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(54) Title: METHODS AND COMPOSITIONS COMPRISING PURIFIED RECOMBINANT POLYPEPTIDES

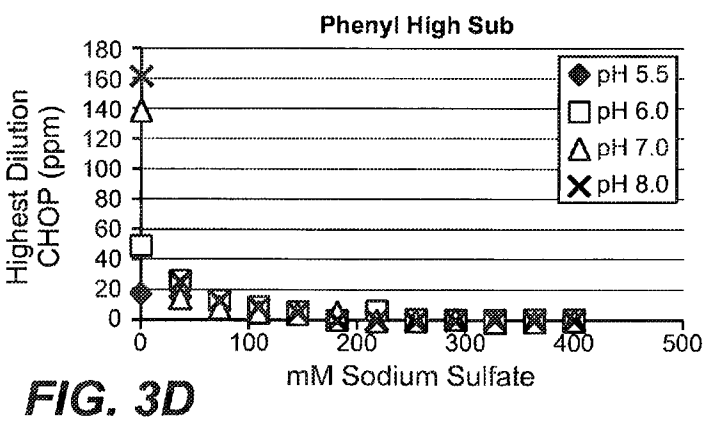

FIG. 3D

(57) Abstract: Purified recombinant polypeptides isolated from Chinese hamster ovary host cells, including antibodies, such as therapeutic antibodies, and methods of making and using such polypeptides are provided.

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METHODS AND COMPOSITIONS COMPRISING PURIFIED RECOMBINANT POLYPEPTIDES

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of priority of provisional U.S. Application No. 61/877,517 filed September 13, 2013, which is hereby incorporated by reference in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on August 28, 2014, is named 2014.AUG.28 P5704R1-WO Sequence Listing.txt and is 34,811 bytes in size.

FIELD

[0003] Purified recombinant polypeptides isolated from Chinese hamster ovary host cells, including antibodies, such as therapeutic antibodies, and methods of making and using such polypeptides are provided.

BACKGROUND

[0004] A number of drugs are on the market or in development for treating asthma and other respiratory disorders. One of the targets for asthma therapy is IL-13. IL-13 is a pleiotropic TH2 cytokine produced by activated T cells, NKT cells, basophils, eosinophils, and mast cells, and it has been strongly implicated in the pathogenesis of asthma in preclinical models. IL-13 antagonists, including anti-IL-13 antibodies, have previously been described. Certain such antibodies have also been developed as human therapeutics. Recently, several studies have shown clinical activity of monoclonal antibodies against IL-13 in the treatment of asthma (*See, e.g.,* Corren et al., 2011, *N. Engl. J. Med.* 365, 1088-1098; Gauvreau et al., 2011, *Am. J. Respir. Crit. Care Med.* 183, 1007-1014; Ingram and Kraft, 2012, *J. Allergy Clin. Immunol.* 130, 829-42; Webb, 2011, *Nat Biotechnol* 29, 860-863). Of these, lebrikizumab, a humanized IgG4 antibody that neutralizes IL-13 activity, improved lung function in asthmatics who were symptomatic despite treatment with, for the majority, inhaled corticosteroids and a long-acting beta2-adrenergic receptor agonist (Corren et al., 2011, *N. Engl. J. Med.* 365, 1088-1098).

[0005] In addition, IL-13 has been implicated in numerous other allergic and fibrotic disorders. For example, such diseases and/or conditions mediated by IL13 include, but are not limited to, allergic asthma, non-allergic (intrinsic) asthma, allergic rhinitis, atopic dermatitis, allergic

ulcerative colitis, RSV infection, uveitis, scleroderma, and osteoporosis.

[0006] For recombinant biopharmaceutical proteins to be acceptable for administration to human patients, it is important that residual impurities resulting from the manufacture and purification process are removed from the final biological product. These process components include culture medium proteins, immunoglobulin affinity ligands, viruses, endotoxin, DNA, and host cell proteins. These host cell impurities include process-specific host cell proteins (HCPs), which are process-related impurities/contaminants in the biologics derived from recombinant DNA technology. While HCPs are typically present in the final drug substance in small quantities (in parts-per-million or nanograms per milligram of the intended recombinant protein), it is recognized that HCPs are undesirable and their quantities should be minimized. For example, the U.S. Food and Drug Administration (FDA) requires that biopharmaceuticals intended for in vivo human use should be as free as possible of extraneous impurities, and requires tests for detection and quantitation of potential contaminants/impurities, such as HCPs.

[0007] Procedures for purification of proteins from cell debris initially depend on the site of expression of the protein. Some proteins are secreted directly from the cell into the surrounding growth media; others are made intracellularly. For the latter proteins, the first step of a purification process involves lysis of the cell, which can be done by a variety of methods, including mechanical shear, osmotic shock, or enzymatic treatments. Such disruption releases the entire contents of the cell into the homogenate, and in addition produces subcellular fragments that are difficult to remove due to their small size. These are generally removed by centrifugation or by filtration. The same problem arises with directly secreted proteins due to the natural death of cells and release of intracellular host cell proteins in the course of the protein production run.

[0008] Once a solution containing the protein of interest is obtained, its separation from the other proteins produced by the cell is usually attempted using a combination of different chromatography techniques. Typically, these techniques separate mixtures of proteins on the basis of their charge, degree of hydrophobicity, or size. Several different chromatography resins are available for each of these techniques, allowing accurate tailoring of the purification scheme to the particular protein involved. The essence of each of these separation methods is that proteins can be caused either to move at different rates down a long column, achieving a physical separation that increases as they pass further down the column, or to adhere selectively to the separation medium, being then differentially eluted by different solvents. In some cases, the desired protein is separated from impurities when the impurities specifically adhere to the

through."

[0009] Ion-exchange chromatography, named for the exchangeable counterion, is a procedure applicable to purification of ionizable molecules. Ionized molecules are separated on the basis of the non-specific electrostatic interaction of their charged groups with oppositely charged molecules attached to the solid phase support matrix, thereby retarding those ionized molecules that interact more strongly with solid phase. The net charge of each type of ionized molecule, and its affinity for the matrix, varies according to the number of charged groups, the charge of each group, and the nature of the molecules competing for interaction with the charged solid phase matrix. These differences result in resolution of various molecule types by ion-exchange chromatography. In typical protein purification using ion exchange chromatography, a mixture of many proteins derived from a host cell, such as in mammalian cell culture, is applied to an ion-exchange column. After non-binding molecules are washed away, conditions are adjusted, such as by changing pH, counter ion concentration and the like in step- or gradient-mode, to release from the solid phase a non-specifically retained or retarded ionized protein of interest and separating it from proteins having different charge characteristics. Anion exchange chromatography involves competition of an anionic molecule of interest with the negative counter ion for interaction with a positively charged molecule attached to the solid phase matrix at the pH and under the conditions of a particular separation process. By contrast, cation exchange chromatography involves competition of a cationic molecule of interest with the positive counter ion for a negatively charged molecule attached to the solid phase matrix at the pH and under the conditions of a particular separation process. Mixed mode ion exchange chromatography (also referred to as multimodal ion exchange chromatography) involves the use of a combination of cation and anion exchange chromatographic media in the same step. In particular, "mixed mode" refers to a solid phase support matrix to which is covalently attached a mixture of cation exchange, anion exchange, and hydrophobic interaction moieties.

[0010] Hydroxyapatite chromatography of proteins involves the non-specific interaction of the charged amino or carboxylate groups of a protein with oppositely charged groups on the hydroxyapatite, where the net charge of the hydroxyapatite and protein are controlled by the pH of the buffer. Elution is accomplished by displacing the non-specific protein-hydroxyapatite pairing with ions such as Ca2+ or Mg2+. Negatively charged protein groups are displaced by negatively charged compounds, such as phosphates, thereby eluting a net-negatively charged protein.

separation of molecules, such as proteins, based on differences in their surface hydrophobicity. Hydrophobic groups of a protein interact non-specifically with hydrophobic groups coupled to the chromatography matrix. Differences in the number and nature of protein surface hydrophobic groups results in differential retardation of proteins on a HIC column and, as a result, separation of proteins in a mixture of proteins.

[0012] Affinity chromatography, which exploits a specific structurally dependent (i.e., spatially complementary) interaction between the protein to be purified and an immobilized capture agent, is a standard purification option for some proteins, such as antibodies. Protein A, for example, is a useful adsorbent for affinity chromatography of proteins, such as antibodies, which contain an Fc region. Protein A is a 41kD cell wall protein from Staphylococcus aureas which binds with a high affinity (about 10^{-8}M to human IgG) to the Fc region of antibodies.

[0013] Purification of recombinant polypeptides is typically performed using bind and elute chromatography (B/E) or flow-through (F/T) chromatography. These are briefly described below.

[0014] Bind and Elute Chromatography (B/E): Under B/E chromatography the product is usually loaded to maximize dynamic binding capacity (DBC) to the chromatography material and then wash and elution conditions are identified such that maximum product purity is attained in the eluate.

[0015] Various B/E methods for use with protein A affinity chromatography, including various intermediate wash buffers, have been described. For example, US Patent Nos. 6,127,526 and 6,333,398 describe an intermediate wash step during Protein A chromatography using hydrophobic electrolytes, *e.g.*, tetramethylammonium chloride (TMAC) and tetraethylammonium chloride (TEAC), to remove the contaminants, but not the immobilized Protein A or the protein of interest, bound to the Protein A column. US Patent No. 6,870,034 describes additional methods and wash buffers for use with protein A affinity chromatography.

