and gallium (Ga^{3+}). Thus, in one embodiment, the container and/or support comprises metal ions immobilized upon its surface, or a portion thereof, wherein the metal ions are selected from the group consisting of nickel (Ni^{2+}), zinc (Zn^{2+}), copper (Cu^{2+}), iron (Fe^{3+}), cobalt (Co^{2+}), calcium (Ca^{2+}), aluminum (Al^{3+}), magnesium (Mg^{2+}), manganese (Mn^{2+}), and gallium (Ga^{3+}). Preferably, the metal ion is nickel, copper, cobalt, or zinc. Most preferably, the metal ion is nickel.

[0033] A variety of proteins that contain a metal chelating peptide may be purified in this way. In one embodiment, the metal chelating peptide may have the formula His-X, wherein X is selected from the group consisting of Gly, His, Tyr, Trp, Val, Leu, Ser, Lys, Phe, Met, Ala, Glu, Ile, Thr, Asp, Asn, Gln, Arg,

Cys, and Pro, as described more fully in Smith, et al. (1986) U.S. Patent No. 4,569,794, incorporated herein by reference. The metal chelating peptide may also have the formula $(\text{His-X})_n$, wherein X is selected from the group consisting of Asp, Pro, Glu, Ala, Gly, Val, Ser, Leu, Ile, or Thr, and n is 3 to 6, as described more fully in Sharma, et al. (1997) U.S. Patent No. 5,594,115, incorporated herein by reference. In another embodiment, the metal chelating peptide includes a poly(His) tag of the formula $(\text{His})_y$, wherein y is at least 2-6, as described more fully in Dobeli, et al. (1994) U.S. Patent No. 5,310,663, incorporated herein by reference. Other examples of metal chelating peptides will be known to those in the art.

[0034] In one embodiment, the capture ligand is a metal chelate as described in WO 01/81365. More specifically, in this embodiment the capture ligand is a metal chelate derived from metal chelating composition (1):



[0035] (1)

[0036] wherein

[0037] Q is a carrier;

[0038] S^1 is a spacer;

[0039] L is -A-T-CH(X)- or -C(=O)-;

[0040] A is an ether, thioether, selenoether, or amide linkage;

[0041] T is a bond or substituted or unsubstituted alkyl or alkenyl;

[0042] X is $-(\text{CH}_2)_k\text{CH}_3$, $-(\text{CH}_2)_k\text{COOH}$, $-(\text{CH}_2)_k\text{SO}_3\text{H}$, $-(\text{CH}_2)_k\text{PO}_3\text{H}_2$, $-(\text{CH}_2)_k\text{N}(\text{J})_2$, or $-(\text{CH}_2)_k\text{P}(\text{J})_2$, preferably $-(\text{CH}_2)_k\text{COOH}$ or $-(\text{CH}_2)_k\text{SO}_3\text{H}$;

[0043] k is an integer from 0 to 2;

[0044] J is hydrocarbyl or substituted hydrocarbyl;

[0045] Y is $-\text{COOH}$, $-\text{H}$, $-\text{SO}_3\text{H}$, $-\text{PO}_3\text{H}_2$, $-\text{N}(\text{J})_2$, or $-\text{P}(\text{J})_2$, preferably, $-\text{COOH}$;

[0046] Z is -COOH, -H, -SO₃H, -PO₃H₂, -N(J)₂, or -P(J)₂, preferably, -COOH; and

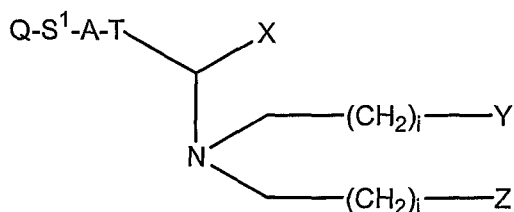
[0047] i is an integer from 0 to 4, preferably 1 or 2.

[0048] In general, the carrier, Q, may comprise any solid or soluble material or compound capable of being derivatized for coupling. Solid (or insoluble) carriers may be selected from a group including agarose, cellulose, methacrylate co-polymers, polystyrene, polypropylene, paper, polyamide, polyacrylonitrile, polyvinylidene, polysulfone, nitrocellulose, polyester, polyethylene, silica, glass, latex, plastic, gold, iron oxide and polyacrylamide, but may be any insoluble or solid compound able to be derivatized to allow coupling of the remainder of the composition to the carrier, Q. Soluble carriers include proteins, nucleic acids including DNA, RNA, and oligonucleotides, lipids, liposomes, synthetic soluble polymers, proteins, polyamino acids, albumin, antibodies, enzymes, streptavidin, peptides, hormones, chromogenic dyes, fluorescent dyes, fluorochemicals or any other detection molecule, drugs, small organic compounds, polysaccharides and any other soluble compound able to be derivatized for coupling the remainder of the composition to the carrier, Q. In one embodiment, the carrier, Q, is the container of the present invention. In another embodiment, the carrier, Q, is a body provided within the container of the present invention.

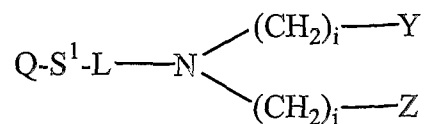
[0049] The spacer, S¹, which flanks the carrier comprises a chain of atoms which may be saturated or unsaturated, substituted or unsubstituted, linear or cyclic, or straight or branched. Typically, the chain of atoms defining the spacer, S¹, will consist of no more than about 25 atoms; stated another way, the backbone of the spacer will consist of no more than about 25 atoms. More preferably, the chain of atoms defining the spacer, S¹, will consist of no more than about 15 atoms, and still more preferably no more than about 12 atoms. The chain of atoms defining the spacer, S¹, will typically be selected from the group consisting of carbon, oxygen, nitrogen, sulfur, selenium, silicon and phosphorous and preferably from the group consisting of carbon, oxygen,

nitrogen, sulfur and selenium. In addition, the chain atoms may be substituted or unsubstituted with atoms other than hydrogen such as hydroxy, keto ($=O$), or acyl such as acetyl. Thus, the chain may optionally include one or more ether, thioether, selenoether, amide, or amine linkages between hydrocarbyl or substituted hydrocarbyl regions. Exemplary spacers, S^1 , include methylene, alkyleneoxy $-(CH_2)_aO-$, alkyleneethioether $-(CH_2)_aS-$, alkylene-selenoether $-(CH_2)_aSe-$, alkyleneamide $-(CH_2)_aNR^1C(=O)-$, alkylene-carbonyl $-(CH_2)_aC(=O)-$, and combinations thereof wherein a is generally from 1 to about 20 and R^1 is hydrogen or hydrocarbyl, preferably alkyl. In one embodiment, the spacer, S^1 , is a hydrophilic, neutral structure and does not contain any amine linkages or substituents or other linkages or substituents which could become electrically charged during the purification of a polypeptide.

[0050] As noted above, the linker, L , may be $-A-T-CH(X)-$ or $-C(=O)-$. When L is $-A-T-CH(X)-$, the chelating composition corresponds to the formula:



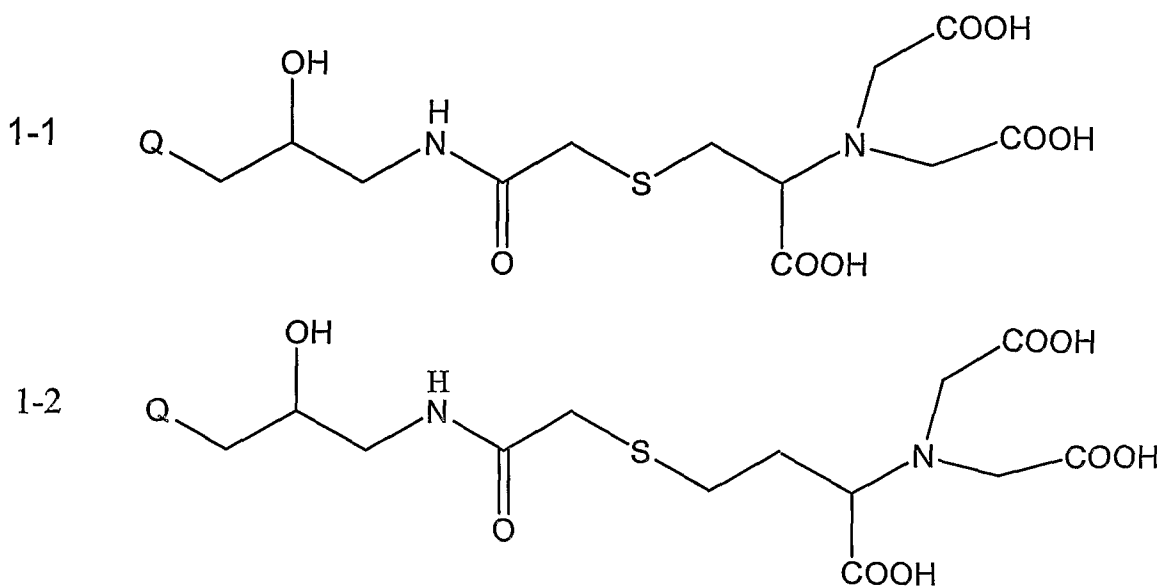
[0051] wherein Q , S^1 , A , T , X , Y , and Z are as previously defined. In this embodiment, the ether ($-O-$), thioether ($-S-$), selenoether ($-Se-$) or amide ($-NR^1C(=O)-$) or ($-C(=O)NR^1-$) wherein R^1 is hydrogen or hydrocarbyl) linkage is separated from the chelating portion of the molecule by a substituted or unsubstituted alkyl or alkenyl region. If other than a bond, T is preferably substituted or unsubstituted C_1 to C_6 alkyl or substituted or unsubstituted C_2 to C_6 alkenyl. More preferably, A is $-S-$, T is $-(CH_2)_n-$, and n is an integer from 0 to 6, typically 0 to 4, and more typically 0, 1 or 2. When L is $-C(=O)-$, the chelating composition corresponds to the formula:

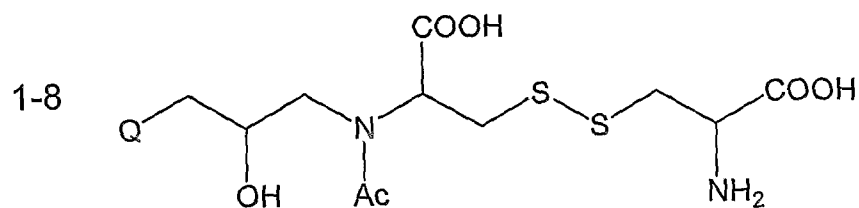
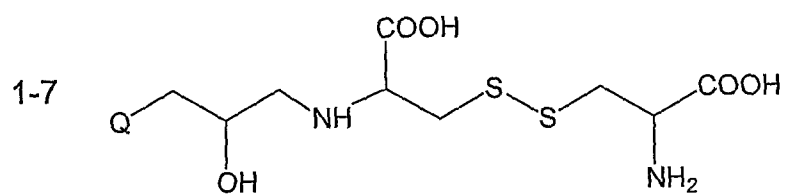
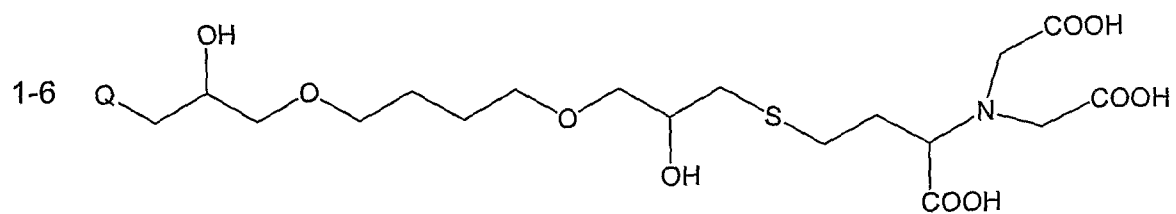
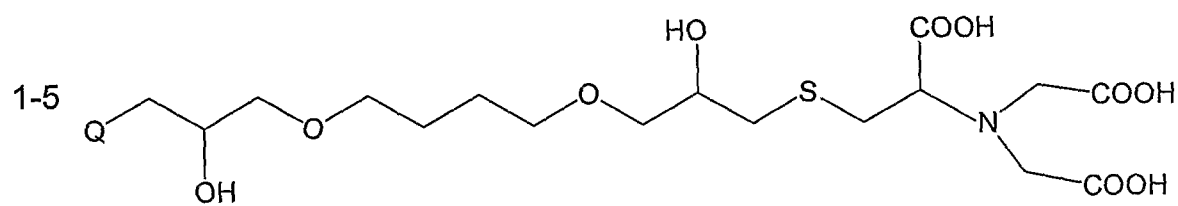
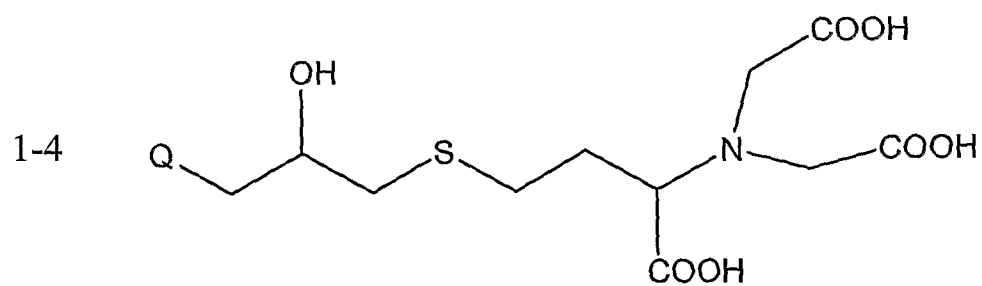
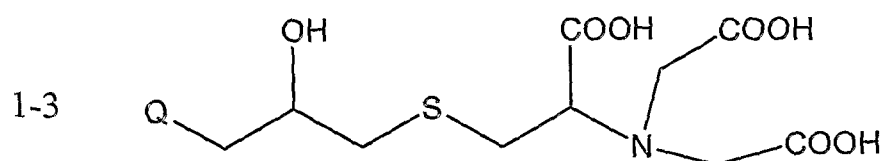


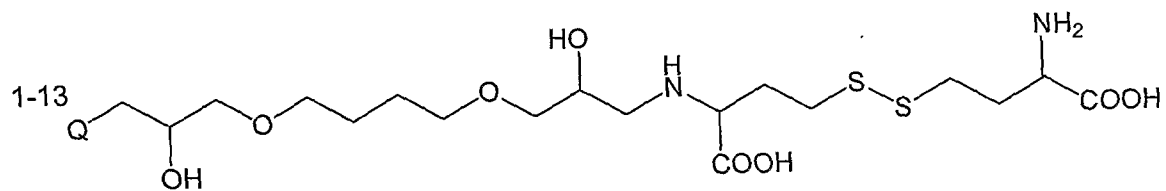
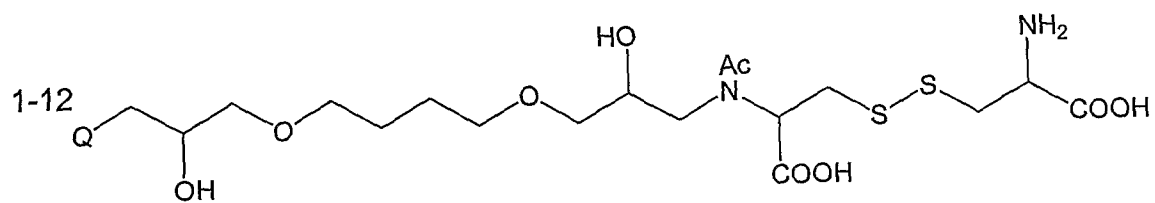
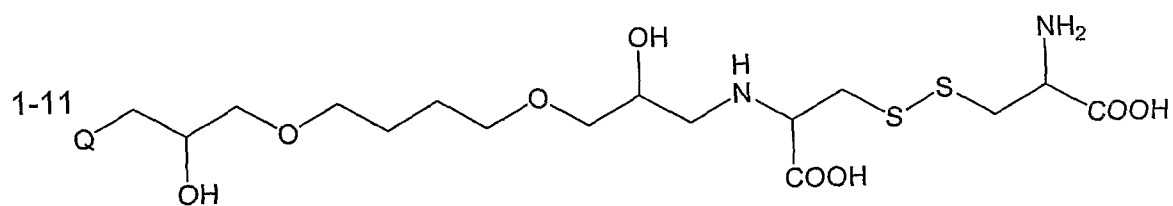
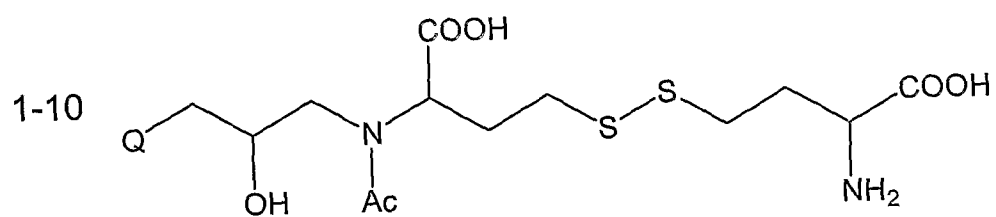
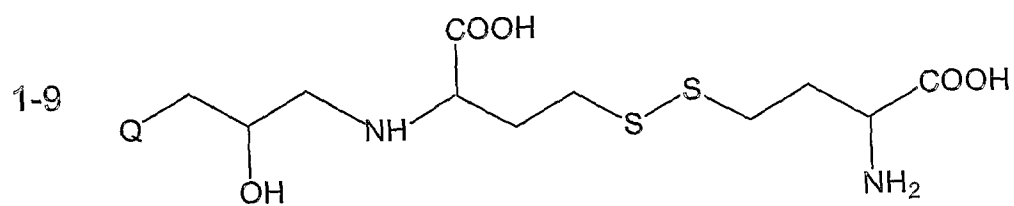
[0052] wherein Q, S¹, i, Y, and Z are as previously defined.