[0016] Flow Through Chromatography (F/T): Using F/T chromatography, load conditions are identified where impurities strongly bind to the chromatography material while the product flows through. F/T chromatography allows high load density for standard monoclonal antibody preparations (MAbs).

[0017] In recombinant anti-IL13 MAb preparations and certain other recombinant polypeptides produced in CHO cells, we identified an enzyme, phospholipase B-like 2, as a single CHOP species present in excess of available antibodies in a total CHOP ELISA assay. As used herein, "PLB2" and "PLBL2" and "PLBD2" are used interchangeably and refer to the enzyme

publications on PLBL2 include Lakomek, K. et al., BMC Structural Biology 9:56 (2009); Deuschi, et al., FEBS Lett 580:5747-5752 (2006). PLBL2 is synthesized as a pre-pro-enzyme with parent MW of about 66,000. There is an initial leader sequence which is removed and potential 6 mannose-6-phosphate (M-6-P) groups are added during post-translational modification. M-6-P is a targeting modification that directs this enzyme to the lysosome via the M-6-P receptor. PLBL2 contains 6 cysteines, two of which have free sulfhydrals, and four form disulfide bonds. In acidic environments, PLBL2 is further clipped into the N- and C-terminal fragments having 32,000 and 45,000 MW, respectively. By analogy with other lysosomal enzymes, this cleavage is an activating step, allowing and access of the substrate to the active site.

[0018] There is about 80% PLBL2 amino acid sequence homology between hamster and human forms of the enzyme. The enzyme activity is thought to be to cleave either fatty acid chain from the phospholipids that make up cell membranes. There are other phospholipases with different substrate cleavage specificities. Similar enzymatic activities exist in microorganisms, where they are often a virulence factor. Although microorganisms have a similar enzymatic activity, the protein generating this activity is different, and there is low sequence homology between microbial and mammalian PLBL2 enzymes. Phospholipases produce free fatty acids (FFA) as one product of the substrate hydrolysis. Free fatty acids are themselves a potential immune-signaling factor. Dehydrogenation converts FFA to arachadonic acid which potentially participates in inflammation cascades involving eicosanoids.

[0019] Having identified PLBL2 as a single HCP (CHOP) in recombinant anti-IL13 MAb preparations and certain other recombinant polypeptides produced in CHO cells, we developed reagents, methods, and kits for the specific, sensitive, and quantitative determination of PLBL2 levels in anti-IL-13 Mab preparations (and other recombinant polypeptide products) and at various stages of purification. These are briefly described in the Examples below and also in US Provisional Patent Application Nos. 61/877,503 and 61/991,228. In addition, there was the formidable challenge of developing a large-scale, robust, and efficient process for the purification of anti-IL13 MAb (and other recombinant polypeptide products) resulting in MAb of sufficient purity (including removal of PLBL2) for human therapeutic use including late-stage clinical and commercial use. The invention described herein meets certain of the above-described needs and provides other benefits.

[0020] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

SUMMARY

[0021] The invention is based, at least in part, on the development of improved processes for the purification of recombinant polypeptides produced in Chinese hamster ovary (CHO) cells that provide purified product with substantially reduced levels of hamster PLBL2. Recombinant polypeptides purified according to the methods of the invention, including therapeutic antibodies such as an anti-IL13 antibody, may have reduced immunogenicity when administered to human subjects.

[0022] Accordingly, in one aspect, compositions comprising an anti-IL13 monoclonal antibody purified from CHO cells comprising the anti-IL13 antibody and a residual amount of hamster PLBL2 are provided. In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7. In certain embodiments, the anti-IL13 antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10. In certain embodiments, the anti-IL13 antibody comprises a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having

acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10 and a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the amount of hamster PLBL2 in the composition is quantified using an immunoassay or a mass spectrometry assay. In certain embodiments, the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA. In certain embodiments, the mass spectrometry assay is LC-MS/MS.

[0023] In another aspect, anti-IL13 monoclonal antibody preparations isolated and purified from CHO cells by a process comprising a hydrophobic interaction chromatography (HIC) step are provided. In certain embodiments, the purified preparation comprises the anti-IL13 antibody and a residual amount of hamster PLBL2. In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin. In certain embodiments, the HIC step comprises operating a resin-containing column in flow-through mode. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 50 mM sodium acetate pH 5.0. In certain embodiments, the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected between 0.5 OD to 1.5 OD. In certain embodiments, the flow-through is collected for a maximum of 8 column volumes. In certain embodiments, the process further comprises an affinity chromatography step. In certain embodiments, the affinity chromatography is protein A chromatography. In certain embodiments, the process further comprises an ion exchange chromatography step. In certain embodiments, the ion exchange chromatography is anion exchange chromatography. In certain embodiments, the anti-IL13

ID NO.: 1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7. In certain embodiments, the anti-IL13 antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10. In certain embodiments, the anti-IL13 antibody comprises a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7 and a light chain variable region having the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10 and a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the amount of hamster PLBL2 is quantified using an immunoassay or a mass spectrometry assay. In certain embodiments, the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA. In certain embodiments, the mass spectrometry assay is LC-MS/MS.

[0024] In yet another aspect, purified anti-IL13 monoclonal antibody preparations isolated from CHO cells are provided. In certain embodiments, the antibody preparation is purified by a process comprising a first Protein A affinity chromatography step, a second anion exchange chromatography step, and a third hydrophobic interaction chromatography (HIC) step thereby producing a purified preparation, In certain embodiments, the purified preparation comprises the anti-IL13 antibody and a residual amount of hamster PLBL2. In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between

or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the affinity chromatography step comprises MABSELECT SURE™ resin, the anion exchange chromatography step comprises Q SEPHAROSE™ Fast Flow, and the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (high sub). In certain embodiments, the affinity chromatography step comprises operating a MABSELECT SURE™ resin-containing column in bind-elute mode, the anion exchange chromatography step comprises operating a Q SEPHAROSE™ Fast Flow resin-containing column in bind-elute mode, and the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode. In certain embodiments, the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7. In certain embodiments, the anti-IL13 antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10. In certain embodiments, the anti-IL13 antibody comprises a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7 and a light chain variable region having the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10 and a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the amount of hamster PLBL2 is quantified using an immunoassay or a mass spectrometry assay. In certain embodiments, the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA. In certain embodiments, the mass spectrometry assay is LC-MS/MS.

[0025] In still yet another aspect, methods of purifying a recombinant polypeptide produced in CHO cells, wherein the method provides a purified preparation comprising the recombinant polypeptide and residual amount of hamster PLBL2 are provided. In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2

ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the recombinant polypeptide is selected from a growth factor, a cytokine, an antibody, an antibody fragment, and an immunoadhesin. In certain embodiments, the recombinant polypeptide is an antibody. In certain embodiments, the antibody is a humanized monoclonal antibody. In certain embodiments, the antibody is IgG1, or IgG2, or IgG3, or IgG4. In certain embodiments, the antibody is IgG1. In certain embodiments, the antibody is IgG2. In certain embodiments, the antibody is IgG3. In certain embodiments, the antibody is IgG4. In certain embodiments, the methods comprise a hydrophobic interaction chromatography (HIC) step. In certain embodiments, the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin.

[0026] In certain embodiments of the above purification methods, the purified antibody is anti-IL13. In certain embodiments, the antibody is lebrikizumab. In certain embodiments, the HIC step comprises operating a resin-containing column in flow-through mode. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 50 mM sodium acetate pH 5.0. In certain embodiments, the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected between 0.5 OD to 1.5 OD. In certain embodiments, the flow-through is collected for a maximum of 8 column volumes. In certain embodiments, the methods further comprise an affinity chromatography step. In certain embodiments, the affinity chromatography is protein A chromatography. In certain embodiments, the methods further comprise an ion exchange chromatography step. In certain embodiments, the ion exchange chromatography is anion exchange chromatography. In certain embodiments, the methods comprise a first Protein A affinity chromatography step, a second anion exchange chromatography step, and a third hydrophobic interaction chromatography (HIC) step. In certain embodiments, the affinity chromatography step comprises MABSELECT SURE™ resin, the anion exchange chromatography step comprises Q SEPHAROSE™ Fast Flow, and the HIC step comprises

chromatography step comprises operating a MABSELECT SURE™ resin-containing column in bind-elute mode, the anion exchange chromatography step comprises operating a Q SEPHAROSE™ Fast Flow resin-containing column in bind-elute mode, and the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode. In certain embodiments, the amount of hamster PLBL2 is quantified using an immunoassay or a mass spectrometry assay. In certain embodiments, the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA. In certain embodiments, the mass spectrometry assay is LC-MS/MS.

[0027] In certain embodiments of the above purification methods, the purified antibody is anti-Abeta. In certain embodiments, the anti-Abeta antibody is crenezumab. In certain embodiments, the anti-Abeta antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.:23, CDR-H2 having the amino acid sequence of SEQ ID NO.:24, and CDR-H3 having the amino acid sequence of SEQ ID NO.:25, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.:26, CDR-L2 having the amino acid sequence of SEQ ID NO.:27, and CDR-L3 having the amino acid sequence of SEQ ID NO.:28. In certain embodiments, the anti-Abeta antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:29. In certain embodiments, the anti-Abeta antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.:30. In certain embodiments, the anti-Abeta antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:29 and a light chain variable region having the amino acid sequence of SEQ ID NO.:30. In certain embodiments, the HIC step comprises operating a resin-containing column in flow-through mode. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 150 mM sodium acetate pH 5.0. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 150 mM sodium acetate pH 4.0. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 150 mM sodium acetate, 240 mM sodium sulfate pH 4.0. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 150 mM sodium acetate, 240 mM sodium sulfate pH 5.0. In certain embodiments, the load density is 300 g/L. In certain embodiments, the load density is 100 g/L. In certain embodiments, the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected

the methods further comprise an affinity chromatography step. In certain embodiments, the affinity chromatography is protein A chromatography. In certain embodiments, the methods further comprise a mixed mode chromatography step. In certain embodiments, the methods comprise a first Protein A affinity chromatography step, a second mixed mode chromatography step, and a third hydrophobic interaction chromatography (HIC) step. In certain embodiments, the affinity chromatography step comprises MABSELECT SURE™ resin, the mixed mode chromatography step comprises CAPTO™ Adhere, and the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (high sub). In certain embodiments, the affinity chromatography step comprises operating a MABSELECT SURE™ resin-containing column in bind-elute mode, the mixed mode chromatography step comprises operating a CAPTO™ Adhere resin-containing column in flow-through mode, and the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode. In certain embodiments, the amount of hamster PLBL2 is quantified using an immunoassay or a mass spectrometry assay. In certain embodiments, the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA. In certain embodiments, the mass spectrometry assay is LC-MS/MS.

[0028] In yet a further aspect of the above purification methods, the purified antibody is IgG1. In some embodiments, the antibody is anti-IL17 A/F. In some embodiments, the anti-IL17 A/F antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.:15, CDR-H2 having the amino acid sequence of SEQ ID NO.:16, and CDR-H3 having the amino acid sequence of SEQ ID NO.:17, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.:18, CDR-L2 having the amino acid sequence of SEQ ID NO.:19 and CDR-L3 having the amino acid sequence of SEQ ID NO.:20. In certain embodiments, the anti-IL17 A/F antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:21. In certain embodiments, the anti-IL17 A/F antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.:22. In certain embodiments, the anti-IL17 A/F antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:21 and a light chain variable region having the amino acid sequence of SEQ ID NO.:22. In certain embodiments, the HIC chromatography step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 50 mM sodium acetate pH 5.5. In certain embodiments, the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected beginning at 0.5 OD and for 10 column volumes. In certain embodiments, the methods further

is protein A chromatography. In certain embodiments, the methods further comprise a cation exchange chromatography step. In some embodiments, the methods comprise a first Protein A affinity chromatography step and a second cation exchange chromatography step prior to the hydrophobic interaction chromatography (HIC) step. In some embodiments, the affinity chromatography step comprises MABSELECT SURE™ resin, the cation exchange chromatography step comprises POROS 50 HS resin, and the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (high sub) resin. In some embodiments, the affinity chromatography step comprises operating a MABSELECT SURE™ resin-containing column in bind-elute mode; the cation exchange chromatography step comprises operating a POROS 50 HS resin-containing column in bind-elute mode, and the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode.

[0029] In still yet another aspect, anti-Abeta monoclonal antibody preparations purified from CHO cells by a process comprising a hydrophobic interaction chromatography (HIC) step are provided. In certain embodiments, the purified preparation comprises the anti-Abeta antibody and a residual amount of hamster PLBL2. In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin. In certain embodiments, the HIC step comprises operating a resin-containing column in flow-through mode. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 150 mM sodium acetate pH 5.0. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of

embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 150 mM sodium acetate, 240 mM sodium sulfate pH 4.0. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 150 mM sodium acetate, 240 mM sodium sulfate pH 5.0. In certain embodiments, the load density is 300 g/L. In certain embodiments, the load density is 100 g/L. In certain embodiments, the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected between 0.5 OD and for 10 column volumes. In certain embodiments, the process further comprises an affinity chromatography step. In certain embodiments, the affinity chromatography is protein A chromatography. In certain embodiments, the process further comprises a mixed mode chromatography step. In certain embodiments, the anti-Abeta antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 23, CDR-H2 having the amino acid sequence of SEQ ID NO.: 24, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 25, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 26, CDR-L2 having the amino acid sequence of SEQ ID NO.: 27, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 28. In certain embodiments, the anti-Abeta antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 29. In certain embodiments, the anti-Abeta antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 30. In certain embodiments, the anti-Abeta antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 29 and a light chain variable region having the amino acid sequence of SEQ ID NO.: 30. In certain embodiments, the amount of hamster PLBL2 is quantified using an immunoassay or a mass spectrometry assay. In certain embodiments, the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA. In certain embodiments, the mass spectrometry assay is LC-MS/MS.

[0030] In one aspect, anti-IL17 A/F monoclonal antibody preparations isolated and purified from CHO cells by a process comprising a hydrophobic interaction chromatography (HIC) step are provided. In certain embodiments, the purified preparation comprises the anti- IL17 A/F antibody and a residual amount of hamster PLBL2. In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the

PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin. In certain embodiments, the HIC step comprises operating a resin-containing column in flow-through mode. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 50 mM sodium acetate pH 5.5. In certain embodiments, the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected between 0.5 OD and for 10 column volumes. In certain embodiments, the process further comprises an affinity chromatography step. In certain embodiments, the affinity chromatography is protein A chromatography. In certain embodiments, the process further comprises a cation exchange chromatography step. In certain embodiments, the anti- IL17 A/F antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 15, CDR-H2 having the amino acid sequence of SEQ ID NO.: 16, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 17, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 18, CDR-L2 having the amino acid sequence of SEQ ID NO.: 19, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 20. In certain embodiments, the anti- IL17 A/F antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 21. In certain embodiments, the anti- IL17 A/F antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 22. In certain embodiments, the anti- IL17 A/F antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 21 and a light chain variable region having the amino acid sequence of SEQ ID NO.: 32. In certain embodiments, the amount of hamster PLBL2 is quantified using an immunoassay or a mass spectrometry assay. In certain embodiments, the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA. In certain embodiments, the mass spectrometry assay is LC-MS/MS.

[0031] In still another aspect, compositions comprising an anti-Abeta monoclonal antibody purified from CHO cells comprising the anti-Abeta antibody and a residual amount of hamster PLBL2 are provided. In certain embodiments, the amount of hamster PLBL2 is less than 20

embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the anti-Abeta antibody is crenezumab. In certain embodiments, the anti-Abeta antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.:23, CDR-H2 having the amino acid sequence of SEQ ID NO.:24, and CDR-H3 having the amino acid sequence of SEQ ID NO.:25, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.:26, CDR-L2 having the amino acid sequence of SEQ ID NO.:27, and CDR-L3 having the amino acid sequence of SEQ ID NO.:28. In certain embodiments, the anti-Abeta antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:29. In certain embodiments, the anti-Abeta antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.:30. In certain embodiments, the anti-Abeta antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:29 and a light chain variable region having the amino acid sequence of SEQ ID NO.:30.

[0032] In yet still another aspect, compositions comprising an anti-IL17 A/F monoclonal antibody purified from CHO cells comprising the anti-IL17 A/F antibody and a residual amount of hamster PLBL2 are provided. In certain embodiments, the composition comprises the anti-IL17 A/F antibody and a residual amount of hamster PLBL2, wherein the amount of hamster PLBL2 is less than 20 ng/mg, or less than 15 ng/mg, or less than 10 ng/mg, or less than 8 ng/mg, or less than 5 ng/mg, or less than 3 ng/mg, or less than 2 ng/mg, or less than 1 ng/mg, or less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than

certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the anti-IL17 A/F antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.:15, CDR-H2 having the amino acid sequence of SEQ ID NO.:16, and CDR-H3 having the amino acid sequence of SEQ ID NO.:17, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.:18, CDR-L2 having the amino acid sequence of SEQ ID NO.:19, and CDR-L3 having the amino acid sequence of SEQ ID NO.:20. In certain embodiments, the anti-IL17 A/F antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:21. In certain embodiments, the anti-IL17 A/F antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.:22. In certain embodiments, the anti-IL17 A/F antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:21 and a light chain variable region having the amino acid sequence of SEQ ID NO.:22.

[0033] In one aspect, methods of treating an IL-13-mediated disorder comprising administering a treatment composition comprising an anti-IL13 monoclonal antibody purified from CHO cells and a residual amount of hamster PLBL2 are provided. In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.:

acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7. In certain embodiments, the anti-IL13 antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10. In certain embodiments, the anti-IL13 antibody comprises a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7 and a light chain variable region having the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10 and a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the treatment composition is administered subcutaneously once every four weeks. In certain embodiments, the treatment composition is administered subcutaneously once every eight weeks. In certain embodiments, the treatment composition is administered subcutaneously once every 12 weeks. In certain embodiments, the patient is treated once every four weeks for at least one month. In certain embodiments, the patient is treated once every four weeks for at least three months. In certain embodiments, the patient is treated once every four weeks for at least six months. In certain embodiments, the patient is treated once every four weeks for at least nine months. In certain embodiments, the patient is treated once every four weeks for at least 12 months. In certain embodiments, the patient is treated once every four weeks for at least 18 months. In certain embodiments, the patient is treated once every four weeks for at least two years. In certain embodiments, the patient is treated once every four weeks for more than two years. In certain embodiments, the IL-13-mediated disorder is asthma. In certain embodiments, the IL-13-mediated disorder is idiopathic pulmonary fibrosis. In certain embodiments, the IL-13-mediated disorder is atopic dermatitis. In certain embodiments, the IL-13-mediated disorder is selected from allergic asthma, non-allergic asthma, allergic rhinitis, allergic conjunctivitis, eczema, urticaria, food allergies, chronic obstructive pulmonary disease, ulcerative colitis, RSV infection, uveitis, scleroderma, and osteoporosis.