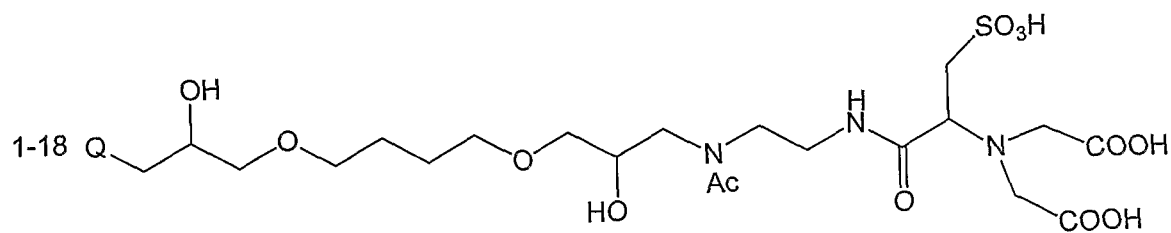
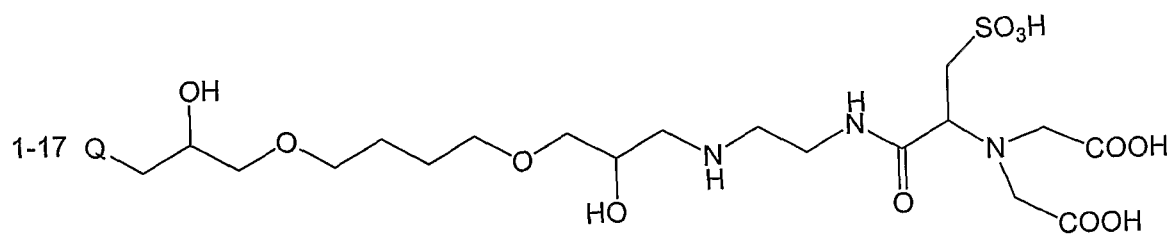
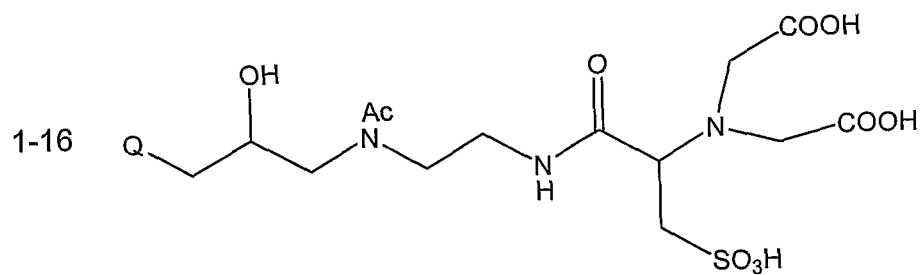
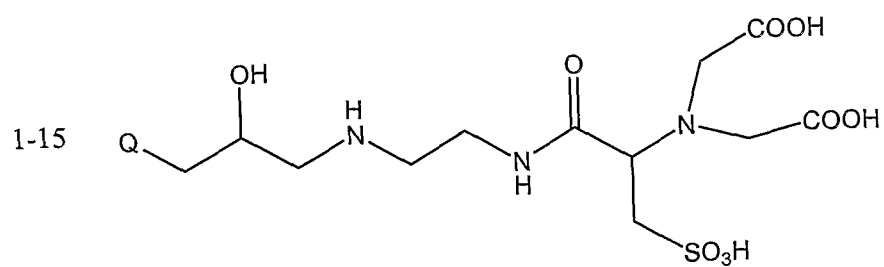
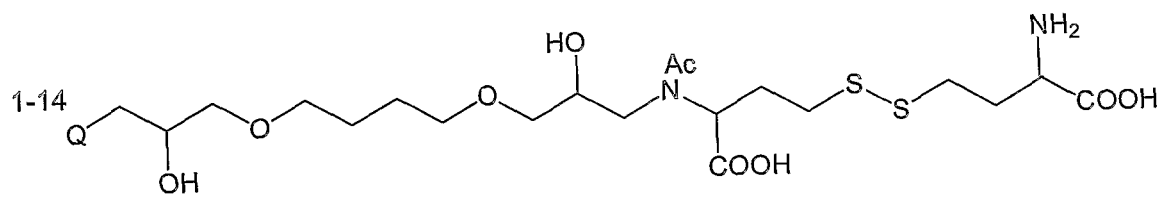
[0053] In a preferred embodiment, the sequence -S¹-L-, in combination, is a chain of no more than about 35 atoms selected from the group consisting of carbon, oxygen, sulfur, selenium, nitrogen, silicon and phosphorous, more preferably only carbon, oxygen sulfur and nitrogen, and still more preferably only carbon, oxygen and sulfur. To reduce the prospects for non-specific binding, nitrogen, when present, is preferably in the form of an amide moiety. In addition, if the carbon chain atoms are substituted with anything other than hydrogen, they are preferably substituted with hydroxy or keto. In a preferred embodiment, L comprises a portion (sometimes referred to as a fragment or residue) derived from an amino acid such as cystine, homocystine, cysteine, homocysteine, aspartic acid, cysteic acid or an ester thereof such as the methyl or ethyl ester thereof.

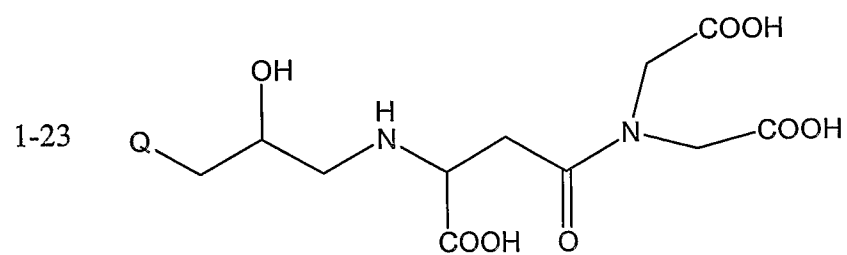
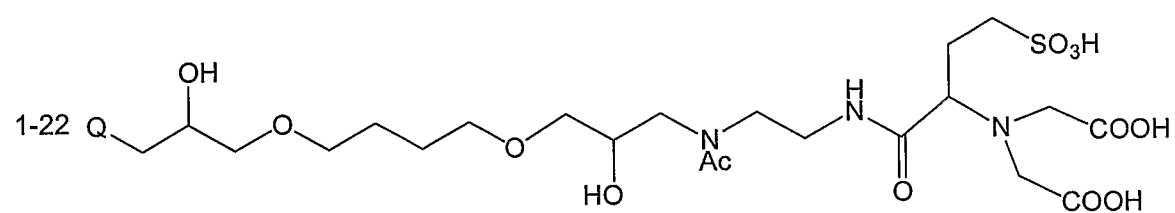
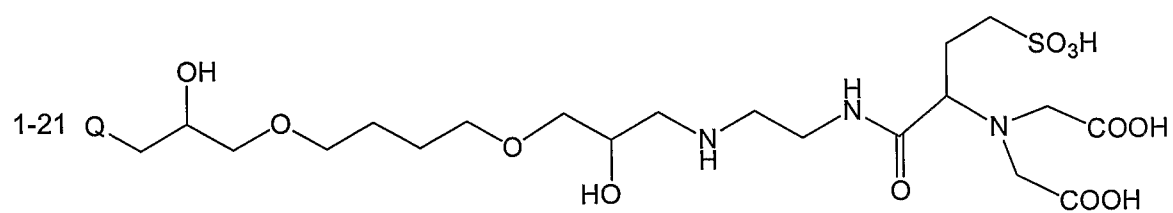
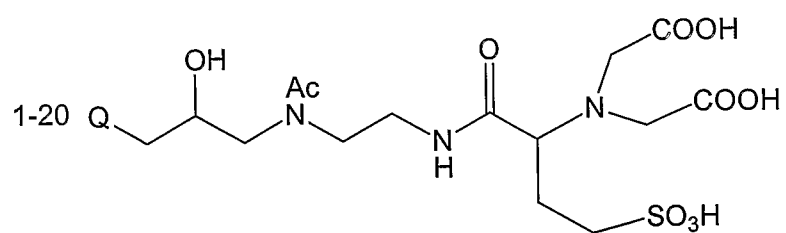
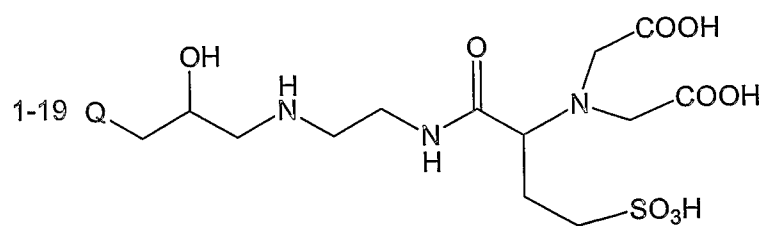
[0054] Exemplary chelating compositions include the following:

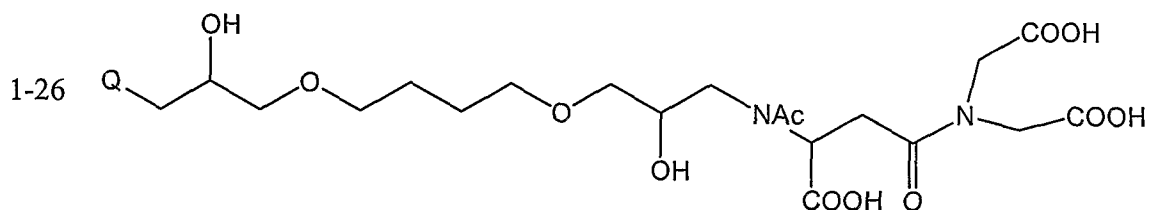
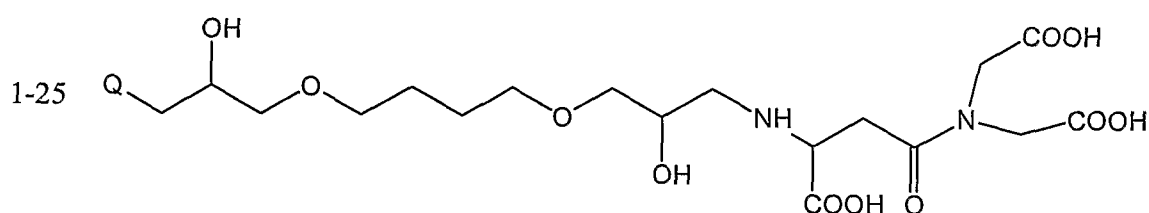
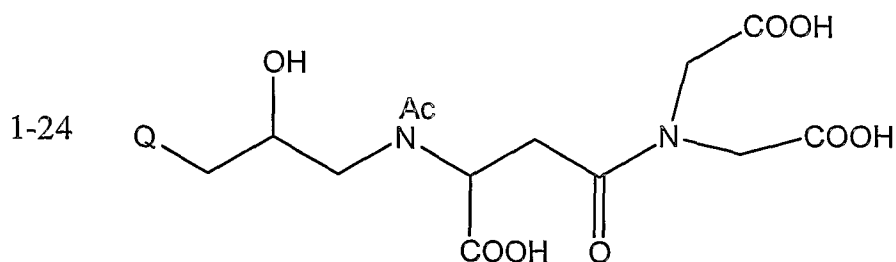






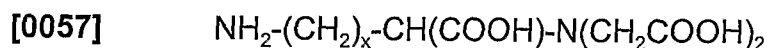






[0055] wherein Q is a carrier and Ac is acetyl.

[0056] In another embodiment, the capture ligand, is a metal chelate of the type described in U.S. Patent No. 5,047,513. More specifically, in this embodiment the capture ligand is a metal chelate derived from nitrilotriacetic acid derivatives of the formula:



[0058] wherein x is 2, 3 or 4. In this embodiment, the nitrilotriacetic acid derivative is immobilized on any of the previously described carriers, Q.

[0059] In these embodiments in which the capture ligand is a metal chelate as described in WO 01/81365 or U.S. Patent No. 5,047,513, the metal

chelate preferably contains a metal ion selected from among nickel (Ni^{2+}), zinc (Zn^{2+}), copper (Cu^{2+}), iron (Fe^{3+}), cobalt (Co^{2+}), calcium (Ca^{2+}), aluminum (Al^{3+}), magnesium (Mg^{2+}), and manganese (Mn^{2+}). In a particularly preferred embodiment, the metal chelate comprises nickel (Ni^{2+}).

[0060] Another common purification technique that can be used in the context of the present invention is the use of an immunogenic capture system. In such systems, an epitope tag on a protein or peptide allows the protein to which it is attached to be purified based upon the affinity of the epitope tag for a corresponding ligand (e.g., antibody) immobilized on a support. One example of such a tag is the sequence Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys, or DYKDDDDK (SEQ. ID. NO. 1); antibodies having specificity for this sequence are sold by Sigma-Aldrich (St. Louis, MO) under the FLAG[®] trademark. Another example of such a tag is the sequence Asp-Leu-Tyr-Asp-Asp-Asp-Asp-Lys, or DLYDDDDK (SEQ. ID. NO. 2); antibodies having specificity for this sequence are sold by Invitrogen (Carlsbad, CA). Another example of such a tag is the 3X FLAG[®] sequence Met-Asp-Tyr-Lys-Asp-His-Asp-Gly-Asp-Tyr-Lys-Asp-His-Asp-Ile-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys (SEQ. ID. NO. 3); antibodies having specificity for this sequence are sold by Sigma-Aldrich (St. Louis, MO). Thus, in one embodiment, the container comprises immobilized antibodies which have specificity for SEQ. ID. NO. 1; in another embodiment, the container comprises immobilized antibodies which have specificity for SEQ. ID. NO. 2. In another embodiment, the container comprises immobilized antibodies which have specificity for SEQ. ID. NO. 3. For example, in one embodiment, an ANTI-FLAG[®] M1, M2, or M5 antibody, sold by Sigma-Aldrich (St. Louis, MO), is immobilized on the interior surface of the container, or a portion thereof, and/or a bead or other support within the container.

[0061] Other tags may also be used to purify recombinant proteins based on their affinity for a corresponding ligand attached to a substrate. Some examples of such other tags include c-myc, maltose binding protein (MBP), influenza A virus haemagglutinin (HA), and β -galactosidase, among others. By

attaching the corresponding ligand to the containers and/or solid supports of the present invention, recombinant proteins containing these affinity tags may be purified from other proteins and cellular debris, as described herein. Non-recombinant proteins may be purified in a similar manner, by attaching a ligand with affinity for the protein or peptide sequence, or a part of that sequence, to the containers and/or supports of the present invention. The selection of an appropriate ligand is within the ability of one skilled in the art.

[0062] In another embodiment, proteins containing glutathione-S-transferase (GST) can be purified by contacting the proteins with immobilized glutathione. The proteins are purified as a result of the affinity of the GST for its substrate. Such systems are more fully described in, for example, U.S. Patent No. 5,654,176, incorporated herein by reference. Thus, in another embodiment, the glutathione is immobilized on the interior surface, or a portion thereof, of the container and/or a bead or other support within the container.

[0063] Proteins may also be purified by using biotin or biotin analogs in combination with avidin, streptavidin, or the derivatives of avidin or streptavidin. For example, in one embodiment, when streptavidin is immobilized on the containers and/or supports of the present invention, biotin labeled proteins can be purified based on the affinity of biotin for streptavidin. Similarly, a protein containing a streptavidin tag, such as those described in U.S. Patent No. 5,506,121, herein incorporated by reference, may be purified based on the affinity of the tag for streptavidin. In another embodiment, when biotin is immobilized on the containers and/or solid supports of the present invention, proteins containing avidin or streptavidin tags may be purified based on the affinity of biotin for avidin and streptavidin. The use of avidin/biotin or biotin/streptavidin affinity purification techniques is well known in the art, and described in, for example, Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*, 3rd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001.

[0064] Proteins and DNA or RNA may also be purified using ion exchange chromatography or hydrophobic chromatography. In ion exchange chromatography, a charged particle immobilized on a solid support binds reversibly to a protein or DNA that has a surface charge. For example, the ion-exchange capture ligand may contain a nitrogen group, a carboxyl group, a phosphate group, or a sulfonic acid group. Examples of ion-exchanger capture ligands include diethylaminoethyl (DEAE), diethyl[2-hydroxypropyl]aminoethyl (QAE), carboxymethyl (CM), and sulfopropyl (SP), and phosphoryl. In hydrophobic chromatography, a protein or DNA with hydrophobic groups on its surface is purified based on hydrophobic interactions with an insoluble hydrophobic group immobilized on a solid support. Examples of hydrophobic ligands are silica, phenyl, hexyl, octyl, and C18 groups. Thus, in one embodiment, charged particles are immobilized on the surface of the containers and/or supports of the present invention. In another embodiment, insoluble hydrophobic groups, are immobilized on the surface of the containers and/or supports of the present invention.

[0065] Other suitable capture ligands include, for example, hormones, amino acids, proteins, peptides, polypeptides, lectins, enzymes, enzyme substrates, enzyme inhibitors, cofactors, nucleotides, oligonucleotides (e.g., oligo dT), polynucleotides, carbohydrates, sugars, oligosaccharides, drugs, and dyes.

[0066] A variety of other purification techniques are known in the art and may be used in conjunction with the containers and methods of the present invention. Some such techniques are described in, e.g., Kenney & Fowell, *Methods in Molecular Biology*, Vol. 11, *Practical Protein Chromatography* (1992); Hanson & Ryden, *Protein Purification: Principles, High Resolution Methods, and Applications* (1989); Dean, et al., *Affinity Chromatography: A Practical Approach* (1987); Hermanson, et al., *Immobilized Affinity Ligand Techniques* (1992); and Jakoby & Wilchek, *Affinity Techniques, Enzyme Purification, Part B*, in *Methods in Enzymology*, Vol. 34 (1974).