[0034] In another aspect, administration of a treatment composition to a patient according to any of the methods described above is less immunogenic for hamster PLBL2 compared to administration of a reference composition, wherein the reference composition comprises an anti-

of hamster PLBL2 of greater than 30 ng/mg. In certain embodiments, the amount of hamster PLBL2 in the reference composition is greater than 50 ng/mg. In certain embodiments, the amount of hamster PLBL2 in the reference composition is greater than 100 ng/mg. In certain embodiments, the amount of hamster PLBL2 in the reference composition is greater than 200 ng/mg. In certain embodiments, the amount of hamster PLBL2 in the reference composition is greater than 300 ng/mg. In certain embodiments, the amount of hamster PLBL2 in the reference composition is between 30 ng/mg and 300 ng/mg, or between 30 ng/mg and 200 ng/mg, or between 30 ng/mg and 100 ng/mg, or between 30 ng/mg and 50 ng/mg.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] Figure 1 shows total CHOP levels in caprylic acid-treated Protein A pools of anti-IL13 MAb as described in Example 2. (A) Caprylic acid precipitation of Protein A pool at pH 4.5; (B) Caprylic acid precipitation of Protein A pool at pH 5.0. CHOP levels in ng/mg are indicated along the vertical axis; percentage of caprylic acid is shown along the horizontal axis, each bar represents the value from 2-fold serial dilution.

[0036] Figure 2 shows total CHOP levels in additive-treated HCCF anti-IL13 MAb following Protein A chromatography which was followed by cation exchange chromatography on POROS® 50HS as described in Example 2. Corrected CHOP levels in ng/ml are shown on the vertical axis; the additive (control, 0.6M guanidine, or 0.6M arginine) is indicated on the horizontal axis, each bar represents the value from 2-fold serial dilution as indicated.

[0037] Figure 3 shows total CHOP levels in UFDF pools of anti-IL13 MAb subjected to different HIC resins under varying salt and pH conditions as described in Example 2. (A) OCTYL-SEPHAROSE® Fast Flow resin; (B) PHENYL SEPHAROSE™ 6 Fast Flow (low sub) resin; (C) BUTYL-SEPHAROSE® 4 Fast Flow resin; (D) PHENYL SEPHAROSE™ 6 Fast Flow (high sub) resin; highest dilution CHOP (in ppm) is shown on the vertical axis and sodium sulfate concentration is shown on the horizontal axis; pH (5.5, 6.0, 7.0, or 8.0 is indicated by the legend.

DETAILED DESCRIPTION

[0038] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton et al., Dictionary of Microbiology and Molecular Biology 2nd ed., J. Wiley & Sons (New York, N.Y. 1994), and March, Advanced Organic Chemistry Reactions,

skilled in the art with a general guide to many of the terms used in the present application.

CERTAIN DEFINITIONS

[0039] For purposes of interpreting this specification, the following definitions will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa. In the event that any definition set forth below conflicts with any document incorporated herein by reference, the definition set forth below shall control.

[0040] As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a protein" or an "antibody" includes a plurality of proteins or antibodies, respectively; reference to "a cell" includes mixtures of cells, and the like.

[0041] The term "detecting" is used herein in the broadest sense to include both qualitative and quantitative measurements of a target molecule. Detecting includes identifying the mere presence of the target molecule in a sample as well as determining whether the target molecule is present in the sample at detectable levels.

[0042] A "sample" refers to a small portion of a larger quantity of material. Generally, testing according to the methods described herein is performed on a sample. The sample is typically obtained from a recombinant polypeptide preparation obtained, for example, from cultured host cells. A sample may be obtained from, for example but not limited to, harvested cell culture fluid, from an in-process pool at a certain step in a purification process, or from the final purified product.

[0043] The term "product" as described herein is the substance to be purified by various chromatographic methods; for example, a polypeptide.

[0044] The term "polypeptide" or "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art. The terms "polypeptide" and "protein" as used herein specifically encompass antibodies.

[0045] "Purified" polypeptide (*e.g.*, antibody or immunoadhesin) means that the polypeptide has been increased in purity, such that it exists in a form that is more pure than it exists in its natural

Purity is a relative term and does not necessarily mean absolute purity.

[0046] The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising a polypeptide fused to a "tag polypeptide." The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the polypeptide to which it is fused. The tag polypeptide preferably also is fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (in certain instances, between about 10 and 20 amino acid residues).

[0047] "Active" or "activity" for the purposes herein refers to form(s) of a polypeptide which retain a biological and/or an immunological activity of interest, wherein "biological" activity refers to a biological function (either inhibitory or stimulatory) caused by the polypeptide other than the ability to induce the production of an antibody against an antigenic epitope possessed by the polypeptide and an "immunological" activity refers to the ability to induce the production of an antibody against an antigenic epitope possessed by the polypeptide.

[0048] The term "antagonist" is used in the broadest sense, and includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of a native polypeptide, e.g., a cytokine. In a similar manner, the term "agonist" is used in the broadest sense and includes any molecule that mimics a biological activity of a native polypeptide. Suitable agonist or antagonist molecules specifically include agonist or antagonist antibodies or antibody fragments, fragments or amino acid sequence variants of native polypeptides, and the like. Methods for identifying agonists or antagonists of a polypeptide may comprise contacting a polypeptide with a candidate agonist or antagonist molecule and measuring a detectable change in one or more biological activities normally associated with the polypeptide.

[0049] A polypeptide "which binds" an antigen of interest, *e.g.* a tumor-associated polypeptide antigen target, is one that binds the antigen with sufficient affinity such that the polypeptide is useful as an assay reagent, a diagnostic and/or therapeutic agent in targeting a sample containing the antigen, a cell or tissue expressing the antigen, and does not significantly cross-react with other polypeptides.

[0050] With regard to the binding of a polypeptide to a target molecule, the term "specific binding" or "specifically binds to" or is "specific for" a particular polypeptide or an epitope on a particular polypeptide target means binding that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a

structure that does not have binding activity. For example, specific binding can be determined by competition with a control molecule that is similar to the target, for example, an excess of non-labeled target. In this case, specific binding is indicated if the binding of the labeled target to a probe is competitively inhibited by excess unlabeled target.

[0051] The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies *(e.g.* bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity. The term "immunoglobulin" (Ig) is used interchangeable with antibody herein.

[0052] Antibodies are naturally occurring immunoglobulin molecules which have varying structures, all based upon the immunoglobulin fold. For example, IgG antibodies have two "heavy" chains and two "light" chains that are disulphide-bonded to form a functional antibody. Each heavy and light chain itself comprises a "constant" (C) and a "variable" (V) region. The V regions determine the antigen binding specificity of the antibody, whilst the C regions provide structural support and function in non-antigen-specific interactions with immune effectors. The antigen binding specificity of an antibody or antigen-binding fragment of an antibody is the ability of an antibody to specifically bind to a particular antigen.

[0053] The antigen binding specificity of an antibody is determined by the structural characteristics of the V region. The variability is not evenly distributed across the 110-amino acid span of the variable domains. Instead, the V regions consist of relatively invariant stretches called framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-12 amino acids long. The variable domains of native heavy and light chains each comprise four FRs, largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (*see* Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

[0054] Each V region typically comprises three complementarity determining regions ("CDRs", each of which contains a "hypervariable loop"), and four framework regions. An antibody

desired antigen, will therefore typically include the three CDRs, and at least three, preferably four, framework regions interspersed there between to hold and present the CDRs in the appropriate conformation. Classical four chain antibodies have antigen binding sites which are defined by V_H and V_L domains in cooperation. Certain antibodies, such as camel and shark antibodies, lack light chains and rely on binding sites formed by heavy chains only. Single domain engineered immunoglobulins can be prepared in which the binding sites are formed by heavy chains or light chains alone, in absence of cooperation between V_H and V_L.

[0055] The term "hypervariable region" when used herein refers to certain amino acid residues of an antibody that are responsible for antigen binding. The hypervariable region may comprise amino acid residues from a "complementarity determining region" or "CDR" as discussed above (*e.g.*, around about residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the V_L, and around about 31-35B (H1), 50-65 (H2) and 95-102 (H3) in the V_H (Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)) and/or those residues from a "hypervariable loop" (*e.g.* residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the V_L, and 26-32 (H1), 52A-55 (H2) and 96-101 (H3) in the V_H (Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)).

[0056] "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

[0057] "Antibody fragments" comprise a portion of an intact antibody, preferably comprising the antigen binding region thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; tandem diabodies (taDb), linear antibodies(*e.g.*, U.S. Patent No. 5,641,870, Example 2; Zapata *et al.*, *Protein Eng.* 8(10):1057-1062 (1995)); one-armed antibodies, single variable domain antibodies, minibodies, single-chain antibody molecules; multispecific antibodies formed from antibody fragments (*e.g.*, including but not limited to, Db-Fc, taDb-Fc, taDb-CH3, (scFV)4-Fc, di-scFv, bi-scFv, or tandem (di,tri)-scFv); and Bi-specific T-cell engagers (BiTEs).

[0058] Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-binding sites and is still capable of cross-linking antigen.

[0059] "Fv" is the minimum antibody fragment that contains a complete antigen-recognition and antigen-binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. It is in this configuration that the three

surface of the V_H-V_L dimer. Collectively, the six hypervariable regions confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0060] The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear at least one free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments that have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0061] The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

[0062] Depending on the amino acid sequence of the constant domain of their heavy chains, antibodies can be assigned to different classes. There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy chain constant domains that correspond to the different classes of antibodies are called α, δ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0063] "Single-chain Fv" or "scFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. In some embodiments, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains that enables the scFv to form the desired structure for antigen binding. For a review of scFv *see* Plückthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[0064] The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (V_H) connected to a light chain variable domain (V_L) in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are

Natl. Acad. Sci. USA, 90:6444-6448 (1993).

[0065] The term "multispecific antibody" is used in the broadest sense and specifically covers an antibody that has polyepitopic specificity. Such multispecific antibodies include, but are not limited to, an antibody comprising a heavy chain variable domain (V_H) and a light chain variable domain (V_L), where the V_HV_L unit has polyepitopic specificity, antibodies having two or more V_L and V_H domains with each V_HV_L unit binding to a different epitope, antibodies having two or more single variable domains with each single variable domain binding to a different epitope, full length antibodies, antibody fragments such as Fab, Fv, dsFv, scFv, diabodies, bispecific diabodies, triabodies, tri-functional antibodies, antibody fragments that have been linked covalently or non-covalently. "Polyepitopic specificity" refers to the ability to specifically bind to two or more different epitopes on the same or different target(s). "Monospecific" refers to the ability to bind only one epitope. According to one embodiment the multispecific antibody is an IgG antibody that binds to each epitope with an affinity of 5 μM to 0.001 pM, 3 μM to 0.001 pM, 1 μM to 0.001 pM, 0.5 μM to 0.001 pM, or 0.1 μM to 0.001 pM.

[0066] The expression "single domain antibodies" (sdAbs) or "single variable domain (SVD) antibodies" generally refers to antibodies in which a single variable domain (VH or VL) can confer antigen binding. In other words, the single variable domain does not need to interact with another variable domain in order to recognize the target antigen. Examples of single domain antibodies include those derived from camelids (lamas and camels) and cartilaginous fish (*e.g.*, nurse sharks) and those derived from recombinant methods from humans and mouse antibodies (Nature (1989) 341:544-546; Dev Comp Immunol (2006) 30:43-56; Trend Biochem Sci (2001) 26:230-235; Trends Biotechnol (2003):21:484-490; WO 2005/035572; WO 03/035694; Febs Lett (1994) 339:285-290; WO00/29004; WO 02/051870).

[0067] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variants that may arise during production of the monoclonal antibody, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed

antibodies to be used in accordance with the methods provided herein may be made by the hybridoma method first described by Kohler *et al., Nature* 256:495 (1975), or may be made by recombinant DNA methods (*see, e.g.*, U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al., Nature* 352:624-628 (1991) and Marks *et al., J. Mol. Biol.* 222:581-597 (1991), for example.

[0068] The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison *et al., Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984)). Chimeric antibodies of interest herein include "primatized" antibodies comprising variable domain antigen-binding sequences derived from a non-human primate *(e.g.* Old World Monkey, such as baboon, rhesus or cynomolgus monkey) and human constant region sequences (US Pat No. 5,693,780).

[0069] "Humanized" forms of non-human *(e.g.,* murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence, except for FR substitution(s) as noted above. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region, typically that of a human immunoglobulin. For further details, *see* Jones *et al., Nature* 321:522-525 (1986); Riechmann *et al., Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992).

domains as well as an Fc region. The constant domains may be native sequence constant domains (*e.g.* human native sequence constant domains) or amino acid sequence variant thereof. Preferably, the intact antibody has one or more effector functions.

[0071] "Native antibodies" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

[0072] "Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

[0074] The terms "anti-IL-13 antibody" and "an antibody that binds to IL-13" refer to an antibody that is capable of binding IL-13 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting IL-13. In some embodiments, the extent of binding of an anti-IL-13 antibody to an unrelated, non-IL-13 protein is less than about 10% of the binding of the antibody to IL-13 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to IL-13 has a dissociation constant (Kd) of $\leq 1\mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g. 10-8 M or less, e.g. from 10-8 M to 10-13 M, e.g., from 10-9 M to 10-13 M). In certain embodiments, an anti-IL-13 antibody binds to an epitope of IL-13 that is conserved among IL-13 from different species.

[0075] "IL-13 mediated disorder" means a disorder associated with excess IL-13 levels or activity in which atypical symptoms may manifest due to the levels or activity of IL-13 locally and/or systemically in the body. Examples of IL-13 mediated disorders include: cancers (e.g., non-Hodgkin's lymphoma, glioblastoma), atopic dermatitis, allergic rhinitis, asthma, fibrosis, inflammatory bowel disease, Crohn's disease, lung inflammatory disorders (including pulmonary fibrosis such as IPF), COPD, and hepatic fibrosis.

[0076] The term "respiratory disorder" includes, but is not limited to, asthma (e.g., allergic and non-allergic asthma (e.g., due to infection, e.g., with respiratory syncytial virus (RSV), e.g., in younger children)); bronchitis (e.g., chronic bronchitis); chronic obstructive pulmonary disease (COPD) (e.g., emphysema (e.g., cigarette-induced emphysema); conditions involving airway inflammation, eosinophilia, fibrosis and excess mucus production, e.g., cystic fibrosis, pulmonary fibrosis, and allergic rhinitis. Examples of diseases that can be characterized by

chronic bronchitis, bronchiectasis, and cystic fibrosis.

[0077] The term "therapeutic agent" refers to any agent that is used to treat a disease. A therapeutic agent may be, for example, a polypeptide(s) (e.g., an antibody, an immunoadhesin or a peptibody), an aptamer or a small molecule that can bind to a protein or a nucleic acid molecule that can bind to a nucleic acid molecule encoding a target (i.e., siRNA), and the like.

[0078] A "naked antibody" is an antibody (as herein defined) that is not conjugated to a heterologous molecule, such as a cytotoxic moiety or radiolabel.

[0079] The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0080] The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

[0081] An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, *see, e.g.*, Flatman et al., *J. Chromatogr.* B 848:79-87 (2007).

[0082] The term "sequential" as used herein with regard to chromatography refers to having a first chromatography followed by a second chromatography. Additional steps may be included between the first chromatography and the second chromatography.

[0083] The term "continuous" as used herein with regard to chromatography refers to having a first chromatography material and a second chromatography material either directly connected or some other mechanism which allows for continuous flow between the two chromatography materials.

polypeptide product. Impurities and contaminants include, without limitation: host cell materials, such as CHOP, including single CHOP species; leached Protein A; nucleic acid; a variant, fragment, aggregate or derivative of the desired polypeptide; another polypeptide; endotoxin; viral contaminant; cell culture media component, etc. In some examples, the contaminant may be a host cell protein (HCP) from, for example but not limited to, a bacterial cell such as an *E. coli* cell, an insect cell, a prokaryotic cell, a eukaryotic cell, a yeast cell, a mammalian cell, an avian cell, a fungal cell.

[0085] The terms "Chinese hamster ovary cell protein" and "CHOP" are used interchangeably to refer to a mixture of host cell proteins ("HCP") derived from a Chinese hamster ovary ("CHO") cell culture. The HCP or CHOP is generally present as an impurity in a cell culture medium or lysate (e.g., a harvested cell culture fluid ("HCCF")) comprising a protein of interest such as an antibody or immunoadhesin expressed in a CHO cell.) The amount of CHOP present in a mixture comprising a protein of interest provides a measure of the degree of purity for the protein of interest. HCP or CHOP includes, but is not limited to, a protein of interest expressed by the host cell, such as a CHO host cell. Typically, the amount of CHOP in a protein mixture is expressed in parts per million relative to the amount of the protein of interest in the mixture. It is understood that where the host cell is another mammalian cell type, an E. coli, a yeast, an insect cell, or a plant cell, HCP refers to the proteins, other than target protein, found in a lysate of the host cell.

[0086] The term "parts per million" or "ppm" are used interchangeably herein to refer to a measure of purity of the protein of interest purified by a method of the invention. The units ppm refer to the amount of HCP or CHOP in nanograms/milliliter per protein of interest in milligrams/milliliter (i.e., CHOP ppm = (CHOP ng/ml)/(protein of interest mg/ml), where the proteins are in solution). Where the proteins are dried (such as by lyophilization), ppm refers to (CHOP ng)/(protein of interest mg)). Impurities may also be expressed as "ng/mg" which is used interchangeably with ppm.

[0087] By "purifying" a polypeptide from a composition comprising the polypeptide and one or more impurities is meant increasing the degree of purity of the polypeptide in the composition by removing (completely or partially) at least one impurity from the composition.

[0088] A "purification step" may be part of an overall purification process resulting in a "homogeneous" composition, which is used herein to refer to a composition comprising less than 100ppm HCP (100 ng/mg) in a composition comprising the protein of interest, or less than 90ppm (90 ng/mg), or less than 80ppm (80 ng/mg), or less than 70ppm (70 ng/mg), or less than

30ppm (30 ng/mg), or less than 20ppm (20 ng/mg), or less than 10ppm (10 ng/mg), or less than 5ppm (5 ng/mg), or less than 3ppm (3 ng/mg) or less than 1 ppm (1 ng/mg). In certain embodiments, the HCP is a single HCP species. In one embodiment, the single HCP species is hamster PLBL2.