[0067] Once the cellular component is bound to the capture ligand, cellular debris may be washed away, e.g., by using water or buffer. After washing, the bound cellular component may then be released from its association with the capture ligand and removed for characterization or quantitation. Release of the target cellular component may be accomplished using a variety of elution techniques including changes in pH or temperature, or through competitive binding. Specific elution techniques will vary, depending on which capture system is used, but will be readily apparent to those skilled in the art. Alternatively, the captured component may be detected while still attached to the immobilized ligand. A variety of analytical techniques are known, including, for example, ELISA, enzymatic analysis, and protein detection, among others.

3. Polymeric Coatings

[0068] In one embodiment, the capture ligands are bound directly to the interior surface of the container. Alternatively, the capture ligands may be bound to a polymeric matrix which overlies the container surface. Stated differently, the capture ligands may be bound directly to the polymeric matrix which, in turn, is bound to or otherwise immobilized on the interior surface of the container. For example, the capture ligand may be a metal chelating composition which is bound to a derivatized dextran polymer matrix which overlies a polystyrene or other plastic substratum. Polymeric matrices may thus be used to increase the effective surface area (by having a matrix which presents a greater surface area than the underlying substratum), thereby enabling an increased density of capture ligands. Alternatively, or in addition, the polymeric matrix may be more or less hydrophobic than the container wall and thereby present a surface which is desirably more (or alternatively less) hydrophilic than the natural surface of the substratum.

[0069] The polymeric coating may be formed or applied by a variety of methods. For example, the polymeric coating may be formed by *in situ*

polymerization; in this approach, a mixture of monomers are dissolved in solvent with an initiator and, after activation, polymerization is carried out on the surface of the container wall. Alternatively, a fully-grown polymer may be immobilized on the surface of the container wall. Such approaches are described, for example, in Sundberg et al., U.S. Patent No. 5,624,711.

[0070] In a preferred embodiment in which a polymeric coating is applied, the polymeric coating is derived from a mixture of two polymers which are bound to the container wall. In general, one or both of such polymers contains a reactive group, which when activated, chemically bonds a polymer molecule containing such reactive groups to the container wall and/or crosslinks the molecule with itself or with other polymer molecules. In addition, one or both of such polymers may contain activatable groups which provide points of attachment for the capture ligands described herein. Such polymeric coatings and the means for their formation are generally described in U.S. Patent Application Pub. No. 2003/0032013 A1.

[0071] The density of the polymer matrix on the substrate may be controlled by, *inter alia*, selection and amounts of the particular polymer and reactive groups employed. The molecular weight of the polymer, the number and type of reactive group and the number and molecular weight of the capture ligands may be selected and adjusted, as detailed further below. The polymer matrix may be attached to all of the substrate or to only a part of the substrate. For example, only a portion of the wall of a container or only a fraction of the wells of a multiwell plate may be provided with the polymer matrix.

Polymeric Matrices Formed From Polymer Mixtures

[0072] Containers comprising a polymer matrix may be prepared by contacting the container substrate with a polymer composition comprising a plurality of polymer molecules having repeating units, wherein at least some of the polymer molecules have at least one reactive group covalently attached thereto, wherein at least some of the polymer molecules have at least one

capture ligand (or activatable group) covalently attached thereto, wherein the polymer molecules have an average molecular weight of at least 100 kDa, and wherein at least 25% of the polymer molecules have at least one reactive group and at least one capture ligand covalently attached thereto. The reactive groups are activated to covalently bind at least some of the polymer molecules directly to the container substrate and to induce cross-linking between polymer molecules to form a polymer matrix attached to the container substrate.

[0073] In general, the polymeric matrix may comprise natural polymers (or a derivative thereof), synthetic polymers (or a derivative thereof), a blend of natural polymers (or derivative(s) thereof), a blend of synthetic polymers (or derivative(s) thereof), or a blend of one or more natural polymers (or derivative(s) thereof) and one or more synthetic polymers (or derivative(s) thereof). In general, a natural polymer is a branched or linear polymer produced in a biological system. Examples of natural polymers include, but are not limited to, oligosaccharides, polysaccharides, peptides, proteins, glycogen, dextran, heparin, amylopectin, amylose, pectin, pectic polysaccharides, starch, DNA, RNA, and cellulose. A particular modified natural polymer that may be used is a dextran-lysine derivative produced by covalently inserting lysine into variable linear positions along the dextran molecule using periodate oxidation and reductive amination or other methods known to those of skill in the art. In contrast, synthetic polymers are branched or linear polymers that are manmade. Examples of synthetic polymers include plastics, elastomers, and adhesives, oligomers, homopolymers, and copolymers produced as a result of addition, condensation or catalyst driven polymerization reactions, i.e., condensation polymerization. Whether natural or synthetic, the polymer may be derivatized or modified by oxidation, or by the covalent attachment of photo-reactive groups, affinity ligands, ion exchange ligands, hydrophobic ligands, other natural or synthetic polymers, or spacer molecules.

[0074] The polymeric matrix may thus comprise one or more of several distinct polymer types. Exemplary polymers include, but are not limited to,

cellulose-based products such as hydroxyethyl cellulose, hydroxypropyl cellulose, carboxymethyl cellulose, cellulose acetate, and cellulose butyrate; acrylics such as those polymerized from hydroxyethyl acrylate, hydroxyethyl methacrylate, glyceryl acrylate, glyceryl methacrylate, acrylic acid, methacrylic acid, acrylamide, and methacrylamide; vinyls such as polyvinyl pyrrolidone and polyvinyl alcohol; nylons such as polycaprolactam, polylauryl lactam, polyhexamethylene adipamide, and polyhexamethylene dodecanediamide; polyurethanes; polylactic acids; linear polysaccharides such as amylose, dextran, chitosan, heparin, and hyaluronic acid; and branched polysaccharides such as amylopectin, hyaluronic acid and hemicelluloses. Blends of two or more different polymer molecules can be used. For example, in one embodiment the polymer molecules are a mixture of dextran and heparin. In another embodiment dextran is mixed with poly Lys-Gly (1 lysine per 20 glycine).

[0075] In general, the polymer molecules preferably have an average molecular weight (total molecular weight of polymer, including covalently attached functional groups) of at least 100 kDa. In some embodiments, the polymer molecules have an average molecular weight of 300 kDa to 6,000 kDa. In some embodiments, the polymer molecules have an average molecular weight of 400 kDa to 3,000 kDa. In another embodiment, the polymer molecules have an average molecular weight of 500 kDa to 2,000 kDa, wherever the average molecular weight is the weight average molar mass (M_w) value of a polymer as measured by gel filtration chromatography using multi-angle light scattering and refractive index detection. The average M_w of the polymer distribution of all chain lengths present is based upon the selection of the peak as measured by the refractive index, starting and ending peak selection criteria of a refractive index value that is three times the refractive index baseline. As shown by example a preferred polymer may have an average M_w of 1,117 kDa with a molecular weight range from 112 kDa to 19,220 kDa.

[0076] In one embodiment, the polymeric matrix is formed by immobilizing a mixture of polymers wherein a subset of the polymer molecules in

the mixture contain capture ligand(s) or activatable group(s) enabling the subsequent covalent attachment of capture ligands and a different subset of the polymer molecules have at least one reactive group covalently attached thereto (for attaching the polymers to the container wall and crosslinking as previously described). This interaction of the reactive group between polymer molecule enables the formation of the three-dimensional matrix. The reactive group reacts either thermochemically or photochemically (polymers that contain a photo-reactive group are referred to as being photolabeled).

[0077] When the polymer molecules have capture ligands (or activatable groups) covalently attached, the ratio of capture ligands (or activatable groups) to polymer repeating units is preferably about 1:1 capture to about 1:100, respectively. For example, in one embodiment the ratio of capture ligands (or activatable groups) to polymer repeating units is preferably about 1:1 capture to about 1:20, respectively. When the polymer molecules have reactive groups covalently attached, the ratio of reactive groups to polymer repeat units is preferably less than about 1:600, more preferably, the ratio of reactive groups to polymer repeat units is preferably less than about 1:200 respectively.

[0078] Exemplary reactive groups include, but are not limited to, reactive groups used in the preparation of chromatography media which include: epoxides, oxiranes, N-hydroxysuccinimide, aldehydes, hydrazines, maleimides, mercaptans, amino groups, alkylhalides, isothiocyanates, carbodiimides, diazo compounds, tosyl chloride, tosyl chloride, and trichloro-S-triazine. Preferred reactive groups are α , β unsaturated ketone photo-reactive groups. Exemplary photo-reactive groups include aryl azides, diazaarenes, beta-carbonyldiazo, and benzophenones. The reactive species are nitrenes, carbenes, and radicals. These reactive species are generally capable of covalent bond formation. Preferred photo-reactive groups are photoactivatable, unsaturated ketones such as acetophenones, benzophenones, and derivatives thereof. A photo-reactive group when contacted with light may become activated, and capable of covalently attaching to the surface of a solid substrate. For example, the photo-

reactive groups may be activated by exposure to UV light from about 3 Joules/cm² to about 6 Joules/cm² depending on the intensity of light and duration of exposure time. The exposure times may range from as low as 0.5 sec/cm² to approximately 32 min/cm² depending on the intensity of the light source. In a preferred embodiment, the photo-reactive groups are activated by exposure to light for 0.5 sec/cm² to 5 sec/cm² at about 1,000 mWatts/cm² to about 5,000 mWatts/cm², or from about 1,000 mWatts/cm² to about 3,000 mWatts/cm², or from about 1,500 mWatts/cm² to about 2,500 mWatts/cm².

[0079] In one embodiment, capture ligands and/or reactive groups are covalently attached to the polymer molecules via a spacer. When used in connection with the formation of a polymer matrix, a spacer is a molecule or combination of covalently bonded molecules that connect the polymer molecule and either one or more of a capture ligand or reactive group. The spacer can be the same or different from any polymer, polymer composition, or polymer matrix. Those of skill in the art will know that many types of spacers are available and the selection and use is dependent upon the intended application of the polymer matrix, e.g., a lysine molecule or a aminocaproic acid molecule.

[0080] The spacer can be covalently attached to the photo-reactive group by a number of different chemistries including amide formation. For example, the use of the hydrocarbon spacer dramatically enhances polymer matrix stability performance. A photo-reactive group with a spacer may be coupled to a portion of a primary amine of the preferred polymer dextran by an amide bond at a controlled ratio relative to total monomer, glucose. Examples of photo-reactive groups with a spacer include, but are not limited to, benzobenzoic aminocaproic, N-Succinimidyl-N'-(4-azido-salicyl)-6-aminocaproate, N-Succinimidyl-(4-azido-2-nitrophenyl)-aminobutyrate, and N-Succinimidyl-(4-azido-2-nitrophenyl)-6-aminocaproate. These photo-reactive groups with spacers may be reacted with a polymer to produce a spacer that now includes the lysine as well as the original spacer attached to the photo-reactive group.

The spacer can also be manufactured by incorporating multiple molecules such as lysine and aminocaproic acid prior to attaching the photo-reactive group containing or not containing an additional spacer. An example of a reactive group covalently attached to a polymer molecule is a spacer comprising a moiety or residue of lysine bound to one or more chemical entities of the reactive group, by the loss of a reactive hydrogen from the amino group. In one embodiment, the density of primary amines contributed by the lysine spacers represents the density of desired capture ligand and reactive group. Modified polymers containing primary amines or other moieties such as spacers in a range of one moiety per every 1 to 100 polymer repeating units may be made by procedures known in the art. Modification of these moieties to selectively incorporate the desired amount of reactive groups is also known. For example, the density of the primary amines contributed by the lysine spacers is on average 1 for every 12 repeating glucose units of the dextran polymer. This density is very high relative to the desired incorporation of photo-reactive groups, e.g., less than one photo-reactive group per 200 repeating monomers. The concentration of primary amines in solution during polymer manufacture might be 4.5 $\mu\text{moles/mL}$, whereas the desired incorporation of photo-reactive groups would represent 0.09 $\mu\text{moles/mL}$. Therefore, in this instance, there would be a 50-fold excess of primary amine to the required photo-reactive group incorporation via a reactive ester. At this concentration of amine, the addition of photo-reactive group via a reactive ester at the desired level of incorporation results in greater than 90% efficiency of incorporation. By varying the amount of photo-reactive group containing a reactive ester any incorporation level less than 1 reactive group per 200 monomers can be consistently achieved. The method required to efficiently convert each of the remaining spacer moieties or amines to capture ligand attachment points is known in the art. A several fold excess of an amine reactive, e.g., reactive ester, derivatization reagent is used for the attachment of the capture ligand, either directly in one step or through multiple steps. In some cases, the derivatization reagent will present an additional

reactive group which, depending on its reactivity, will dictate the stoichiometry for subsequent capture ligand attachment. When lower ligand density is desired the initial amine reactive derivatization reagent will be lowered accordingly. In some instances free amines remaining after selective modifications will generally be derivatized by acetylation.

[0081] The first step in coating a surface of a substrate is contacting the polymer composition with the substrate surface to be coated. The method used to contact the polymer composition with the container surface depends on the dimensions and shape of the surface to be coated. The container may be made from a variety of natural and synthetic materials, such as those listed above. The container surface can be derivatized prior to coating. Pre-derivatization can be done by any method known by one of skill in the art, including silanization of silica and glass and plasma treatment of polystyrene or polypropylene to incorporate amines, carboxyl groups, alcohols, aldehydes and other reactive groups or by chemical modification of the surface to change its chemical composition.

[0082] If necessary, the surface of the substrate may be chemically modified to facilitate covalent bonding with the reactive groups carried on the polymer molecules. Such modifications include treating the substrate surface with a hydrocarbon, or plasma-treating the surface. An illustrative example of a chemical modification is the silanization of glass. In a preferred embodiment a MALDI plate is dipped into a 1 mg/mL solution of parafilm dissolved in chloroform and dried.

[0083] When coating a multiwell plate, tube or a surface or a portion thereof, larger than 0.1 mm square, the polymer composition may be contacted with the container surface by pouring, micropipeting, or transferring the polymer composition onto the portions of the container or plate, e.g., wells, to be coated. In the alternative, the portion of the plate, tube, container surface, or support larger than 2 mm square to be coated may also be coated by dipping the portion

of the surface into a solution of the polymer composition so as to place the container surface in contact with the polymer composition.

[0084] The amount of polymer that attaches to the container surface may be adjusted or controlled by varying the polymer composition concentration and volume added to the substrate. Once the polymer composition is placed in contact with the surface, the polymer composition may be dried on the container surface prior to activating the reactive groups, for example, evaporated to dryness by incubation in the dark at 20-50 °C with air flow. The polymer composition can also be evaporated using lyophilization or by any other drying means, including air drying, to remove the solvent. A variety of drying methods may be used provided that there is no premature activation of the reactive groups in response to the drying step. The substrate is considered sufficiently dry when no moisture is detectable visibly. During the drying, the polymer molecules of the polymer composition orient themselves so as to bind with the substrate surface or interact with each other to promote inter and intra-crosslinking with other polymers of the polymer composition.

[0085] The dried coated solid surface is then treated to induce the reactive groups to covalently bond to the substrate. In the case of the photo-reactive groups, they may be activated by irradiation. Activation is the application of an external stimulus that causes reactive groups to bond to the substrate. Specifically, a covalent bond is formed between the substrate and the reactive group, e.g., carbon-carbon bond formation.

[0086] There are many UV irradiation systems capable of delivering the total energy (dosage measured in Joules) required to bond the photo-activated polymer to a hydrocarbon rich substrate. Irradiation may be provided by a mercury lamp which has a distinct and known wavelength pattern of irradiation. The intensity of irradiation requires Joules to fall in the range of 3-6 Joules/cm². Joule measurements encompass the time factor (1 Joule = watt X second). In one embodiment, the irradiation is provided by an electrodeless mercury lamp powered by microwave radiation. One six inch, 500 watt/in. lamp has a rated

power output of 2,500 mWatts/cm² measured in the UVA range at about 2 inches distance of lamp to substrate. The lamp can be successfully run at 80% power or approximately 2,000 mWatts/cm². Sample plates prepared using a standard low intensity UV irradiation box having an intensity of irradiation (UVA/UVB, approximately 250 to 350 nm) measured at approximately 9.0 mWatts/cm² and requiring greater than 10 Joules/cm² (10,000 mJoules) total energy to provide good bonding. This requires an incubation time of the sample plates in the irradiation box of greater than 20 minutes. Plates processed using an electrodeless mercury lamp (2,000 mWatts/cm²) irradiation system requires only 1.75 sec/cm² for a total energy dosage of 3.5 Joules/cm². The higher intensity irradiation more efficiently activates the photo-active groups and consequently a lower overall energy dosage is required.

[0087] In one embodiment, activation may be done with a UVA/UVB light irradiating at 9.0 mWatts/cm² for approximately 30 minutes to a total energy of approximately 15,000 mJoules/cm². In a preferred embodiment, activation may be done by exposure to UVA/UVB light irradiating at 2,000 mWatts/cm² to a total energy of from about 3 Joules/cm² to about 4 Joules/cm². The amount of incubation time and the total energy used may vary according to the photo-reactive group bound to the polymer. In the most preferred embodiment, activation may be done by photoirradiation using a Fusion UV Conveyor System using a mercury electrodeless lamp irradiating at 2,000 mWatts/cm² with the conveyor belt set at 8 feet/minute with the lamp power at 400 watts/in. A radiometer, IL290 Light Bug, is run through the conveyor belt to verify the desired energy in the range of 3,000-4,000 mJoules/cm². The multiwell plates, for example, are photoirradiated at about 800 plates per hour, or about 1 plate per 4 to 5 seconds.