[0089] The "composition" to be purified herein comprises the polypeptide of interest and one or more impurities or contaminants. The composition may be "partially purified" (i.e. having been subjected to one or more purification steps or may be obtained directly from a host cell or organism producing the polypeptide (e.g. the composition may comprise harvested cell culture fluid).

[0090] The terms "Protein A" and "ProA" are used interchangeably herein and encompasses Protein A recovered from a native source thereof, Protein A produced synthetically (e.g. by peptide synthesis or by recombinant techniques), and variants thereof which retain the ability to bind proteins which have a CH2/CH3 region, such as an Fc region. Protein A can be purchased commercially from various sources. Protein A is generally immobilized on a solid phase support material. The term "ProA" also refers to an affinity chromatography resin or column containing chromatographic solid support matrix to which is covalently attached Protein A.

[0091] The term "chromatography" refers to the process by which a solute of interest in a mixture is separated from other solutes in a mixture as a result of differences in rates at which the individual solutes of the mixture migrate through a stationary medium under the influence of a moving phase, or in bind and elute processes.

[0092] The term "affinity chromatography" and "protein affinity chromatography" are used interchangeably herein and refer to a protein separation technique in which a protein of interest or antibody of interest is reversibly and specifically bound to a biospecific ligand. Typically, the biospecific ligand is covalently attached to a chromatographic solid phase material and is accessible to the protein of interest in solution as the solution contacts the chromatographic solid phase material. The protein of interest (e.g., antibody, enzyme, or receptor protein) retains its specific binding affinity for the biospecific ligand (antigen, substrate, cofactor, or hormone, for example) during the chromatographic steps, while other solutes and/or proteins in the mixture do not bind appreciably or specifically to the ligand. Binding of the protein of interest to the immobilized ligand allows contaminating proteins or protein impurities to be passed through the chromatographic medium while the protein of interest remains specifically bound to the immobilized ligand on the solid phase material. The specifically bound protein of interest is then removed in active form from the immobilized ligand with low pH, high pH, high salt, competing

free of the contaminating proteins or protein impurities that were earlier allowed to pass through the column. Any component can be used as a ligand for purifying its respective specific binding protein, e.g. antibody.

[0093] The terms "non-affinity chromatography" and "non-affinity purification" refer to a purification process in which affinity chromatography is not utilized. Non-affinity chromatography includes chromatographic techniques that rely on non-specific interactions between a molecule of interest (such as a protein, e.g. antibody) and a solid phase matrix.

[0094] The term "specific binding" as used herein in the context of chromatography, such as to describe interactions between a molecule of interest and a ligand bound to a solid phase matrix, refers to the generally reversible binding of a protein of interest to a ligand through the combined effects of spatial complementarity of protein and ligand structures at a binding site coupled with electrostatic forces, hydrogen bonding, hydrophobic forces, and/or van der Waals forces at the binding site. The greater the spatial complementarity and the stronger the other forces at the binding site, the greater will be the binding specificity of a protein for its respective ligand. Non-limiting examples of specific binding includes antibody-antigen binding, enzyme-substrate binding, enzyme-cofactor binding, metal ion chelation, DNA binding protein-DNA binding, regulatory protein-protein interactions, and the like. Typically, in affinity chromatography specific binding occurs with an affinity of about 10^{-4} to 10^{-8} M in free solution.

[0095] The term "non-specific binding" as used herein in the context of chromatography, such as to describe interactions between a molecule of interest and a ligand or other compound bound to a solid phase matrix, refers to binding of a protein of interest to the ligand or compound on a solid phase matrix through electrostatic forces, hydrogen bonding, hydrophobic forces, and/or van der Waals forces at an interaction site, but lacking structural complementarity that enhances the effects of the non-structural forces. Examples of non-specific interactions include, but are not limited to, electrostatic, hydrophobic, and van der Waals forces as well as hydrogen bonding.

[0096] A "salt" is a compound formed by the interaction of an acid and a base. Exemplary salts include, but are not limited to, acetate (e.g. sodium acetate), citrate (e.g. sodium citrate), chloride (e.g. sodium chloride), sulphate (e.g. sodium sulphate), or a potassium salt.

[0097] As used herein, "solvent" refers to a liquid substance capable of dissolving or dispersing one or more other substances to provide a solution. Solvents include aqueous and organic solvents, where certain organic solvents include a non-polar solvent, ethanol, methanol, isopropanol, acetonitrile, hexylene glycol, propylene glycol, and 2,2-thiodiglycol.

polysorbates 20 or 80); poloxamers (e.g. poloxamer 188); Triton; sodium dodecyl sulfate (SDS); sodium laurel sulfate; sodium octyl glycoside; lauryl-, myristyl-, linoleyl-, or stearyl-sulfobetaine; lauryl-, myristyl-, linoleyl- or stearyl-sarcosine; linoleyl-, myristyl-, or cetyl-betaine; lauroamidopropyl-, cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-betaine (e.g. lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl oleyl-taurate; and the MONAQUAT(tm) series (Mona Industries, Inc., Paterson, New Jersey), polysorbate, such as polysorbate 20 (TWEEN 20(r)) or polysorbate 80 (TWEEN 80(r)).

[0099] A "polymer" herein is a molecule formed by covalent linkage of two or more monomers, where the monomers are not amino acid residues. Examples of polymers include, but are not limited to, polyethyl glycol, polypropyl glycol, and copolymers (e.g. PLURONICS™, PF68 etc), polyethylene glycol (PEG), e.g. PEG 400 and PEG 8000.

[00100] The term "ion-exchange" and "ion-exchange chromatography" refers to the chromatographic process in which a solute of interest (such as a protein) in a mixture interacts with a charged compound linked (such as by covalent attachment) to a solid phase ion exchange material such that the solute of interest interacts non-specifically with the charged compound more or less than solute impurities or contaminants in the mixture. The contaminating solutes in the mixture elute from a column of the ion exchange material faster or slower than the solute of interest or are bound to or excluded from the resin relative to the solute of interest. "Ion-exchange chromatography" specifically includes cation exchange, anion exchange, and mixed mode chromatography.

[00101] The phrase "ion exchange material" refers to a solid phase that is negatively charged (i.e. a cation exchange resin) or positively charged (i.e. an anion exchange resin). The charge may be provided by attaching one or more charged ligands to the solid phase, e.g. by covalent linking. Alternatively, or in addition, the charge may be an inherent property of the solid phase (e.g. as is the case for silica, which has an overall negative charge).

[00102] By "solid phase" is meant a non-aqueous matrix to which one or more charged ligands can adhere. The solid phase may be a purification column, a discontinuous phase of discrete particles, a membrane, or filter etc. Examples of materials for forming the solid phase include polysaccharides (such as agarose and cellulose); and other mechanically stable matrices such as silica (e.g. controlled pore glass), poly(styrenedivinyl)benzene, polyacrylamide, ceramic particles and derivatives of any of the above.

which thus has free cations for exchange with cations in an aqueous solution passed over or through the solid phase. A negatively charged ligand attached to the solid phase to form the cation exchange resin may, e.g., be a carboxylate or sulfonate. Commercially available cation exchange resins include, but are not limited to, carboxy-methyl-cellulose, sulphopropyl (SP) immobilized on agarose (e.g. SP-SEPHAROSE FAST FLOW(or SP-SEPHAROSE HIGH PERFORMANCE) and sulphonyl immobilized on agarose (e.g. S-SEPHAROSE FAST FLOW), and POROS®HS.

[00104] A "mixed mode ion exchange resin" refers to a solid phase which is covalently modified with cationic, anionic, and hydrophobic moieties. Mixed mode ion exchange is also referred to as "multimodal ion exchange." Commercially available mixed mode ion exchange resin are available, e.g., BAKERBOND ABX containing weak cation exchange groups, a low concentration of anion exchange groups, and hydrophobic ligands attached to a silica gel solid phase support matrix. Additional exemplary mixed mode ion exchange resins include, but are not limited to, CAPTO™ Adhere resin, QMA resin, CAPTO™ MMC resin, MEP HyperCel resin, HEA HyperCel resin, PPA HyperCel resin, or ChromaSorb membrane or Sartobind STIC. In some embodiments, the mixed mode material is CAPTO™ Adhere resin.

[00105] The term "anion exchange resin" is used herein to refer to a solid phase which is positively charged, e.g. having one or more positively charged ligands, such as quaternary amino groups, attached thereto. Commercially available anion exchange resins include DEAE cellulose, QAE SEPHADEX and FAST Q SEPHAROSE™ and Q SEPHAROSE™ FAST FLOW.

[00106] A "buffer" is a solution that resists changes in pH by the action of its acid-base conjugate components. Various buffers which can be employed depending, for example, on the desired pH of the buffer are described in Buffers. A Guide for the Preparation and Use of Buffers in Biological Systems, Gueffroy, D., ed. Calbiochem Corporation (1975). In certain instances, the buffer has a pH in the range from about 2 to about 9, alternatively from about 3 to about 8, alternatively from about 4 to about 7 alternatively from about 5 to about 7. Non-limiting examples of buffers that will control the pH in this range include MES, MOPS, MOPSO, Tris, HEPES, phosphate, acetate, citrate, succinate, and ammonium buffers, as well as combinations of these.

[00107] The term "hydrophobic interaction chromatography" or "HIC" is used herein to refer to a chromatographic process that separates molecule based on their hydrophobicity. Exemplary resins that can be used for HIC include, but are not limited to phenyl-, butyl-, octyl-

Performance, PHENYL SEPHAROSE™ 6 Fast Flow (low sub), and PHENYL SEPHAROSE™ 6 Fast Flow (high sub). Typically, sample molecules in a high salt buffer are loaded onto the HIC column. The salt in the buffer interacts with water molecules to reduce the solvation of the molecules in solution, thereby exposing hydrophobic regions in the sample molecules which are consequently adsorbed by the HIC column. The more hydrophobic the molecule, the less salt needed to promote binding. Typically, a decreasing salt gradient is used to elute samples from the column. As the ionic strength decreases, the exposure of the hydrophilic regions of the molecules increases and molecules elute from the column in order of increasing hydrophobicity. Sample elution may also be achieved by the addition of mild organic modifiers or detergents to the elution buffer.

[00108] The "loading buffer" is that which is used to load the composition comprising the polypeptide molecule of interest and one or more impurities onto the ion exchange resin. The loading buffer has a conductivity and/or pH such that the polypeptide molecule of interest (and generally one or more impurities) is/are bound to the ion exchange resin or such that the protein of interest flows through the column while the impurities bind to the resin.

[00109] The "intermediate buffer" is used to elute one or more impurities from the ion exchange resin, prior to eluting the polypeptide molecule of interest. The conductivity and/or pH of the intermediate buffer is/are such that one or more impurity is eluted from the ion exchange resin, but not significant amounts of the polypeptide of interest.

[00110] The term "wash buffer" when used herein refers to a buffer used to wash or re-equilibrate the ion exchange resin, prior to eluting the polypeptide molecule of interest. In certain instances, for convenience, the wash buffer and loading buffer may be the same, but this is not required.

[00111] The "elution buffer" is used to elute the polypeptide of interest from the solid phase. The conductivity and/or pH of the elution buffer is/are such that the polypeptide of interest is eluted from the ion exchange resin.

[00112] A "regeneration buffer" may be used to regenerate the ion exchange resin such that it can be re-used. The regeneration buffer has a conductivity and/or pH as required to remove substantially all impurities and the polypeptide of interest from the ion exchange resin.

[00113] The term "conductivity" refers to the ability of an aqueous solution to conduct an electric current between two electrodes. In solution, the current flows by ion transport. Therefore, with an increasing amount of ions present in the aqueous solution, the solution will have a higher conductivity. The unit of measurement for conductivity is milliSeimens per

conductivity of a solution may be altered by changing the concentration of ions therein. For example, the concentration of a buffering agent and/or concentration of a salt (e.g. NaCl or KCl) in the solution may be altered in order to achieve the desired conductivity.

[00114] The "pI" or "isoelectric point" of a polypeptide refer to the pH at which the polypeptide's positive charge balances its negative charge. pI can be calculated from the net charge of the amino acid residues or sialic acid residues of attached carbohydrates of the polypeptide or can be determined by isoelectric focusing.

[00115] By "binding" a molecule to an ion exchange material is meant exposing the molecule to the ion exchange material under appropriate conditions (pH/conductivity) such that the molecule is reversibly immobilized in or on the ion exchange material by virtue of ionic interactions between the molecule and a charged group or charged groups of the ion exchange material.

[00116] By "washing" the ion exchange material is meant passing an appropriate buffer through or over the ion exchange material.

[00117] To "elute" a molecule (e.g. polypeptide or impurity) from an ion exchange material is meant to remove the molecule therefrom by altering the ionic strength of the buffer surrounding the ion exchange material such that the buffer competes with the molecule for the charged sites on the ion exchange material.

[00118] "Ultrafiltration" is a form of membrane filtration in which hydrostatic pressure forces a liquid against a semipermeable membrane. Suspended solids and solutes of high molecular weight are retained, while water and low molecular weight solutes pass through the membrane. In some examples, ultrafiltration membranes have pore sizes in the range of 1 to 100 nm. The terms "ultrafiltration membrane" and "ultrafiltration filter" may be used interchangeably.

[00119] "Diafiltration" is a method that incorporates ultrafiltration membranes to remove salts or other microsolutes from a solution. Small molecules are separated from a solution while retaining larger molecules in the retentate. The process selectively utilizes permeable (porous) membrane filters to separate the components of solutions and suspensions based on their molecular size.

[00120] As used herein, "filtrate" refers to that portion of a sample that passes through the filtration membrane.

[00121] As used herein, "retentate" refers to that portion of a sample that is substantially retained by the filtration membrane.

to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[00123] A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[00124] As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies are used to delay development of a disease or to slow the progression of a disease.

[00125] Reference to "about" a value or parameter herein includes (and describes) variations that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X".

ANTI-IL13 ANTIBODIES

[00126] In some embodiments, isolated and purified antibodies that bind IL-13 are provided. Exemplary anti-IL13 antibodies are known and include, for example, but not limited to, lebrikizumab, IMA-026, IMA-638 (also referred to as, anrukinzumab, INN No. 910649-32-0; QAX-576), tralokinumab (also referred to as CAT-354, CAS No. 1044515-88-9); AER-001, ABT-308 (also referred to as humanized 13C5.5 antibody. Examples of such anti-IL13 antibodies and other inhibitors of IL13 are disclosed, for example, in WO 2005/062967, WO2008/086395, WO2006/085938, US 7,615,213, US 7,501,121, WO2007/036745, WO2010/073119, WO2007/045477. In one embodiment, the anti-IL13 antibody is a humanized IgG4 antibody. In one embodiment, the anti-IL13 antibody is lebrikizumab. In one embodiment, the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 (SEQ ID NO.: 1), CDR-H2 (SEQ ID NO.: 2), and CDR-H3 (SEQ ID NO.: 3). In one embodiment, the anti-IL13 antibody comprises three light chain CDRS, CDR-L1 (SEQ ID NO.: 4), CDR-L2 (SEQ ID NO.: 5), and CDR-L3 (SEQ ID NO.: 6). In one embodiment, the anti-IL13 antibody comprises three heavy chain CDRs and three light chain CDRs, CDR-H1 (SEQ ID NO.: 1),

(SEQ ID NO.: 5), and CDR-L3 (SEQ ID NO.: 6). In one embodiment, the anti-IL13 antibody comprises a variable heavy chain region, VH, having an amino acid sequence selected from SEQ ID NOs. 7 and 8. In one embodiment, the anti-IL13 antibody comprises a variable light chain region, VL, having the amino acid sequence of SEQ ID NO.: 9. In one embodiment, the anti-IL13 antibody comprises a variable heavy chain region, VH, having an amino acid sequence selected from SEQ ID NOs. 7 and 8 and a variable light chain region, VL, having an amino acid sequence of SEQ ID NO.: 9. In one embodiment, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10 or SEQ ID NO.: 11 or SEQ ID NO.: 12 or SEQ ID NO.: 13. In one embodiment, the anti-IL13 antibody comprises a light chain having the amino acid sequence of SEQ ID NO.: 14. In one embodiment, the anti-IL13 antibody comprises a heavy chain having an amino acid sequence selected from SEQ ID NO.: 10, SEQ ID NO.: 11, SEQ ID NO.: 12, and SEQ ID NO.: 13 and a light chain having the amino acid sequence of SEQ ID NO.: 14.

[00127] In another aspect, an anti-IL-13 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO.: 8. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-IL-13 antibody comprising that sequence retains the ability to bind to human IL-13. In certain embodiments, a total of 1 to 10 amino acids have been substituted, altered inserted and/or deleted in SEQ ID NO.: 8. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the CDRs (i.e., in the FRs). Optionally, the anti-IL13 antibody comprises the VH sequence in SEQ ID NO.: 8, including post-translational modifications of that sequence.

[00128] In another aspect, an anti-IL-13 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-IL-13 antibody comprising that sequence retains the ability to bind to IL-13. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO.: 9. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the CDRs (i.e., in the FRs).

post-translational modifications of that sequence.

[00129] In yet another embodiment, the anti-IL-13 antibody comprises a VL region having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO.: 9 and a VH region having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO.: 8.

[00130] The table below shows the amino acid sequences of the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3 regions of lebrikizumab, along with VH, VL, heavy chain sequences and light chain sequences. As indicated in Table 1 below, VH and the heavy chain may include an N-terminal glutamine and the heavy chain may also include a C-terminal lysine. As is well known in the art, N-terminal glutamine residues can form pyroglutamate and C-terminal lysine residues can be clipped during manufacturing processes.

Table 1. Anti-IL13 antibody (lebrikizumab) amino acid sequences.