[0088] The concentration of the polymer composition can be adjusted by changing the amount of total polymer per milliliter of solvent. In the case where a higher concentration of polymer composition or polymer matrix per square cm would be advantageous, less solvent can be used to solvate the polymer

molecules of the composition. In the case where a lower concentration of polymer composition or polymer matrix per square cm would be advantageous, more solvent can be used to solvate the polymer molecules of the composition. In other words, adjusting the concentration of the polymer composition between 0.02 and 1.0 mg/mL solvent and coating a solid surface, such as a multiwell plate, would produce a surface having a selectable range of total bound polymer matrix. The polymer composition can be completely soluble or contain suspended insoluble polymer. The solvents that may be used to make the polymer composition include water, alcohols, ketones, and mixtures of any or all of these. The solvent(s) are preferably compatible with the substrate being used. Since the polymers of the composition may crosslink between each other, it is possible that a fluid-like solution of the composition may change into a gel. In the alternative, the solution may be produced in the form of a slurry. Examples of solvents that may be used in the composition include water, alcohols, ketones, and mixtures of any or all of these.

[0089] Non-bound polymers may be removed by incubating in a suitable solution to dissolve and remove unbound polymer. For example, multiwell plates may be incubated with MOPS buffer overnight at 25 °C, washed with MPTS buffer and distilled water three times each, washed with hibitane solution, air dried, packaged and stored below ambient temperature (2-8°C). The remaining polymers form the polymer matrix.

[0090] The resulting polymer-coated substrate preferably contains the polymer matrix in a density of at least 2 $\mu\text{g}/\text{cm}^2$, more preferably, in a density of 4 $\mu\text{g}/\text{cm}^2$ to 30 $\mu\text{g}/\text{cm}^2$, and, for some embodiment, in a density of 6 $\mu\text{g}/\text{cm}^2$ to 15 $\mu\text{g}/\text{cm}^2$. The density of capture ligands (or activatable groups) in the polymer matrix may thus be controlled by controlling the number and/or molecular weight of the capture ligands covalently attached to the polymer molecules. Generally the density of capture ligands (or activatable groups) in the polymer matrix is preferably at least 1 nanomole/ cm^2 . In some embodiments, the density of the capture ligands (or activatable groups) is about 1.2 nanomoles/ cm^2 to about

185 nanomoles/cm². In another embodiment, the density of the capture ligands (or activatable groups) is about 1.5 nanomoles/cm² to about 90 nanomoles/cm², or about 1.8 nanomoles/cm² to about 15 nanomoles/cm². As a result, the polymer matrix may thereby enable binding target molecules having a molecular weight of less than 3.5 kDa in an amount of at least 1 nanomole/cm².

[0091] In a preferred embodiment, the polymer molecules contacted with the container substrate have at least one capture ligand (or activatable group) covalently attached thereto and at least some of the polymer molecules have no reactive group covalently attached thereto. The percentage of polymer molecules having both reactive groups and capture ligands covalently attached may be 25% to 80%. In another embodiment the percentage of both reactive groups and capture ligands attached may be from 40% to 75%. In yet another embodiment, the percentage of both reactive groups and capture ligands attached may be from 50% to 60%. In a preferred embodiment, the percentage of polymer molecules having both reactive groups and capture ligands covalently attached thereto may be approximately 50%. The use of a mixture of polymer molecules, with and without reactive groups, enhances the highly functional formation of a three dimensional polymer matrix.

[0092] If desired, the capture ligands in the formed polymer matrix may be derivatized, e.g., by noncovalently or covalently attaching the capture ligands either by the addition of a different capture ligand or chemical modification of the existing capture ligand, thereby further enabling the high capacity capture of a larger variety of target molecules.

[0093] In one embodiment, the container is a multiwell polystyrene plate, the polymer coating is derived from a mixture of dextran polymers, the capture ligand is a nickel chelate, and the polymer matrix has a capture ligand density of 1.5 nanomoles/cm² to 7.5 nanomoles/cm². In other embodiments, the capture ligand is a Gallium or Iron chelate or the capture ligand is glutathione.

[0094] In another embodiment, the container is a multiwell polypropylene plate, the polymer coating is derived from a mixture of dextran polymers, and the capture ligand is an oligonucleotide.

[0095] In yet another embodiment, the container is a multiwell polystyrene plate, the polymer coating is derived from a mixture of dextran polymers, the capture ligand is streptavidin, and the polymer matrix has a capture ligand density of $1.5 \mu\text{g}/\text{cm}^2$ to $7.5 \mu\text{g}/\text{cm}^2$.

[0096] Additionally, in another embodiment, the container is a multiwell polystyrene plate, the polymer coating is derived from a mixture of dextran polymers, the capture ligand is selected from the group consisting of protein A, protein G, protein L, or a mixture thereof, and the polymer matrix has a capture ligand density of $1.5 \mu\text{g}/\text{cm}^2$ to $7.5 \mu\text{g}/\text{cm}^2$.

[0097] In another embodiment, the container is a polypropylene column, the polymer coating is derived from a mixture of dextran polymers, and the capture ligand is a nickel chelate.

[0098] A container comprising a polymer matrix can be used in combination with the lytic reagents described in greater detail elsewhere herein to lyse cells and isolate target cellular components from the resulting solutions. The lytic reagent may be provided within the container in any suitable manner, such as those described below. In one embodiment, the lytic reagent is adsorbed onto at least a portion of the polymer matrix. In another embodiment, the lytic reagent resides within the container as a free-flowing powder, on top of the polymer matrix. A solution comprising host cells may then be added to the container comprising the polymer matrix and the lytic reagent. Once some or all of the cellular components have been released from a host cell by the lytic reagent, the target cellular component may be isolated from the cellular solution by the capture ligand present in the polymer matrix.

[0099] The polymer matrix may be constructed to enable binding target molecules having a molecular weight of 3.5 kDa to 500 kDa in an amount of $0.5 \mu\text{g}/\text{cm}^2$ to $20 \mu\text{g}/\text{cm}^2$, a molecular weight of 10 kDa to 500 kDa in an amount

of 1 $\mu\text{g}/\text{cm}^2$ to 20 $\mu\text{g}/\text{cm}^2$, a molecular weight of 10 kDa to 350 kDa in an amount of 2 $\mu\text{g}/\text{cm}^2$ to 20 $\mu\text{g}/\text{cm}^2$, a molecular weight of 10 kDa to 350 kDa in an amount of 3 $\mu\text{g}/\text{cm}^2$ to 15 $\mu\text{g}/\text{cm}^2$. In some embodiments, the polymer matrix is capable of binding target molecules with a molecular weight of 10 kDa to 350 kDa in an amount of 4 $\mu\text{g}/\text{cm}^2$ to 10 $\mu\text{g}/\text{cm}^2$. In certain embodiments the polymer matrix is capable of binding polypeptide target molecules having a molecular weight up to 350 kDa in an amount of at least 2 $\mu\text{g}/\text{cm}^2$ of polymer matrix.

4. Lytic Reagent

[0100] To aid in the extraction or extraction and isolation of a cellular component, such as a peptide, protein, or nucleic acid, from a host cell, the containers of the present invention comprise a lytic reagent. In one embodiment, the lytic reagent is of a composition and in a concentration which causes the membrane of the host cell to rupture and release its contents into a solution containing the lytic reagent. In another embodiment, the lytic reagent merely renders the membrane sufficiently permeable to release some, but not necessarily all of its cellular components.

[0101] The lytic reagent may be provided within the container by a variety of manners. In one embodiment, the lytic reagent is adsorbed (as a dry composition) to the interior surface of the container (or, alternatively, to a polymeric coating overlying the container surface, if present). In one such embodiment, for example, the lytic reagent is adsorbed to at least a portion of the sidewall formation of the container. In another embodiment, the lytic reagent is adsorbed to at least a portion of the bottom of the container. In another embodiment, the lytic reagent is adsorbed to at least a portion of each of the bottom and the sidewall formation of the container. Optionally, if the container comprises a polymer matrix, the lytic reagent may be adsorbed to at least a portion of the surface of the polymer matrix. In another embodiment, the lytic reagent is adsorbed to another body, for example, a support such as a bead, rod, mesh (such as a filter) or other porous body which is loosely contained

within the volume of the container or affixed to the interior surface of the container. Such supports as well as the container itself may be comprised of, for example, polystyrene, polypropylene, polyethylene, glass, nylon, polyacrylamides, celluloses, nitrocellulose, other plastic polymers, metals, magnetite, or other synthetic substances. In another embodiment, the lytic reagent is adsorbed to at least a portion of the interior surface of the container and to a body, for example, a support such as a bead, rod, mesh (such as a filter) or other porous body which is loosely contained within the volume of the container or affixed to the interior surface of the container.

[0102] The ratio of the surface area of the surfaces coated with lytic reagent (i.e., the sum of the surface area of the coated interior surface and/or coated bodies contained within the volume of the container) may be controlled in accordance with one aspect of the present invention. In one embodiment, the surface area to volume ratio, $SA:V$, wherein SA is the surface area of the coated interior surface of the container and the surface of any coated bodies contained within the volume of the container and V is the volume of the container, is less than about $4 \text{ mm}^2/\mu\text{l}$. In another embodiment, this surface area to volume ratio does not exceed about $3 \text{ mm}^2/\mu\text{l}$. In another embodiment, this surface area to volume ratio does not exceed about $2 \text{ mm}^2/\mu\text{l}$. In another embodiment, this surface area to volume ratio does not exceed about $1 \text{ mm}^2/\mu\text{l}$.

[0103] The coating of the lytic reagent on the interior surface of the container and/or bodies contained within the volume of the container will typically be adsorbed as a dry material, e.g., a composition having a moisture content of not more than about 5 wt.%. Alternatively, the lytic reagent may be provided in the form of a gel or paste, i.e., a material which has a viscosity of greater than about 10,000 centipoise, coated on the interior surface or a portion of the interior surface of the container, or additionally on included bodies.

[0104] In one alternative embodiment, the lytic reagent is provided to and resides within the container as a mass of material, e.g., a matrix, granule(s), tablet(s), or free-flowing powder, rather than as an adsorbed layer on the interior

surface of the container or bodies contained within the volume of the container. Thus, for example, the lytic reagent may be a lyophilized matrix or a lyophilized powder which is placed within the container independently of the capture ligand; in one embodiment, a mass of lyophilized lytic reagent is placed upon a layer of resin having capture ligand bound thereto. In general, finer particles tend to dissolve more rapidly than larger particles. To minimize the risk of loss and/or contamination of the lytic reagent, it may be preferred to provide a lid over the mouth of the container.

[0105] In another alternative embodiment, the lytic reagent may be present in the container as a dissolved or slurried component. To avoid undesired dilution of any solutions or suspensions containing the host cell, in this embodiment the liquid in which the lytic reagent is dissolved or slurried preferably contains a high concentration of the lytic reagent, e.g., greater than about 10% by weight. In another embodiment, the concentration of the lytic reagent is greater than about 20% by weight. Again, to minimize the risk of loss and/or contamination of the lytic reagent, it may be preferred to cover the container with a lid.

[0106] In general, the lytic reagent may be any composition or combination of compositions which chemically or enzymatically induces a cell to release a target cellular component from a host cell. In addition, the lytic reagent may optionally provide protection for that component, such as protection from degradation. The lytic reagent may thus comprise a detergent, a lytic enzyme, a chaotropic reagent, or combinations thereof. The lytic reagent may further comprise buffers, anti-foaming agents, bulking agents, processing enzymes, enzymatic inhibitors, or other additives that aid in the extraction and isolation of cellular components, such as peptides, proteins, or nucleic acids.

[0107] In one embodiment, the lytic reagent comprises a detergent. A variety of detergents may be used herein, including anionic, cationic, non-ionic, and zwitterionic detergents. Exemplary detergents include chenodeoxycholic acid; chenodeoxycholic acid sodium salt; cholic acid; dehydrocholic acid;

deoxycholic acid; deoxycholic acid methyl ester; digitonin; digitoxigenin; N,N-dimethyldodecylamine oxide; docusate sodium salt; glycochenodeoxycholic acid sodium salt; glycocholic acid hydrate; glycocholic acid sodium salt hydrate; glycodeoxycholic acid monohydrate; glycodeoxycholic acid sodium salt; glycolithocholic acid 3-sulfate disodium salt; glycolithocholic acid ethyl ester; N-lauroylsarcosine sodium salt; N-lauroylsarcosine; lithium dodecyl sulfate; lugol solution; Niaproof 4, Type 4 (i.e., 7-ethyl-2-methyl-4-undecyl sulfate sodium salt; sodium 7-ethyl-2-methyl-4-undecyl sulfate); 1-octanesulfonic acid sodium salt; sodium 1-butanesulfonate; sodium 1-decanesulfonate; sodium 1-dodecanesulfonate; sodium 1-heptanesulfonate anhydrous; sodium 1-nonanesulfonate; sodium 1-propanesulfonate monohydrate; sodium 2-bromoethanesulfonate; sodium cholate hydrate; sodium choleate; sodium deoxycholate; sodium deoxycholate monohydrate; sodium dodecyl sulfate; sodium hexanesulfonate anhydrous; sodium octyl sulfate; sodium pentanesulfonate anhydrous; sodium taurocholate; sodium taurodeoxycholate; saurochenodeoxycholic acid sodium salt; taurodeoxycholic acid sodium salt monohydrate; taurohyodeoxycholic acid sodium salt hydrate; tauroolithocholic acid 3-sulfate disodium salt; tauroursodeoxycholic acid sodium salt; Trizma[®] dodecyl sulfate (i.e., tris(hydroxymethyl)aminomethane lauryl sulfate); ursodeoxycholic acid, alkyltrimethylammonium bromide; benzalkonium chloride; benzyldimethylhexadecylammonium chloride; benzyldimethyltetradecylammonium chloride; benzyldodecyldimethylammonium bromide; benzyltrimethylammonium tetrachloroiodate; cetyltrimethylammonium bromide; dimethyldioctadecylammonium bromide; dodecylethyldimethylammonium bromide; dodecyltrimethylammonium bromide; ethylhexadecyldimethylammonium bromide; Girard's reagent T; hexadecyltrimethylammonium bromide; N,N',N'-polyoxyethylene(10)-N-tallow-1,3-diaminopropane; thonzonium bromide; trimethyl(tetradecyl)ammonium bromide, BigCHAP (i.e., N,N-bis[3-(D-gluconamido)propyl]cholamide); bis(polyethylene glycol bis[imidazolyl carbonyl]); polyoxyethylene alcohols, such

as Brij® 30 (polyoxyethylene(4) lauryl ether), Brij® 35 (polyoxyethylene(23) lauryl ether), Brij® 35P, Brij® 52 (polyoxyethylene 2 cetyl ether), Brij® 56 (polyoxyethylene 10 cetyl ether), Brij® 58 (polyoxyethylene 20 cetyl ether), Brij® 72 (polyoxyethylene 2 stearyl ether), Brij® 76 (polyoxyethylene 10 stearyl ether), Brij® 78 (polyoxyethylene 20 stearyl ether), Brij® 78P, Brij® 92 (polyoxyethylene 2 oleyl ether); Brij® 92V (polyoxyethylene 2 oleyl ether), Brij® 96V, Brij® 97 (polyoxyethylene 10 oleyl ether), Brij® 98 (polyoxyethylene(20) oleyl ether), Brij® 58P, and Brij® 700 (polyoxyethylene(100) stearyl ether); Cremophor® EL (i.e., polyoxyethylenglyceroltriricinoleat 35; polyoxyl 35 castor oil); decaethylene glycol monododecyl ether; decaethylene glycol mono hexadecyl ether; decaethylene glycol mono tridecyl ether; N-decanoyl-N-methylglucamine; n-decyl α -D-glucopyranoside; decyl β -D-maltopyranoside; digitonin; n-dodecanoyl-N-methylglucamide; n-dodecyl α -D-maltoside; n-dodecyl β -D-maltoside; heptaethylene glycol monodecyl ether; heptaethylene glycol monododecyl ether; heptaethylene glycol monotetradecyl ether; n-hexadecyl β -D-maltoside; hexaethylene glycol monododecyl ether; hexaethylene glycol monohexadecyl ether; hexaethylene glycol monooctadecyl ether; hexaethylene glycol monotetradecyl ether; Igepal® CA-630 (i.e., nonylphenyl-polyethylenglykol, (octylphenoxy)polyethoxyethanol, octylphenyl-polyethylene glycol); methyl-6-O-(N-heptylcarbamoyle)- α -D-glucopyranoside; nonaethylene glycol monododecyl ether; N-nonanoyl-N-methylglucamine; octaethylene glycol monodecyl ether; octaethylene glycol monododecyl ether; octaethylene glycol monohexadecyl ether; octaethylene glycol monooctadecyl ether; octaethylene glycol monotetradecyl ether; octyl- β -D-glucopyranoside; pentaethylene glycol monodecyl ether; pentaethylene glycol monododecyl ether; pentaethylene glycol monohexadecyl ether; pentaethylene glycol monohexyl ether; pentaethylene glycol monooctadecyl ether; pentaethylene glycol monooctyl ether; polyethylene glycol diglycidyl ether; polyethylene glycol ether W-1; polyoxyethylene 10 tridecyl ether; polyoxyethylene 100 stearate; polyoxyethylene 20 isohexadecyl ether; polyoxyethylene 20 oleyl ether; polyoxyethylene 40 stearate; polyoxyethylene 50

stearate; polyoxyethylene 8 stearate; polyoxyethylene bis(imidazolyl carbonyl); polyoxyethylene 25 propylene glycol stearate; saponin from quillaja bark; sorbitan fatty acid esters, such as Span[®] 20 (sorbitan monolaurate), Span[®] 40 (sorbitane monopalmitate), Span[®] 60 (sorbitane monostearate), Span[®] 65 (sorbitane tristearate), Span[®] 80 (sorbitane monooleate), and Span[®] 85 (sorbitane trioleate); various alkyl ethers of polyethylene glycols, such as Tergitol[®] Type 15-S-12, Tergitol[®] Type 15-S-30, Tergitol[®] Type 15-S-5, Tergitol[®] Type 15-S-7, Tergitol[®] Type 15-S-9, Tergitol[®] Type NP-10 (nonylphenol ethoxylate), Tergitol[®] Type NP-4, Tergitol[®] Type NP-40, Tergitol[®] Type NP-7, Tergitol[®] Type NP-9 (nonylphenol polyethylene glycol ether), Tergitol[®] MIN FOAM 1x, Tergitol[®] MIN FOAM 2x, Tergitol[®] Type TMN-10 (polyethylene glycol trimethylnonyl ether), Tergitol[®] Type TMN-6 (polyethylene glycol trimethylnonyl ether), Triton[®] 770, Triton[®] CF-10 (benzyl-polyethylene glycol tert-octylphenyl ether), Triton[®] CF-21, Triton[®] CF-32, Triton[®] DF-12, Triton[®] DF-16, Triton[®] GR-5M, Triton[®] N-42, Triton[®] N-57, Triton[®] N-60, Triton[®] N-101 (i.e., polyethylene glycol nonylphenyl ether; polyoxyethylene branched nonylphenyl ether), Triton[®] QS-15, Triton[®] QS-44, Triton[®] RW-75 (i.e., polyethylene glycol 260 mono(hexadecyl/octadecyl) ether and 1-octadecanol), Triton[®] SP-135, Triton[®] SP-190, Triton[®] W-30, Triton[®] X-15, Triton[®] X-45 (i.e., polyethylene glycol 4-tert-octylphenyl ether; 4-(1,1,3,3-tetramethylbutyl)phenyl-polyethylene glycol), Triton[®] X-100 (t-octylphenoxypolyethoxyethanol; polyethylene glycol tert-octylphenyl ether; 4-(1,1,3,3-tetramethylbutyl)phenyl-polyethylene glycol), Triton[®] X-102, Triton[®] X-114 (polyethylene glycol tert-octylphenyl ether; (1,1,3,3-tetramethylbutyl)phenyl-polyethylene glycol), Triton[®] X-165, Triton[®] X-305, Triton[®] X-405 (i.e., polyoxyethylene(40) isooctylcyclohexyl ether; polyethylene glycol tert-octylphenyl ether), Triton[®] X-705-70, Triton[®] X-151, Triton[®] X-200, Triton[®] X-207, Triton[®] X-301, Triton[®] XL-80N, and Triton[®] XQS-20; tetradecyl- β -D-maltoside; tetraethylene glycol monodecyl ether; tetraethylene glycol monododecyl ether; tetraethylene glycol monotetradecyl ether; triethylene glycol monodecyl ether; triethylene glycol monododecyl ether; triethylene glycol

monohexadecyl ether; triethylene glycol mono-octyl ether; triethylene glycol monotetradecyl ether; polyoxyethylene sorbitan fatty acid esters, such as TWEEN® 20 (polyethylene glycol sorbitan monolaurate), TWEEN® 20 (polyoxyethylene (20) sorbitan monolaurate), TWEEN® 21 (polyoxyethylene (4) sorbitan monolaurate), TWEEN® 40 (polyoxyethylene (20) sorbitan monopalmitate), TWEEN® 60 (polyethylene glycol sorbitan monostearate; polyoxyethylene (20) sorbitan monostearate), TWEEN® 61 (polyoxyethylene (4) sorbitan monostearate), TWEEN® 65 (polyoxyethylene (20) sorbitan tristearate), TWEEN® 80 (polyethylene glycol sorbitan monooleate; polyoxyethylene (20) sorbitan monooleate), TWEEN® 81 (polyoxyethylene (5) sorbitan monooleate), and TWEEN® 85 (polyoxyethylene (20) sorbitan trioleate); tyloxapol; n-undecyl β -D-glucopyranoside, CHAPS (i.e., 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate); CHAPSO (i.e., 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate); N-dodecylmaltoside; α -dodecyl-maltoside; β -dodecyl-maltoside; 3-(decyldimethylammonio)propanesulfonate inner salt (i.e., SB3-10); 3-(dodecyldimethylammonio)propanesulfonate inner salt (i.e., SB3-12); 3-(N,N-dimethylmyristylammonio)propanesulfonate (i.e., SB3-14); 3-(N,N-dimethyloctadecylammonio)propanesulfonate (i.e., SB3-18); 3-(N,N-dimethyloctylammonio)propanesulfonate inner salt (i.e., SB3-8); 3-(N,N-dimethylpalmitylammonio)propanesulfonate (i.e., SB3-16); MEGA-8; MEGA-9; MEGA-10; methylheptylcarbonyl glucopyranoside; N-nonanoyl N-methylglucamine; octyl-glucopyranoside; octyl-thioglucopyranoside; octyl- β -thioglucopyranoside; 3-(4-heptyl) phenyl 3-hydroxy propyl dimethylammonio propane sulfonate (i.e., C7BzO); 3-[N,N-dimethyl(3-myristoylaminopropyl)ammonio]propanesulfonate (i.e., ASB-14); and deoxycholic acid, and various combinations thereof.

[0108] In one embodiment, the lytic reagent will be one or more detergent selected from the group consisting of CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate), octyl- β -thioglucopyranoside, octyl-glucopyranoside, C7BzO (3-(4-heptyl) phenyl 3-

hydroxy propyl) dimethylammonio propane sulfonate), ASB-14 (3-[N,N-dimethyl(3-myristoylamino)propyl]ammonio]propanesulfonate), Triton® X-100, α -dodecyl-maltoside, β -dodecyl-maltoside, decaethylene glycol mono hexadecyl ether, decaethylene glycol mono tridecyl ether, deoxycholic acid, sodium dodecyl sulfate, Igepal® CA-630, hexadecyltrimethylammonium bromide, SB3-10 (3-(decyldimethylammonio)propanesulfonate inner salt), SB3-12 (3-(dodecyldimethylammonio)propanesulfonate inner salt), SB3-14 (3-(N,N-dimethylmyristylammonio)propanesulfonate), and n-dodecyl α -D-maltoside.

[0109] In another embodiment, the lytic reagent will be one or more detergent selected from the group consisting of 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, octyl- β -thioglucopyranoside, octyl-glucopyranoside, 3-(4-heptyl) phenyl 3-hydroxy propyl) dimethylammonio propane sulfonate, 3-[N,N-dimethyl(3-myristoylamino)propyl]ammonio]propanesulfonate, 3-(decyldimethylammonio)propanesulfonate inner salt, 3-(dodecyldimethylammonio)propanesulfonate inner salt, 3-(N,N-dimethylmyristylammonio)propanesulfonate, and n-dodecyl α -D-maltoside.

[0110] In another embodiment, the lytic reagent comprises a lytic enzyme. A wide variety of enzymes may be used herein. Exemplary enzymes include beta glucuronidase; glucanase; glucosylase; lysozyme; lyticase; mannanase; mutanolysin; zymolyase, cellulase, chitinase, lysostaphin, pectolyse, streptolysin O, and various combinations thereof. See, e.g., Wolska-Mitaszko, et al., *Analytical Biochem.*, 116:241-47 (1981); Wiseman, *Process Biochem.*, 63-65 (1969); and Andrews & Asenjo, *Trends in Biotech.*, 5:273-77 (1987).

[0111] The type of cell being lysed may affect the choice of enzyme. See Coakley, et al., *Adv. Microb. Physiol.*, 16:279-341 (1977). For example, with regards to proteins or peptides, chitinase, beta glucuronidase, mannanase, and pectolyse are all useful when the host cell is a plant cell. Yeast cells are difficult to disrupt because the cell walls may form capsules or resistant spores.

DNA can be extracted from yeast by using lysing enzymes such as lyticase, chitinase, zymolase, and gluculase to induce partial spheroplast formation; spheroplast are subsequently lysed to release DNA. Lyticase is preferred to digest cell walls of yeast and generate spheroplasts from fungi for transformation. Lyticase hydrolyzes poly(β -1,3-glucose) such as yeast cell-wall glucan.

[0112] Lysozyme and mutanolysin are useful when the host cell is a bacterial cell. Lysozyme hydrolyzes the beta 1-4 glycosidic bond between N-acetylglucosamine and N-acetylmuramic acid in the polysaccharide backbone of peptidoglycan. It is effective in lysing bacteria by hydrolyzing the peptidoglycan which is present in bacterial cell walls.

[0113] In another embodiment, the lytic reagent comprises a chaotrope. In some instances chaotropes alone are sufficient to lyse the host cell. In particular, chaotropes are used when the cellular component is RNA. Examples of chaotropes that may be used herein include urea, guanidine HCl, guanidine thiocyanate, guanidium thiosulfate, and thiourea. Chaotropes may also be used in combination with the detergents, buffers, anti-foaming agents, and other additives described herein.

[0114] In addition to a detergent, lytic enzyme, or chaotrope which is primarily responsible for lysing the host cells, the lytic reagent may comprise one or more buffers to control pH, an anti-foaming agent to prevent excessive foaming or frothing, a bulking agent, enzymatic inhibitors, and other processing enzymes which aid in the purification of the cellular component. Exemplary buffers include TRIS, TRIS-HCl, HEPES, and phosphate. Exemplary anti-foaming agents include Antifoam 204; Antifoam A Concentrate; Antifoam A Emulsion; Antifoam B Emulsion; and Antifoam C Emulsion. Exemplary bulking agents include sodium chloride, potassium chloride, and polyvinylpyrrolidone (PVP). Processing enzymes and enzymatic inhibitors include nucleases, such as Benzonase[®] endonuclease; DNase (e.g., DNase I); RNase (e.g., RNase A); proteases, such as proteinase K; nuclease inhibitors; protease inhibitors, such

as phosphoramidon, pepstatin A, bestatin, E-64, aprotinin, leupeptin, 1,10-phenanthroline, antipain, benzamidine HCl, chymostatin, EDTA, e-aminocaproic acid, trypsin inhibitor, and 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride; and phosphatase inhibitors, such as cantharidin, bromotetramisole, microcystin LR, sodium orthovanadate, sodium molybdate, sodium tartrate, and imidazole; among others. Like lysing enzymes, the choice of processing enzyme and enzymatic inhibitor will also vary depending on several factors, including the type of material to be extracted (e.g., peptides, proteins, nucleic acids, etc.), as well as the type of cell to be lysed (e.g., plant, yeast, bacterial, fungal, mammalian, insect, etc.). For example, nucleases hydrolyze or degrade nucleic acids. It would thus be desirable for the lytic reagent to comprise a nuclease when the cellular component is a protein or peptide, but not when the cellular component is a nucleic acid. Likewise, proteases break down or degrade proteins. It would thus be desirable for the lytic reagent to comprise a protease when the cellular component is a nucleic acid, but not when the cellular component is a protein. Similar reasoning may be applied when selecting other enzymes or inhibitors. Thus, in general, enzymes or inhibitors such as proteases, nuclease inhibitors, and lysozymes are useful when the cellular component is a nucleic acid. Other enzymes or inhibitors, such as Benzonase[®] endonuclease, protease inhibitors, phosphatase inhibitors, DNase, RNase, or other nucleases are useful when the cellular component is a protein or peptide. With regards to nucleic acids, RNase A could be used for the extraction of bacterial and mammalian DNA. DNase I may be used for the extraction of bacterial RNA, yeast RNA, RNA from animal cells and tissues, and RNA from biological fluids. A protease, such as proteinase K, may be used to extract DNA from all cell types.

[0115] When the host cell is a bacterial or animal cell, or the cellular component is a protein or DNA, the lytic reagent will typically comprise a detergent. When the host cell is a yeast cell, the lytic reagent will typically

comprise a detergent, or an enzyme capable of lysing yeast cells, such as lyticase, zymolyase, or other lytic enzymes, such as those previously listed.

[0116] By way of further example, when the cellular component is a protein or peptide, the lytic reagent preferably comprises one or more detergents, lysozymes, nucleases, Benzonase[®] endonuclease, buffers, protease inhibitors, phosphatase inhibitors, or chaotropic reagents, or various combinations thereof. In another embodiment, when the cellular component is DNA, the lytic reagent preferably comprises one or more detergents, lysozymes, nuclease inhibitors, RNase, buffers, or proteases, or various combinations thereof.

[0117] In another embodiment, when the cellular component is RNA, the lytic reagent preferably comprises one or more detergents, chaotropic reagents, or buffers, or various combinations thereof. Enzymes would not be typically used in this application since the chaotrope will inactivate them.

[0118] In one embodiment, the lytic reagent comprises 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, lysozyme, Tris-HCl, and DNase I.

[0119] In another embodiment, the lytic reagent comprises octyl-thioglucopyranoside, protease inhibitors, lysozyme, and Benzonase[®] endonuclease.

[0120] The lytic reagent may thus comprise a variety of different combinations of detergents, enzymes, inhibitors, chaotropes, buffers, anti-foaming agents, bulking agents, and/or other additives that will aid in the extraction and isolation of the cellular component. These lytic reagents and/or components may be native, recombinant, or in any modified or active form. One skilled in the art may readily determine what a preferred lytic reagent comprises, based the cellular component and the type of host cell.

[0121] The amount of the lytic reagent and the relative proportions of each component thereof will vary depending upon the type of host cell, the class of lytic reagents selected and the degree of cell permeation desired in a defined

period of time. Thus, in one embodiment, the concentration of any single detergent is from about 0.01% to about 5% (w/v), and more preferably from about 0.1% to about 2%. In another embodiment, the concentration of each lytic enzyme is from about 0.01 mg/ml to about 0.2 mg/ml. In yet another embodiment, the concentration of buffer is such that the pH of the cellular solution is maintained at about pH 3 to about pH 12, for the duration of the period of time during which extraction or extraction and isolation occurs. In another embodiment, the concentration of protease inhibitor is from about 10 nM to about 10 mM. In another embodiment, the concentration of phosphatase inhibitor is from about 0.01 nM to about 10 mM.

[0122] Regardless of whether the lytic reagent is present in the container as an adsorbed, free-flowing, dissolved or slurried component, when a solution or suspension containing a host cell is added to the container, the lytic reagent will be dissolved or diluted by the suspension containing the host cell, and the host cell is lysed. If the lytic reagent contains all reagents needed for lysis, there is no need to perform multiple pipetting steps to ensure all the needed lytic reagents are present. Furthermore, as noted above, the lytic reagent need not completely solubilize the host cell to be effective. Rather, the host cell need only be lysed to the extent necessary to release some or all of the target product into solution. In addition, the lytic reagent need not lyse all host cells in any particular cellular suspension to be effective, so long as some of the host cells are lysed.

5. Kits

[0123] Advantageously, a container of the present invention may be combined with instructions for use, and reagents for extracting and/or isolating a cellular component from a host cell, and/or reagents for assaying or detecting a captured cellular component, and/or processing buffers or controls, wherein all of this is packaged together and distributed as a kit. In one embodiment, the kit would comprise a single container or, alternatively, a multiwell plate comprising

a plurality of containers; typically, the kit will be sealed. Either way, a lytic reagent is included, and, optionally, a capture ligand may also be included.

[0124] As described herein, the lytic reagent and/or capture ligand may be provided in a container of the present invention in a variety of different manners. For example, the lytic reagent may be coated on a portion of the container, on the bottom of the container, on the sidewall formation, on both the bottom and the sidewall formation of the container, or may be present in the form of a free-flowing powder. Likewise, a supported capture ligand may be positioned on a portion of the container, on the bottom of the container, on the sidewall formation, or on both the bottom and the sidewall formation of the container. In one embodiment, the container further comprises an additional support, such as a bead or mesh, onto which a lytic reagent may be coated and/or a supported capture ligand may be positioned. Alternatively, the container may be a high capacity platform comprising a three dimensional polymer matrix, a capture ligand or activatable group, and a lytic reagent.

[0125] In one embodiment, the container will comprise all reagents necessary for the extraction or extraction and isolation of the cellular component (e.g., polypeptide, protein, RNA or DNA product). The kit may also contain other reagents and equipment useful in releasing or eluting the captured product from the supported capture ligands or three dimensional matrix, as well as various processing buffers.

6. Methods

[0126] In general, the methods of the present invention are directed to the extraction or extraction and isolation of a cellular component, such as a peptide, protein, nucleic acid, or other cellular component, from a host cell. Thus, in one aspect, the present invention is directed to a process for the extraction of a cellular component from a host cell, the process comprising (a) introducing a liquid suspension containing the host cell into a container, the container having a mouth, an interior surface, a volume, V , and a coating of a

lytic reagent on at least a portion of the interior surface, the interior surface comprising a sidewall formation and a bottom, the ratio of the area of the coated interior surface to the volume, V , being less than about $4 \text{ mm}^2/\mu\text{l}$, and (b) lysing the host cell in the container to release the cellular component and form cellular debris. The lytic reagent causes the host cell to release its contents. Lysis may be complete, i.e., all the cellular components (e.g., peptides, proteins, or nucleic acids) are released from the host cell, or partial, i.e., a portion of the cellular components are released from the host cell.

[0127] In another aspect, the present invention is directed to a process for the extraction and isolation of a cellular component from a host cell. In one aspect, the process comprises (a) introducing a liquid suspension containing the host cell into a container, the container having a mouth, an interior surface, a volume, V , a lytic reagent, and a supported, capture ligand, the interior surface comprising a sidewall formation and a bottom, the sidewall formation being between the bottom and the mouth, the mouth serving as the inlet for the introduction of the liquid into and the outlet for the removal of the liquid from the container, (b) lysing the host cell in the container to release the cellular component and form solid cellular debris; and (c) capturing the cellular component with the capture ligand in the presence of the solid cellular debris. In one embodiment, the capture ligand is supported by the interior surface of the container. In another embodiment, the capture ligand is attached to a polymeric matrix coated on the interior surface of the container.

[0128] In another aspect, the process comprises (a) introducing a liquid suspension containing the host cell into a container, the container having a mouth, an interior surface, a volume, V , a lytic reagent, and a supported capture ligand, the interior surface comprising a sidewall formation and a bottom, the sidewall formation being between the bottom and the mouth, the mouth serving as the inlet for the introduction of the liquid into the container, (b) lysing the host cell in the container to release the cellular component and form solid cellular debris; (c) capturing the cellular component with the capture ligand in the

presence of the solid cellular debris, (d) releasing the cellular component from the capture ligand, and (e) recovering the released cellular component. In one embodiment, the capture ligand is supported by the interior surface of the container. In another embodiment, the capture ligand is attached to a polymeric matrix coated on the interior surface of the container.

[0129] Lysis may be complete, i.e., all the cellular components are released from the host cell, or partial, i.e., a portion of the cellular components are released from the host cell. In one embodiment, the cellular debris and other unbound cellular compositions are then washed away, leaving the cellular component attached to the capture ligand. The captured product may then be detected while still attached to the capture ligand. Such detection methods are well known in the art, and include ELISA, protein detection, and enzymatic analysis, among others. In another embodiment, the captured component is recovered by releasing or eluting the captured cellular component from the capture ligand, through the use of reagents such as salts, or by the competitive binding of other reagents with the capture ligands.

[0130] Referring now to Fig. 7, a method of the present invention will be described in the context of a container comprising lytic reagent and capture ligand. The container generally designated as 10 is a column or tube having a generally cylindrical shaft 12 defining an internal chamber, a mouth 13 (which may be covered by upper cap 14), an outlet 15 (which may be covered by lower cap 16). Within the chamber defined by generally cylindrical shaft 12 is a resin bed 18 having capture ligand bound thereto, and a mass of lytic reagent 20 overlying resin bed 18. To support the resin bed in the chamber, container 10 may additionally comprise a porous polyethylene frit (approximately 20 μm pore size). In operation, upper cap 14 is removed and a liquid suspension containing host cells are poured into the column through mouth 13. Lytic reagent 20 is dissolved by the liquid suspension thereby enabling the release of all, or a portion of, the cellular components of the host cell and their capture by capture ligands bound to resin bed 18. After capture of the cellular components, cellular

debris and other components of the liquid suspension are drained from the container via outlet 15; advantageously, a frit or other support means prevents resin 18 from exiting the chamber but allows the cellular debris and other components of the suspension to exit the column. In a preferred embodiment, the column has a 9.1 cm interior column length (or 12.3 cm length, when capped at the bottom and mouth); a diameter of approximately 1 cm at the bottom opening, a diameter of approximately 1.7 cm at the mouth opening; and a total volume of approximately 7.5 ml. In one embodiment, the capture ligand is a nickel chelate covalently attached to a bed of agarose resin and the lytic reagent comprises a free-flowing powder of CHAPS (i.e., 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate), lysozyme, Tris-HCl, and DNase I.

[0131] In another embodiment, the methods described above may be performed in a well or wells of a multiwell plate, such as a 96 well multiwell plate, comprising a lytic reagent and a polymer matrix coating. For example, in one embodiment, the well(s) are coated with a polymer matrix derived from dextran polymer(s) as previously described, to which is attached a capture ligand. In a preferred embodiment, the polymer matrix is derived from a mixture of dextran polymers, and the capture ligand is a nickel chelate. In one embodiment, the lytic reagent is comprised of octyl-thioglucoopyranoside (OTG), protease inhibitors, lysozyme, and Benzonase[®] endonuclease. More specifically, the lytic reagent may be comprised of 2% OTG, 1% protease inhibitor, 2% lysozyme, and 0.02% Benzonase[®] endonuclease. In one embodiment, the lytic reagent is coated onto at least a portion of the surface of the polymer matrix and/or onto the sidewalls of the well(s). Alternatively, or in addition, the lytic reagent may be present in the form of a lyophilized matrix or other mass (e.g., a free-flowing powder) within the well(s). Upon addition of a liquid suspension containing host cells into the well(s), the lytic reagent is dissolved, and the host cells are lysed, as previously described. The target cellular component is then bound by the capture ligand. The captured target cellular component may then optionally be

released and recovered using techniques known in the art and previously described.

[0132] In another aspect, the present invention is directed to a process for the preparation of a multiwell plate for the extraction of a cellular component from a host cell. The process comprises contacting the interior surfaces of a plurality of the wells of the multiwell plate with a liquid containing a lytic reagent, and drying the liquid to form an adsorbed layer of lytic reagent on the interior surfaces of the wells. Any lytic reagent, as described herein, can be used in this manner. As previously discussed, the amount of lytic reagent may vary, but should be sufficient so that the amount of adsorbed lytic reagent will provide the desired level of extraction. Drying may be accomplished by air drying, use of an incubator, or other techniques known in the art.

[0133] Containers for the extraction and isolation of a cellular component from a host cell may be prepared in a similar manner. For example, in one embodiment, the interior surface of a well comprising a supported capture ligand may be contacted with a liquid containing a lytic reagent, and the liquid dried to form an adsorbed layer of lytic reagent on the interior surface of the well. In another embodiment, the interior surface of a well comprising a polymer matrix attached thereto (e.g. a well or wells of a multiwell plate, described above) may be contacted with a liquid containing a lytic reagent, and the liquid dried to form an adsorbed layer of lytic reagent on the surface of the polymer matrix and/or the sidewalls of the well(s). In another embodiment, the interior surface of a column, such as a column comprising a resin with attached capture ligands, as described above, may be contacted with a liquid containing a lytic reagent, and the liquid dried to form an adsorbed layer of lytic reagent on the surface of the resin and/or the sidewalls of the column.

[0134] All publications, patents, patent applications and other references cited in this application are herein incorporated by reference in their entirety as if each individual publication, patent, patent application or other reference were specifically and individually indicated to be incorporated by reference.

7. Definitions

[0135] The term "capture ligand" means any moiety, molecule, receptor, or layer that can be or is immobilized or supported on a container or support and used to isolate a cellular component from cellular debris. Some non-limiting examples of capture ligands that may be used in connection with the present invention include: biotin, streptavidin, various metal chelate ions, antibodies, various charged particles such as those for use in ion exchange chromatography, dye, various affinity chromatography supports, and various hydrophobic groups for use in hydrophobic chromatography.

[0136] The terms "cell debris" and "cellular debris" are used interchangeably herein to describe membrane fragments, organelles, or any other soluble or insoluble cell component other than a target product, that is released from the host cell as a result of cell lysis.

[0137] The term "extraction" means the release of at least some of the target product from the host cell in which it is expressed, as a result of cell lysis.

[0138] The term "host cell" means any prokaryotic or eukaryotic cell that expresses or contains the target product. Host cells may include, for example, bacterial cells, such as *E. coli*; fungal cells, such as yeast cells; plant cells; animal cells, such as mammalian cells; and insect cells.

[0139] The term "isolation" or "purification" means the removal or separation of at least a portion of the target product from at least part of the cellular debris.

[0140] The term "lysis" or "lysing" means rupturing the cell wall and/or cell membrane of a cell so that the target product is released. Lysis may be complete or partial (i.e., the cell wall and/or cell membrane is rendered sufficiently permeable to release some, but not necessarily all of its cellular components).

[0141] The term "target product" means any cellular component, such as a polypeptide, protein, protein fragment, DNA, RNA, other nucleotide sequence,

carbohydrate, lipid, cholesterol, kinase, or other cellular component, that is to be extracted or extracted and isolated from the host cell in which it is expressed or contained (e.g., the "target protein," "target DNA," "target RNA," "target cellular component," etc.). The target product may naturally occur in the host cell, or it may be non-naturally occurring, e.g., a recombinant protein.

[0142] As various changes could be made in the above products and methods without departing from the scope of the invention, it is intended that all matter contained in the above description and in the examples given below, shall be interpreted as illustrative and not in a limiting sense.

EXAMPLES

EXAMPLE 1

Detergent Lysis and Purification by a HIS-Select™ High Capacity Plate using a his- tagged Recombinant Protein

[0143] In this example, a bacteria comprising a protein containing a recombinant his-tag was lysed, and the target protein was purified in one step. The recombinant protein was spiked into *E. coli* cells at different amounts to determine if the protein could be captured while the cells were lysed. Unless otherwise noted, all materials were obtained from Sigma-Aldrich Corporation, St. Louis, MO.

[0144] Dry Lysis Support. The purification of the target protein was done using a HIS-Select™ high capacity (HC) plate (Sigma S5563). These 96 well multiwell plates are coated with a high-density, nickel chelate polymer matrix, such as described above. These plates are used for purification of his-tagged recombinant proteins, and can bind greater than 4 µg protein per well. The main lysing component was 1% octyl-thioglucoopyranoside (OTG) in 20 mM Tris-Cl pH 7.5. Various processing reagents and enzymes were also added to the buffered detergent: i) 1% (v/v) protease inhibitors (Sigma P8849), 2% lysozyme (Sigma

10 mg/ml solution L3790), and 0.02% Benzonase[®] endonuclease (Sigma E1014); ii) 1% protease inhibitors and 2% lysozyme; and iii) 1% protease inhibitors and 0.02% Benzonase[®] endonuclease. The solutions were dispensed into separate wells of a 96 well HIS-Select[™] HC plate, with each well containing 0.1 ml of solution of the buffered detergent plus (i), (ii), or (iii). The solutions were dried onto the wells of the plate in an incubator overnight at 47°C with dry air blowing over the plate. Once dried, the surface area of each well coated with the detergent plus (i), (ii), or (iii) was approximately 134.7 mm².

[0145] Cell Growth. 5-ml sterile terrific broth (TB) media was added to each of three 15-ml round bottom tubes. Ampicillin was added to a final concentration of 0.1 mg/ml to each of the tubes. One colony of non-expressing *E. coli* was added to each of the tubes. The cultures were incubated overnight at 37°C with shaking at 250 rpm.

[0146] E. coli Samples. A purified recombinant 28 kDa protein containing a histidine tag of the sequence His-Asn-His-Arg-His-Lys-His (SEQ. ID. NO. 4) was diluted to 1 mg/ml with sterile TB media. The protein samples were made by spiking in a specified amount of target protein into the non-expressing *E. coli* cultures. Aliquots of 100 µl were added to each well containing dried lysis reagents. Non-expressing *E. coli* cultures were used as a control. The samples were incubated at room temperature with gentle shaking for 2 hours.

[0147] SDS-PAGE Analysis. The plate was washed 4 times with tris buffered saline with 0.05% Tween 20 (TBST), pH 8.0 using a BioMek plate washer. Selected wells were eluted at room temperature with 50 µl of a solution containing 50 mM sodium phosphate, pH 8, 300 mM sodium chloride, and 250 mM imidazole. The samples were mixed 1:1 with Laemmli sample buffer, and a 20 µl sample was electrophoresed through a 4-20% tris-glycine gel (Invitrogen) in 1x Tris-Glycine-SDS buffer. The gel was stained with EZBlue staining reagent (Sigma G1041) followed by silver staining (Sigma #Prot-sil1). The results are given in Figure 1.

[0148] Results and Discussion. Table 1 indicates the lysing reagent and composition of the sample used for each lane in Figure 1. In each of the wells in which the target protein was added the protein was captured and eluted. Higher amounts of protein resulted in higher amounts of target protein captured. The processing aids were beneficial to the amount of target protein bound, especially the presence of the lysozyme in addition to the detergent.

TABLE 1

Lytic Reagent and Sample Composition for SDS-PAGE Analysis

Lane Number	Lytic reagent dried in plate	Composition of Sample 20 μ l of a sample loaded onto the gel that was eluted from the plate with imidazole
1	N/A	Molecular weight markers (Colorburst Sigma C4105)
2	1% OTG, 20 mM Tris-Cl pH 7.5, 2% 10 mg/ml lysozyme, 1% v/v protease inhibitor cocktail (Sigma, P8849) and 0.02% Benzonase [®] endonuclease (Sigma E1014)	3 μ g of pure his- tagged target protein in Terrific broth (TB)
3	1% OTG, 20 mM Tris-Cl pH 7.5, 2% 10 mg/ml lysozyme, 1% v/v protease inhibitor cocktail (Sigma, P8849) and 0.02% Benzonase [®] endonuclease (Sigma E1014)	Non-expressing <i>E. coli</i> cells in TB

Lane Number	Lytic reagent dried in plate	Composition of Sample 20 µl of a sample loaded onto the gel that was eluted from the plate with imidazole
4	1% OTG, 20 mM Tris-Cl pH 7.5	Non-expressing <i>E. coli</i> cells spiked with 3 µg of pure his-tagged target protein in Terrific broth (TB)
5	1% OTG, 20 mM Tris-Cl pH 7.5, 2% 10 mg/ml lysozyme, 1% v/v protease inhibitor cocktail (Sigma, P8849) and 0.02% Benzonase® endonuclease (Sigma E1014)	Non-expressing <i>E. coli</i> cells in TB
6	1% OTG, 20 mM Tris-Cl pH 7.5, 2% 10 mg/ml lysozyme, 1% v/v protease inhibitor cocktail (Sigma, P8849) and 0.02% Benzonase® endonuclease (Sigma E1014)	Non-expressing <i>E. coli</i> extract spiked with 1 µg of pure his-tagged target protein in Terrific broth (TB)
7	1% OTG, 20 mM Tris-Cl pH 7.5, 2% 10 mg/ml lysozyme, 1% v/v protease inhibitor cocktail (Sigma, P8849) and 0.02% Benzonase® endonuclease (Sigma E1014)	Non-expressing <i>E. coli</i> cells spiked with 2 µg of pure his-tagged target protein in Terrific broth (TB)

Lane Number	Lytic reagent dried in plate	Composition of Sample 20 µl of a sample loaded onto the gel that was eluted from the plate with imidazole
8	1% OTG, 20 mM Tris-Cl pH 7.5, 2% 10 mg/ml lysozyme, 1% v/v protease inhibitor cocktail (Sigma, P8849) and 0.02% Benzonase® endonuclease (Sigma E1014)	Non-expressing <i>E. coli</i> cells spiked with 3 µg of pure his-tagged target protein in Terrific broth (TB)
9	1% OTG, 20 mM Tris-Cl pH 7.5, 2% 10 mg/ml lysozyme, 1% v/v protease inhibitor cocktail (Sigma, P8849) and 0.02% Benzonase® endonuclease (Sigma E1014)	Non-expressing <i>E. coli</i> cells spiked with 4 µg of pure his-tagged target protein in Terrific broth (TB)
10	1% OTG, 20 mM Tris-Cl pH 7.5, 2% 10 mg/ml lysozyme, 1% v/v protease inhibitor cocktail (Sigma, P8849) and 0.02% Benzonase® endonuclease (Sigma E1014)	Non-expressing <i>E. coli</i> cells spiked with 5 µg of pure his-tagged target protein in Terrific broth (TB)
11	1% OTG, 20 mM Tris-Cl pH 7.5, 2% 10 mg/ml lysozyme, 1% v/v protease inhibitor cocktail (Sigma, P8849)	Non-expressing <i>E. coli</i> cells spiked with 3 µg of pure his-tagged target protein in Terrific broth (TB)

Lane Number	Lytic reagent dried in plate	Composition of Sample 20 µl of a sample loaded onto the gel that was eluted from the plate with imidazole
12	1% OTG, 20 mM Tris-Cl pH 7.5, 1% v/v protease inhibitor cocktail (Sigma, P8849) and 0.02% Benzonase [®] endonuclease (Sigma E1014)	Non-expressing <i>E. coli</i> cells spiked with 3 µg of pure his-tagged target protein in Terrific broth (TB)

EXAMPLE 2

Detergent Lysis, Capture, and Purification by HIS-Select™ High Capacity Plate Using Recombinant *E. coli* Cells

[0149] In this procedure, a bacteria comprising a protein containing a recombinant his-tag was lysed using various detergents in combination with processing aids, and the target protein was purified in one step.

Unless otherwise noted, all materials were obtained from Sigma-Aldrich Corporation, St. Louis, MO.

[0150] Dry Lysis Support. Various combinations of detergents and processing reagents and enzymes were used to examine a range of lysis reagents. 100 µl of 2% OTG, 2% CHAPS, 4% CHAPS, 2% C7BzO, or 2% ASB-14 was dried onto a 96 well HIS-Select™ high capacity plate (Sigma S5563). Solutions containing these detergents and other processing reagents and enzymes were also made. Each detergent was combined with i) 2% (v/v) protease inhibitor cocktail (Sigma P8849); ii) 2% protease inhibitor cocktail (Sigma P8849) and 0.01% Benzonase[®] endonuclease (Sigma E1014); iii) 2% protease inhibitor cocktail (Sigma P8849) and 0.04% lysozyme; and iv) 2% protease inhibitor cocktail (Sigma P8849), 0.01% Benzonase[®] endonuclease (Sigma E1014), and 0.04% lysozyme. Additional solutions containing i) 2% OTG

or 2% CHAPS and 0.04% lysozyme; ii) 2% OTG or 2% CHAPS and 0.01% Benzonase® endonuclease (Sigma E1014); and iii) 2% OTG or 2% CHAPS and 0.01% Benzonase® endonuclease (Sigma E1014) and 0.04% lysozyme were also made. Each of these solutions were dispensed into 2-3 wells of a HIS-Select™ high capacity plate (Sigma S5563), with each well containing 100 µl of solution. The lytic reagents were dried overnight in a 47°C oven with air blowing over the plate.

[0151] Cell Growth. In a 15 ml round bottom tube, 5-ml sterile TB media was added. Ampicillin was added to a final concentration of 0.1 mg/ml to the tube. One colony of *E. coli* BL21G expressing the his-tagged target protein was added to the tube. The culture was incubated overnight at 37°C with shaking at 250 rpm. One ml of cells from the starter culture was used to inoculate 500-ml autoclaved terrific broth (TB). Ampicillin was added to a final concentration of 0.1 mg/ml to the tube. The culture was incubated for 3 ½ hours at 37°C with shaking at 250 rpm. After 3 ½ hours, the OD at 600 nm was 0.5. Isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to the culture at a final concentration of 1 mM to induce expression of the target protein. The culture was incubated for another 1 ½ hours at 37°C with shaking at 250 rpm.

[0152] E. coli Samples. *E. coli* expressing the his-tagged protein (as used in Example 1) was added to half of the wells containing dried lysis reagents, in 200 µl aliquots. The empty wells were used as controls. The samples were incubated at room temperature for 1 hour with gentle shaking.

[0153] Bicinchoninic Acid (BCA) Protein Assay. The wells were washed 4 times with TBST, pH 8.0 using a BioMek plate washer. 1 mg/ml bovine serum albumin (BSA) was used for the standard curve. 200 µl of BCA working reagent was added to each of the wells. The plate was incubated at 37°C for 30 minutes and read on a plate reader at 562 nm. The results are given in Table 2.

[0154] Results and Discussion. The BCA protein assay indicated that the target protein was successfully captured on the HIS-Select™ high capacity

plate. The various detergent formulations were able to lyse the cells, allowing the protein to be captured. The nonionic detergent OTG, as well as the zwitterionic detergents CHAPS, C7BzO and ASB-14, worked well. The addition of processing aids, especially lysozyme, helped increase the amount of protein bound to the plate.

TABLE 2

Protein amount ($\mu\text{g}/\text{well}$) determined by BCA assay

Detergent	No Add.	Lys.	Benz.	Pr. Inh.	Lys., Benz.	Lys., Pr. Inh.	Benz., Pr. Inh.	Lys., Benz., Pr. Inh.
2% OTG	2.771	4.045	2.607	2.607	4.946	4.912	2.953	6.523
2% CHAPS	2.026	4.704	2.208	2.156	5.31	4.253	1.792	6.593
4% CHAPS	1.908			2.052		4.201	1.896	5.38
2% C7BzO	2.133			3.352		6.939	2.763	9.921
2% ASB-14	2.771			3.109		5.362	2.815	9.539

[0155] Table 2 shows the average amount of protein (μg) per well, for each lytic reagent tested, for the BCA protein assay. Column 1 indicates the detergent used. Column 2 summarizes the results when only the detergent was used. Columns 3-9 summarize the results when lysozyme ("Lys"), Benzonase[®] endonuclease ("Benz"), protease inhibitor cocktail ("Pr. inh."), or various combinations thereof, are used in addition to a detergent.

EXAMPLE 3

Lysis, Capture, and Purification with 2% OTG and HIS-Select[™] High Capacity Plate Using *E. coli* and a Recombinant his-tagged Protein

[0156] In this example, a bacteria comprising a protein containing a recombinant his-tag was lysed using 2% OTG, and the target protein was purified in one step.

[0157] Unless otherwise noted, all materials were obtained from Sigma-Aldrich Corporation, St. Louis, MO.

[0158] Dry Lysis Support. The target protein was purified using a HIS-Select™ high capacity plate (Sigma S5563). These 96 well multiwell plates are used for purification of his-tagged recombinant proteins and can bind greater than 4 µg protein per well. A lysing solution, comprising 2% octyl-thioglucopyranoside (OTG) in 20 mM Tris-Cl pH 7.5, 1% (v/v) protease inhibitors (Sigma P8849), 2% lysozyme (Sigma 10 mg/ml solution L3790), and 0.02% Benzonase® endonuclease (Sigma E1014), was made. Either 50 µl or 100 µl of this solution was dispensed into the wells of the 96 well HIS-Select™ HC plate. The solution was dried onto the wells of the plate in an incubator overnight at 47°C with dry air blowing over the plate.

[0159] Cell Growth. In a 15 ml round bottom tube, 5 ml sterile TB media was added. One colony of non-ampicillin resistant *E. coli* expressing the his-tagged target protein was added to the tube. The culture was incubated overnight at 37°C with shaking at 250 rpm.

[0160] *E. coli* Samples. Purified recombinant 28 kDa protein containing a histidine tag (as described in Example 1) was diluted to 1 mg/ml with sterile TB media. The protein samples were made by spiking in a specified amount of target protein into the non-expressing *E. coli* cultures. Control samples comprised only purified target protein or non-expressing *E. coli* cultures. Aliquots of 100 µl were added to each well containing the dried lytic reagents. The samples were incubated at room temperature with gentle shaking for 2 hours.

[0161] SDS-PAGE Analysis. After incubation, the plate was washed 4 times with TBST, pH 8.0 using a BioMek plate washer. Some of the wells were eluted at room temperature with 50 µl of a solution containing 50 mM sodium phosphate, pH 8, 300 mM sodium chloride, and 250 mM imidazole. The samples were mixed 1:1 with Laemmli sample buffer, and 20 µl was electrophoresed through a 4-20% tris-glycine gel (Invitrogen) in 1x Tris-Glycine-

SDS buffer. The gel was stained with EZBlue staining reagent followed by silver staining. The results are given in Figures 2 and 3, and Table 3.

[0162] Bradford Protein Assay. 1 mg/ml BSA was used for the standard curve. 250 μ l of Bradford reagent was added to each of the wells. The plate was incubated at room temperature for 15 minutes and read on a plate reader at 595 nm. The results are given in Table 5.

[0163] Light Scattering. A 100 μ l aliquot of cell culture was diluted 1:10 with sterile media to determine OD at 550 nm prior to lysing. Duplicate aliquots were read at 550 nm after lysis, for the cell sample containing the 8 μ g spike of target protein. The results are given in Table 4.

[0164] Results and Discussion. The SDS-PAGE samples show that the cells were lysed, and target protein was captured and successfully eluted. The amount of target protein captured increased with increasing amount of target protein added to the cells. The light-scattering data showed a decrease in absorbance at 550 nm for the post-lysis sample, which indicates that the cells were lysed. The Bradford protein assay data done on the samples indicated that there was target protein bound to the plate. The lysis of non-expressing cells showed background protein levels but increasing amounts of target protein gave protein numbers higher than this background level.

TABLE 3

Sample Composition for SDS-PAGE Analysis

Lane Number	Composition of Sample 20 μ l of a sample loaded onto the gel that was eluted from the plate with imidazole
1	Molecular weight markers (Colorburst Sigma C4105)
2	Non-expressing <i>E. coli</i> cells in TB
3	Non-expressing <i>E. coli</i> cells in TB

Lane Number	Composition of Sample 20 μ l of a sample loaded onto the gel that was eluted from the plate with imidazole
4	Non-expressing <i>E. coli</i> cells spiked with 2 μ g of pure his- tagged target protein in Terrific broth (TB)
5	Non-expressing <i>E. coli</i> cells spiked with 4 μ g of pure his- tagged target protein in Terrific broth (TB)
6	Non-expressing <i>E. coli</i> cells spiked with 6 μ g of pure his- tagged target protein in Terrific broth (TB)
7	Non-expressing <i>E. coli</i> cells spiked with 8 μ g of pure his- tagged target protein in Terrific broth (TB)
8	Non-expressing <i>E. coli</i> cells spiked with 10 μ g of pure his- tagged target protein in Terrific broth (TB)
9	Terrific broth (TB)
10	2 μ g of pure his- tagged target protein in Terrific broth (TB)
11	4 μ g of pure his- tagged target protein in Terrific broth (TB)
12	6 μ g of pure his- tagged target protein in Terrific broth (TB)
13	8 μ g of pure his- tagged target protein in Terrific broth (TB)
14	10 μ g of pure his- tagged target protein in Terrific broth (TB)

[0165] Table 3 indicates the composition of the samples for each lane of Figures 2 and 3. All of the samples were applied to a HIS-Select™ HC plate (Sigma S5563) that contained a dried solution of 50 μ l (Figure 2) or 100 μ l (Figure 3) of 2% OTG, 20 mM Tris-Cl pH 7.5, 2% 10 mg/ml lysozyme, 1% v/v protease inhibitor cocktail (Sigma, P8849) and 0.02% Benzonase® endonuclease (Sigma E1014).

TABLE 4
Light Scattering Results

Sample	Absorbance at 550 nm
Non-lysed	0.3774
Sample after lysing	0.0463
Sample after lysing	0.0458

TABLE 5
Protein amount bound to the HIS-Select™ HC plate
per well as determined by Bradford Assay directly in the well.

Amount of target protein loaded per well (μg)	Amount of protein bound in a well using Bradford protein assay (μg/well)					
	50 μl of solution dried in each well			100 μl of solution dried in each well		
	Target protein plus crude <i>E. coli</i>	Target protein only	<i>E. coli</i> only	Target protein plus crude <i>E. coli</i>	Target protein only	<i>E. coli</i> only
0	1.2	1.4	1.4	1.2	1.6	1.6
2	3.2	2.9	---	3.2	3.1	---
4	4.3	3.6	---	4.2	4.3	---
6	4.2	4.5	---	4.6	4.8	---
8	5.3	4.7	---	4.8	4.8	---
10	4.9	5.7	---	5.2	5.6	---

EXAMPLE 4

Detergent Lysis, Capture and Purification of Recombinant Proteins using High Capacity and High Sensitivity HIS-Select™ and ANTI-FLAG® M2 Plates

[0173] In this example, bacterial cells expressing a target protein with a DYKDDDDK (SEQ. ID. NO. 1) and/or his tag were lysed using various detergent(s) in combination with processing aids, and the target protein was purified in one step.

[0174] Unless otherwise noted, all materials were obtained from Sigma-Aldrich Corporation, St. Louis, MO.

[0175] Dry Lysis Support. Various combinations of detergents, processing reagents, and enzymes were used to examine a range of lysis conditions. Detergent lysis solutions containing the following were prepared:

- [0176]** a) 2% SB3-10, 0.2 % C7BzO, 0.2% n-dodecyl α -D-maltoside, 0.2% Triton X-100
- [0177]** b) 2% CHAPS, 1% ASB-14
- [0178]** c) 2% SB3-14, 0.2% C7BzO
- [0179]** d) 2% CHAPS, 1% n-Octyl glucoside
- [0180]** e) 2% SB3-12, 0.2% C7BzO
- [0181]** f) 2% SB3-14, 0.2% ASB-14
- [0182]** g) 1% n-Octyl glucoside, 1% CHAPS, 0.2% n-dodecyl α -D-maltoside
- [0183]** h) 8% CHAPS

[0184] The detergent CHAPS is 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; SB3-10 is 3-(decyldimethylammonio)propanesulfonate inner salt; SB3-12 is 3-(dodecyldimethylammonio)propanesulfonate inner salt; SB3-14 is 3-(N,N-dimethylmyristylammonio)propanesulfonate; C7BzO is 3-(4-heptyl) phenyl 3-hydroxy propyl) dimethylammonio propane sulfonate; and ASB-14 is 3-[N,N-dimethyl(3-myristoylaminopropyl)ammonio]propanesulfonate.

The first seven detergent solutions (a-g) also contained 40 mM Tris-HCl, pH 7.4, 0.04% lysozyme (Sigma L3790), and 0.01% Benzonase[®] endonuclease (Sigma E1014). The 8% CHAPS solution (h) also contained 80 mM Tris-HCl, pH 8.0, 0.04% lysozyme (Sigma L6876), and 0.01% DNase I (Sigma D4527). 100 μ l of each of these detergent solutions was dispensed into 6 wells (half a row) of a HIS-Select[™] high capacity plate (Sigma M5563), HIS-Select[™] high sensitivity plate (Sigma S5688), ANTI-FLAG[®] M2 high capacity plate, and an ANTI-FLAG[®] M2 high sensitivity plate (Sigma P2983). The lytic reagents were dried overnight in an incubator with ambient air running over the plates.

[0185] Cell Growth. 5-ml sterile terrific broth (TB) was added to each of three 15 ml round bottom tubes. Ampicillin was added to a final concentration of 0.1 mg/ml to each of the tubes. A 20 μ l aliquot of a glycerol stock solution of BL21 *E. coli* expressing a target protein with a DYKDDDDK (SEQ. ID. NO. 1) tag was added to the first tube. A 20 μ l aliquot of a glycerol stock solution of BL21 *E. coli* expressing a target protein with a DYKDDDDK (SEQ. ID. NO. 1) /his tag was added to the second tube. A 20 μ l aliquot of a glycerol stock solution of BL21 *E. coli* expressing a target protein with a his tag (as described in Example 1) was added to the third tube. The cultures were incubated overnight at 37°C with shaking at 275 rpm.

[0186] The starter cultures grown overnight were used to inoculate three 500-ml autoclaved terrific broth samples. Ampicillin was added to a final concentration of 0.1 mg/ml to each flask. The cultures were incubated for 4 hours at 37°C with shaking at 275 rpm. Isopropyl β -D-1-thiogalactopyranoside (IPTG) was added to the cultures at a final concentration of 1 mM to induce expression of the target proteins. The cultures were incubated another 3 hours at 37°C with shaking at 275 rpm.

[0187] E. coli Samples. *E. coli* expressing the recombinant proteins grown in the 500 ml shake flasks was added to two columns of each plate that was coated with the lysis reagents, in 200 μ l aliquots. The empty wells were used as controls. The samples were incubated at room temperature for 2 hours with gentle shaking.

[0188] Enzyme Immunodetection Assay for High Sensitivity Plates. The wells were washed 4 times with TBS-T, pH 8.0, followed by 4 washes with deionized water, using a BioTek plate washer. 200 μ l of a horseradish peroxidase (HRP) conjugated antibody specific to the target protein was added to each well. These conjugates were also added to four other wells which did not contain protein for use as blanks. The plates were allowed to incubate with the antibody for 45 minutes at room temperature, and then were washed 4 times

with TBS-T, pH 8.0. 100 μ l of TMB substrate (Sigma T0440) was added to each well and the plates were developed until the color was distinct (approximately 3-5 minutes). At this point, the reaction was stopped by adding 100 μ l of 1M HCl to each well. Absorbance readings were obtained at 450 nm, and the blanks were subtracted to determine corrected A_{450} .

[0189] TCA Precipitation for High Capacity Plates. The wells were washed 4 times with TBS-T, pH 8.0, followed by 4 washes with deionized water, using a BioTek plate washer. 100 μ l of 50 mM sodium phosphate, pH 8.0, 300 mM NaCl, and 250 mM imidazole was aliquoted into each well of the HIS-Select™ high capacity plate. 100 μ l of 0.1 M glycine, pH 3.0, was aliquoted into each well of the ANTI-FLAG® M2 high capacity plate. The plates were allowed to incubate at 37°C for 20 minutes to elute the target proteins. The eluted samples were removed from the plates and placed into clean tubes. Each sample was diluted with 0.2% sodium deoxycholate solution (Sigma D3691) to a final volume of 500 μ l. The samples were briefly vortexed and incubated at room temperature for 10 minutes. 50 μ l of a 100% trichloroacetic acid solution (TCA) (Sigma T6323) was added to each sample, and they were briefly vortexed and incubated on ice for 15 minutes. The samples were centrifuged at 15,000 x g for 10 minutes at room temperature and the supernatants were decanted off. 500 μ l of a 25% acetone solution (Sigma A5351) was added to each tube. The samples were briefly vortexed and centrifuged at 15,000 x g for 5 minutes. The supernatants were decanted off and the protein pellets were dried in a SpeedVac at 30°C for 20 minutes.

[0190] SDS-PAGE Analysis. Each protein pellet was resuspended in 10 μ l of Laemmli sample buffer (Sigma S3401), and titrated to basic pH with 1 M NaOH. The entire sample was electrophoresed through 10-20% Tris-glycine gels (BioRad Cat. #345-0044). The gels were stained with EZ Blue™ (Sigma G1041) gel staining reagent for 1 hour, and destained with deionized water overnight.

[0191] Results and Discussion. The corrected A_{450} readings from the enzyme immunodetection assay indicated that the target protein was successfully captured on the HIS-Select™ and ANTI-FLAG® M2 high sensitivity plates. The various detergent formulations were capable of lysing the cells, allowing the protein to be captured. Figure 4 depicts the corrected absorbance values from the ANTI-FLAG® M2 high sensitivity plate assay, which shows that the proteins with a DYKDDDDK (SEQ. ID. NO. 1) tag were captured, while those proteins without a DYKDDDDK (SEQ. ID. NO. 1) tag were not. Figure 5 contains corrected absorbance values from the HIS-Select™ high sensitivity plate immunodetection assay, and shows that the plate was capable of selectively capturing his-tagged target proteins, while not capturing proteins without a his-tag. Similarly, The SDS-PAGE results in Figure 6 show that the target protein was successfully captured and eluted from the HIS-Select™ high capacity plate. Similar results were obtained from the ANTI-FLAG® M2 high capacity plate. Table 6 indicates the lysing reagent and composition of the sample used for each lane in Figure 6.

TABLE 6

Lytic Reagent and Sample Composition for SDS-PAGE Analysis

Lane Number	Lysis Reagent in Plate	Composition of Sample
1	N/A	Molecular Weight Markers (Sigma Product M3913)
2	N/A	10 μ l <i>E. coli</i> cells expressing ~ 60 kDa his-tagged protein
3	1% SB 3-10, 0.1 % C7BzO, 0.1% n-dodecyl α -D-maltoside, 0.1% Triton X-100, 20 mM Tris-HCl, pH 7.4, 0.02% lysozyme, 0.005 % Benzonase® endonuclease (Sigma E1014)	Sample eluted from HIS-Select™ High Capacity plate with imidazole

Lane Number	Lysis Reagent in Plate	Composition of Sample
4	1% CHAPS, 0.5% ASB-14, 20 mM Tris-HCl, pH 7.4, 0.02% lysozyme, 0.005 % Benzonase® endonuclease (Sigma E1014)	Sample eluted from HIS-Select™ High Capacity plate with imidazole
5	1% SB 3-14, 0.1% C7BzO, 20 mM Tris-HCl, pH 7.4, 0.02% lysozyme, 0.005 % Benzonase® endonuclease (Sigma E1014)	Sample eluted from HIS-Select™ High Capacity plate with imidazole
6	1% CHAPS, 0.5% n-Octyl glucoside, 20 mM Tris-HCl, pH 7.4, 0.02% lysozyme, 0.005 % Benzonase® endonuclease (Sigma E1014)	Sample eluted from HIS-Select™ High Capacity plate with imidazole
7	1% SB 3-12, 0.1% C7BzO, 20 mM Tris-HCl, pH 7.4, 0.02% lysozyme, 0.005 % Benzonase® endonuclease (Sigma E1014)	Sample eluted from HIS-Select™ High Capacity plate with imidazole
8	1% SB 3-14, 0.1% ASB-14, 20 mM Tris-HCl, pH 7.4, 0.02% lysozyme, 0.005 % Benzonase® endonuclease (Sigma E1014)	Sample eluted from HIS-Select™ High Capacity plate with imidazole
9	0.5% n-Octyl glucoside, 0.5% CHAPS, 0.1% n-dodecyl α -D-maltoside, 20 mM Tris-HCl, pH 7.4, 0.02% lysozyme, 0.005 % Benzonase® endonuclease (Sigma E1014)	Sample eluted from HIS-Select™ High Capacity plate with imidazole
10	4% CHAPS, 40 mM Tris-HCl, pH 8.0, 0.02% lysozyme, 0.005 % DNase I (Sigma D4527)	Sample eluted from HIS-Select™ High Capacity plate with imidazole
11	N/A	Molecular Weight Markers (Sigma Product M3913)
12	N/A	10 μ l <i>E. coli</i> cells expressing ~ 24 kDa his-tagged protein
13	1% SB 3-10, 0.1 % C7BzO, 0.1% n-dodecyl α -D-maltoside, 0.1% Triton X-100, 20 mM Tris-HCl, pH 7.4, 0.02% lysozyme, 0.005 % Benzonase® endonuclease (Sigma E1014)	Sample eluted from HIS-Select™ High Capacity plate with imidazole

Lane Number	Lysis Reagent in Plate	Composition of Sample
14	1% CHAPS, 0.5% ASB-14, 20 mM Tris-HCl, pH 7.4, 0.02% lysozyme, 0.005 % Benzonase® endonuclease (Sigma E1014)	Sample eluted from HIS-Select™ High Capacity plate with imidazole
15	1% SB 3-14, 0.1% C7BzO, 20 mM Tris-HCl, pH 7.4, 0.02% lysozyme, 0.005 % Benzonase® endonuclease (Sigma E1014)	Sample eluted from HIS-Select™ High Capacity plate with imidazole
16	1% CHAPS, 0.5% n-Octyl glucoside, 20 mM Tris-HCl, pH 7.4, 0.02% lysozyme, 0.005 % Benzonase® endonuclease (Sigma E1014)	Sample eluted from HIS-Select High Capacity plate with imidazole
17	1% SB 3-12, 0.1% C7BzO, 20 mM Tris-HCl, pH 7.4, 0.02% lysozyme, 0.005 % Benzonase® endonuclease (Sigma E1014)	Sample eluted from HIS-Select™ High Capacity plate with imidazole
18	1% SB 3-14, 0.1% ASB-14, 20 mM Tris-HCl, pH 7.4, 0.02% lysozyme, 0.005 % Benzonase® endonuclease (Sigma E1014)	Sample eluted from HIS-Select™ High Capacity plate with imidazole
19	0.5% n-Octyl glucoside, 0.5% CHAPS, 0.1% n-dodecyl α -D-maltoside, 20 mM Tris-HCl, pH 7.4, 0.02% lysozyme, 0.005 % Benzonase® endonuclease (Sigma E1014)	Sample eluted from HIS-Select™ High Capacity plate with imidazole
20	4% CHAPS, 40 mM Tris-HCl, pH 8.0, 0.02% lysozyme, 0.005 % DNase I (Sigma D4527)	Sample eluted from HIS-Select™ High Capacity plate with imidazole
21	N/A	Molecular Weight Markers (Sigma Product M3913)

WHAT IS CLAIMED IS:

1. A container for the extraction of a cellular component from a host cell, the container having a mouth, an interior surface, a volume, V , and a coating of a lytic reagent on at least a portion of the interior surface, the interior surface comprising a sidewall formation and a bottom, the amount of the lytic reagent in the coating being sufficient for the formation of a lysis solution having the capacity to lyse the host cell when a liquid suspension containing the host cell is introduced into the container, the ratio of the area of the coated interior surface, SA , to the volume, V , being less than about $4 \text{ mm}^2/\mu\text{l}$.
2. A container for the extraction and isolation of a cellular component from a host cell, the container having a mouth, an interior surface, a volume, V , a lytic reagent, and a supported, capture ligand, the sidewall formation being between the bottom and the mouth, the mouth serving as the inlet for the introduction of liquid into and the outlet for the removal of liquid from the container, the interior surface comprising a sidewall formation and a bottom, wherein the capture ligand is supported at a location in the container which allows the capture ligand to contact intact host cells or solid cellular components derived therefrom when a liquid suspension containing the intact host cells or solid cellular components is introduced into the container through its mouth.
3. A multiwell plate for the extraction of a cellular component from a host cell, at least one of the wells of the multiwell plate containing a lytic reagent, wherein the lytic reagent (i) is coated onto at least a portion of the interior surface of the well(s), or (ii) is in the form of a mass of material contained within the well(s).
4. The multiwell plate of claim 3 wherein the well(s) further comprises a capture ligand for the cellular component.

5. The container of claim 1 or 2 or the multiwell plate of claim 3 wherein the lytic reagent is selected from the group consisting of a detergent, a lytic enzyme, a chaotrope, and combinations thereof.

6. The container or multiwell plate of claim 5 wherein the lytic reagent is a detergent and the detergent is selected from the group consisting of 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, octyl- β -thioglucopyranoside, octyl-glucopyranoside, 3-(4-heptyl) phenyl 3-hydroxy propyl dimethylammonio propane sulfonate, 3-[N,N-dimethyl(3-myristoylaminopropyl)ammonio]propanesulfonate, 3-(decyldimethylammonio)propanesulfonate inner salt, 3-(dodecyldimethylammonio)propanesulfonate inner salt, 3-(N,N-dimethylmyristylammonio)propanesulfonate, n-dodecyl α -D-maltoside and combinations thereof.

7. The container or multiwell plate of claim 5 wherein the lytic reagent is a lytic enzyme and the lytic enzyme is selected from the group consisting of beta glucuronidase, glucanase, glusulase, lysozyme, lyticase, mannanase, mutanolysin, zymolase, cellulase, lysostaphin, pectolyase, streptolysin O, and various combinations thereof.

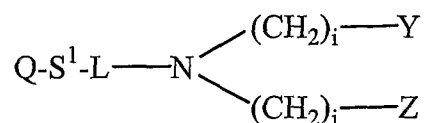
8. The container or multiwell plate of claim 5 wherein the lytic reagent is a chaotrope and the chaotrope is selected from the group consisting of urea, guanidine HCl, guanidine thiocyanate, guanidium thiosulfate, and thiourea, or any combination thereof.

9. The container or multiwell plate of claim 5 wherein the lytic reagent further comprises a buffer, an anti-foaming agent, a bulking agent, a processing enzyme, or an enzymatic inhibitor, or any combination thereof.

10. The container of claim 2 or the multiwell plate of claim 4 wherein the capture ligand is a metal chelate, glutathione, biotin, streptavidin, antibody, charged particle, or insoluble hydrophobic group.

11. The container or multiwell plate of claim 10 wherein the capture ligand is an antibody that has specificity for SEQ. ID. NO. 1, SEQ. ID. NO. 2, or SEQ. ID. NO. 3.

12. The container or multiwell plate of claim 10 wherein the capture ligand is a metal chelate derived from a composition corresponding to the formula:



wherein

Q is a carrier;

S¹ is a spacer;

L is -A-T-CH(X)- or -C(=O)-;

A is an ether, thioether, selenoether, or amide linkage;

T is a bond or substituted or unsubstituted alkyl or alkenyl;

X is -(CH₂)_kCH₃, -(CH₂)_kCOOH, -(CH₂)_kSO₃H, -(CH₂)_kPO₃H₂, -(CH₂)_kN(J)₂, or -(CH₂)_kP(J)₂, preferably -(CH₂)_kCOOH or -(CH₂)_kSO₃H;

k is an integer from 0 to 2;

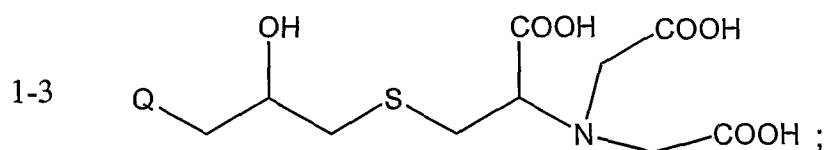
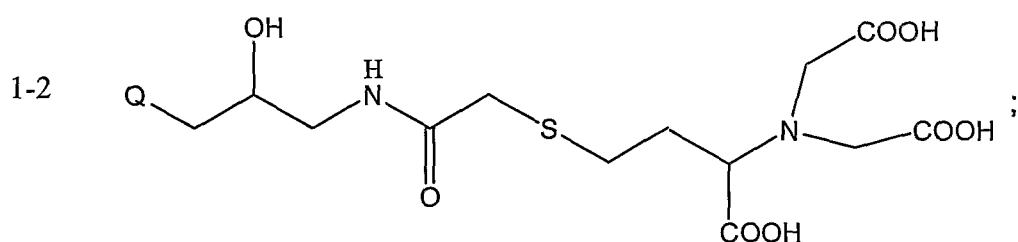
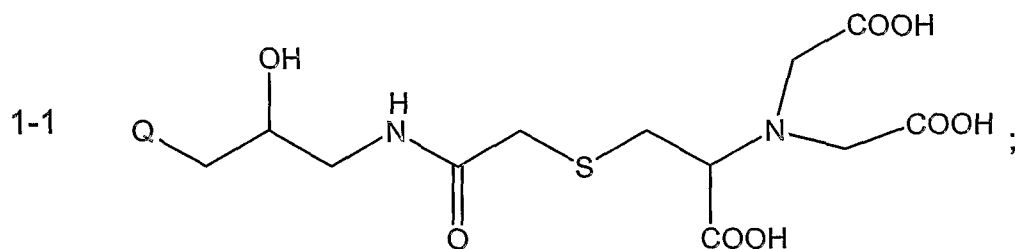
J is hydrocarbyl or substituted hydrocarbyl;

Y is -COOH, -H, -SO₃H, -PO₃H₂, -N(J)₂, or -P(J)₂, preferably, -COOH;

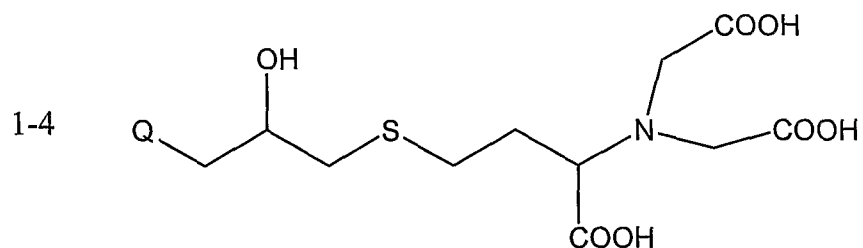
Z is -COOH, -H, -SO₃H, -PO₃H₂, -N(J)₂, or -P(J)₂, preferably, -COOH; and

i is an integer from 0 to 4, preferably 1 or 2.

13. The container or multiwell plate of claim 12 wherein the metal chelate is derived from a composition selected from the group consisting of:



and



wherein Q is a carrier.

14. A process for the extraction of a cellular component from a host cell, the process comprising: (a) introducing a liquid suspension containing the host cell into a container, the container having a mouth, an interior surface, a volume, V, and a coating of a lytic reagent on at least a portion of the interior

surface, the interior surface comprising a sidewall formation and a bottom, the ratio of the area of the coated interior surface, SA, to the volume, V, being less than about 4 mm²/μl, and (b) lysing the host cell in the container to release the cellular component and form cellular debris.

15. A process for the extraction and isolation of a cellular component from a host cell, the process comprising (a) introducing a liquid suspension containing the host cell into a container, the container having a mouth, an interior surface, a volume, V, a lytic reagent, and a supported, capture ligand, the interior surface comprising a sidewall formation and a bottom, the sidewall formation being between the bottom and the mouth, the mouth serving as the inlet for the introduction of the liquid into and the outlet for the removal of the liquid from the container, (b) lysing the host cell in the container to release the cellular component and form solid cellular debris; and (c) capturing the cellular component with the capture ligand in the presence of the solid cellular debris.

16. A process for the preparation of a container or multiwell plate for the extraction of a cellular component from a host cell, the process comprising contacting the interior surfaces of the container or a plurality of the wells of the multiwell plate with a liquid containing a lytic reagent and drying the liquid to form an adsorbed layer of lytic reagent on the interior surfaces of the container or wells.

17. The process of claim 14, 15, or 16 wherein the lytic reagent is selected from the group consisting of a detergent, a lytic enzyme, a chaotrope, and combinations thereof.

18. The process of claim 17 wherein the lytic reagent is a detergent and the detergent is selected from the group consisting of 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, octyl-β-thioglucopyranoside, octyl-glucopyranoside, 3-(4-heptyl) phenyl 3-hydroxy

propyl) dimethylammonio propane sulfonate, 3-[N,N-dimethyl(3-myristoylamino)propyl]ammonio]propanesulfonate, 3-(decyldimethylammonio)propanesulfonate inner salt, 3-(dodecyldimethylammonio)propanesulfonate inner salt, 3-(N,N-dimethylmyristylammonio)propanesulfonate, n-dodecyl α -D-maltoside and combinations thereof.

19. The process of claim 17 wherein the lytic reagent is a lytic enzyme and the lytic enzyme is selected from the group consisting of beta glucuronidase, glucanase, glucosylase, lysozyme, lyticase, mannanase, mutanolysin, zymolase, cellulase, lysostaphin, pectolyase, streptolysin O, and various combinations thereof.

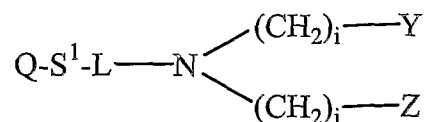
20. The process of claim 17 wherein the lytic reagent is a chaotrope and the chaotrope is selected from the group consisting of urea, guanidine HCl, guanidine thiocyanate, guanidinium thiosulfate, and thiourea, or any combination thereof.

21. The process of claim 17 wherein the lytic reagent further comprises a buffer, an anti-foaming agent, a bulking agent, a processing enzyme, or an enzymatic inhibitor, or any combination thereof.

22. The process of claim 15 wherein the capture ligand is a metal chelate, glutathione, biotin, streptavidin, antibody, charged particle, or insoluble hydrophobic group.

23. The process of claim 22 wherein the capture ligand is an antibody that has specificity for SEQ. ID. NO. 1, SEQ. ID. NO. 2, or SEQ. ID. NO. 3.

24. The process of claim 22 wherein the capture ligand is a metal chelate derived from a composition corresponding to the formula:



wherein

Q is a carrier;

S¹ is a spacer;

L is -A-T-CH(X)- or -C(=O)-;

A is an ether, thioether, selenoether, or amide linkage;

T is a bond or substituted or unsubstituted alkyl or alkenyl;

X is -(CH₂)_kCH₃, -(CH₂)_kCOOH, -(CH₂)_kSO₃H, -(CH₂)_kPO₃H₂, -(CH₂)_kN(J)₂, or -(CH₂)_kP(J)₂, preferably -(CH₂)_kCOOH or -(CH₂)_kSO₃H;

k is an integer from 0 to 2;

J is hydrocarbyl or substituted hydrocarbyl;

Y is -COOH, -H, -SO₃H, -PO₃H₂, -N(J)₂, or -P(J)₂, preferably, -COOH;

Z is -COOH, -H, -SO₃H, -PO₃H₂, -N(J)₂, or -P(J)₂, preferably, -COOH; and

i is an integer from 0 to 4, preferably 1 or 2.

25. A process for the extraction and isolation of a cellular component from a host cell, the process comprising (a) introducing a liquid suspension containing the host cell into a container, the container having a mouth, an interior surface, a volume, V, a lytic reagent, and a supported, capture ligand, the interior surface comprising a sidewall formation and a bottom, the sidewall formation being between the bottom and the mouth, the mouth serving as the inlet for the introduction of the liquid into the container, (b) lysing the host cell in the container to release the cellular component and form solid cellular debris; (c) capturing the cellular component with the capture ligand in the presence of the solid cellular debris; (d) releasing the cellular component from the capture ligand, and (e) recovering the released cellular component.

26. A kit for the extraction and isolation of a cellular component from a host cell, the kit comprising the container of claim 1 or 2 or the multiwell plate of claim 3 and instructions for the extraction and isolation of the cellular component from the host cell.

27. The kit of claim 26 further comprising reagents for assaying or detecting a captured cellular component.

28. The container of claim 2 wherein the container comprises a column having an internal chamber, the chamber comprising a bed of resin having the capture ligand bound thereto and a lyophilized mass comprising the lytic reagent.