CDR-H1 (SEQ ID NO.:1)	Ala Tyr Ser Val Asn
CDR-H2 (SEQ ID NO.:2)	Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys Ser
CDR-H3 (SEQ ID NO.:3)	Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn
CDR-L1 (SEQ ID NO.:4)	Arg Ala Ser Lys Ser Val Asp Ser Tyr Gly Asn Ser Phe Met His
CDR-L2 (SEQ ID NO.:5)	Leu Ala Ser Asn Leu Glu Ser
CDR-L3 (SEQ ID NO.:6)	Gln Gln Asn Asn Glu Asp Pro Arg Thr
VH (SEQ ID NO.:7)	Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ala Tyr Ser Val Asn Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu Ala Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala Gly Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser
VH (SEQ ID NO.:8)	Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ala Tyr Ser Val Asn Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu Ala Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys

	Gly Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser
VL (SEQ ID NO.:9)	Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ser Val Ser Leu Gly Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Lys Ser Val Asp Ser Tyr Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Asn Asn Glu Asp Pro Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
H Chain (SEQ ID NO.:10)	VTLRESGPA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGDGY YPYAMDNWGQ GSLVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTKTYT CNVDHKPSNT KVDKRVESKY GPPCPPCPAP EFLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLG
H Chain (SEQ ID NO.:11)	QVTLRESGPA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGDGY YPYAMDNWGQ GSLVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTKTYT CNVDHKPSNT KVDKRVESKY GPPCPPCPAP EFLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLG
H Chain (SEQ ID NO.:12)	VTLRESGPA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGDGY YPYAMDNWGQ GSLVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTKTYT CNVDHKPSNT KVDKRVESKY GPPCPPCPAP EFLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLGK
H Chain (SEQ ID NO.:13)	QVTLRESGPA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGDGY YPYAMDNWGQ GSLVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTKTYT CNVDHKPSNT KVDKRVESKY GPPCPPCPAP EFLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLGK
L Chain (SEQ ID NO.: 14)	DIVMTQSPDS LSVSLGERAT INCRASKSVD SYGNSFMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQNNEDPR TFGGGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC

OTHER RECOMBINANT POLYPEPTIDES

[00131] Recombinant polypeptides produced in CHO cells may be purified according to the methods described herein to remove or reduce levels of hamster PLBL2 such that only residual

growth factors, cytokines, immunoglobulins, antibodies, peptibodies and the like.

[00132] Certain exemplary antibodies include antibodies to Abeta, antibodies to IL17A/F and antibodies to CMV. Exemplary anti-Abeta antibodies and methods of producing such antibodies have been described previously, for example, in WO2008011348, WO2007068429, WO2001062801, and WO2004071408. Exemplary anti-IL17 A/F antibodies and methods of producing such antibodies have been described previously, for example, in WO 2009136286 and U.S. Patent No. 8,715,669. Exemplary anti-CMV antibodies, including anti-CMV-MSL, and methods of producing such antibodies have been described previously, for example, in WO 2012047732.

[00133] Exemplary polypeptides include include mammalian proteins, such as, *e.g.,* CD4, integrins and their subunits, such as beta7, growth hormone, including human growth hormone and bovine growth hormone; growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; lipoproteins; ct-l-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor, and von Willebrands factor; anti-clotting factors such as Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator, such as urokinase or tissue-type plasminogen activator (t-PA, *e.g.,* Activase®, TNKase®, Retevase®); bombazine; thrombin; tumor necrosis factor-α and -β; enkephalinase; RANTES (regulated on activation normally T-cell expressed and secreted); human macrophage inflammatory protein (MIP-I-a); serum albumin such as human serum albumin; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; DNase; inhibin; activin; vascular endothelial growth factor (VEGF); IgE, receptors for hormones or growth factors; an integrin; protein A or D; rheumatoid factors; a neurotrophic factor such as bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6), or a nerve growth factor such as NGF-β; platelet-derived growth factor (PDGF); fibroblast growth factor such as aFGF and bFGF; epidermal growth factor (EGF); transforming growth factor (TGF) such as TGF–α and TGF-β including TGF-β1, TGF-β2, TGF-β3, TGF-β4, or TGF-β5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(1-3)-IGF-I (brain IGF-I); insulin-like growth factor binding proteins; other CD proteins such as CD3, CD8, CD19 and CD20; erythropoietin (EPO); thrombopoietin (TPO); osteoinductive factors; immunotoxins; a bone morphogenetic protein (BMP); an interferon such as interferon-α, -β, or –γ; colony stimulating factors (CSFs), *e.g.,* M-CSF, GM-CSF, and G-CSF; interleukins (ILs), *e.g.,* IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25,

receptors; surface membrane proteins; decay accelerating factor (DAF); a viral antigen such as, for example, a portion of an HIV envelope; transport proteins; homing receptors; addressins; regulatory proteins; integrins such as CDlla, CDllb, CDllc, CD18, integrin subunits such alpha4, alphaE, beta7; cellular adhesion molecules such as an ICAM, VLA-4 and VCAM; a tumor associated antigen such as HER1, (EGFR), HER2, HER3 or HER4 receptor; Apo2L/TRAIL, and fragments of any of the above listed polypeptides; as well as immunoadhesins and antibodies binding to; and biologically active fragments or variants of any of the above-listed proteins.

[00134] Additional exemplary polypeptides include brain polypeptides, including but not limited to, beta-secretase 1 (BACE1), Abeta, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), tau, apolipoprotein E (ApoE), alpha-synuclein, CD20, huntingtin, prion protein (PrP), leucine rich repeat kinase 2 (LRRK2), parkin, presenilin 1, presenilin 2, gamma secretase, death receptor 6 (DR6), amyloid precursor protein (APP), p75 neurotrophin receptor (p75NTR), P-selectin, and caspase 6, and fragments of any of the above listed polypeptides; as well as immunoadhesins and antibodies binding to; and biologically active fragments or variants of any of the above-listed proteins.

[00135] Further exemplary polypeptides include therapeutic antibodies and immunoadhesins, including, without limitation, antibodies, including antibody fragments, to one or more of the following antigens: HER1 (EGFR), HER2 (e.g., trastuzumab, pertuzumab), HER3, HER4, VEGF (e.g., bevacizumab, ranibizumab), MET (e.g., onartuzumab), CD20 (e.g., rituximab, obinutuzumab, ocrelizumab), CD22, CD11a, CD11b, CD11c, CD18, an ICAM, VLA-4, VCAM, IL-17A and/or F, IgE (e.g., omalizumab), DR5, CD40, Apo2L/TRAIL, EGFL7 (e.g., parsatuzumab), NRP1, integrin beta7 (e.g., etrolizumab), IL-13 (e.g., lebrikizumab), Abeta (e.g., crenezumab, gantenerumab), P-selectin (e.g., inclacumab), IL-6R (e.g., tociluzumab), IFNα (e.g., rontalizumab), M1prime (e.g., quilizumab), mitogen activated protein kinase (MAPK), OX40L, TSLP, Factor D (e.g., lampalizumab) and receptors such as: IL-9 receptor, IL-5 receptor, IL-4receptor alpha, IL-13receptoralpha1 and IL-13receptoralpha2, OX40, TSLP-R, IL-7Ralpha (a co-receptor for TSLP), IL17RB (receptor for IL-25), ST2 (receptor for IL-33), CCR3, CCR4, CRTH2, FcepsilonRI and FcepsilonRII/CD23 (receptors for IgE). Other exemplary antibodies include those selected from, and without limitation, antiestrogen receptor antibody, anti-progesterone receptor antibody, anti-p53 antibody, anticathepsin D antibody, anti-Bcl-2 antibody, anti-E-cadherin antibody, anti-CA125 antibody, anti- CA15-3 antibody, anti-CA19-9 antibody, anti-c-erbB-2 antibody, anti-P-glycoprotein antibody, anti-CEA antibody,

Ki-67 antibody, anti-PCNA antibody, anti-CD3 antibody, anti-CD4 antibody, anti-CD5 antibody, anti-CD7 antibody, anti-CD8 antibody, anti-CD9/p24 antibody, anti-CD10 antibody, anti-CDllc antibody, anti-CD13 antibody, anti-CD14 antibody, anti-CD15 antibody, anti-CD19 antibody, anti-CD23 antibody, anti-CD30 antibody, anti-CD31 antibody, anti-CD33 antibody, anti-CD34 antibody, anti-CD35 antibody, anti-CD38 antibody, anti-CD41 antibody, anti-LCA/CD45 antibody, anti-CD45RO antibody, anti-CD45RA antibody, anti-CD39 antibody, anti-CD100 antibody, anti-CD95/Fas antibody, anti-CD99 antibody, anti-CD106 antibody, anti-ubiquitin antibody, anti-CD71 antibody, anti-c-myc antibody, anti-cytokeratins antibody, anti-vimentins antibody, anti-HPV proteins antibody, anti-kappa light chains antibody, anti-lambda light chains antibody, anti-melanosomes antibody, anti-prostate specific antigen antibody, anti-S-100 antibody, anti-tau antigen antibody, anti-fibrin antibody, anti-keratins antibody and anti-Tn-antigen antibody.

CERTAIN PURIFICATION METHODS

[00136] The protein to be purified using the methods described herein is generally produced using recombinant techniques. Methods for producing recombinant proteins are described, *e.g.*, in US Pat No's 5,534,615 and 4,816,567, specifically incorporated herein by reference. In certain embodiments, the protein of interest is produced in a CHO cell (see, *e.g.* WO 94/11026). Examples of proteins, including anti-IL13 monoclonal antibodies (anti-IL13 MAb), which can be purified using the processes described herein have been described above.

[00137] When using recombinant techniques, the protein can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the protein is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration. Where the protein is secreted into the medium, the recombinant host cells may be separated from the cell culture medium by tangential flow filtration, for example.

[00138] Protein A immobilized on a solid phase is used to purify the anti-IL13 MAb preparation. In certain embodiments, the solid phase is a column comprising a glass, silica, agarose or polystyrene surface for immobilizing the Protein A. In certain embodiments, the solid phase is a controlled pore glass column or a silicic acid column. Sometimes, the column has been coated with a reagent, such as glycerol, in an attempt to prevent nonspecific adherence to the column. The PROSEP A™ column, commercially available from Bioprocessing Limited, is an example of a Protein A controlled pore glass column which is coated with glycerol. Other examples of columns contemplated herein include the POROS® 50 ATM (polystyrene) column

(agarose) column available from GE Healthcare Life Sciences (agarose).

[00139] The solid phase for the Protein A chromatography is equilibrated with a suitable buffer. For example, the equilibration buffer may be 25mM Tris, 25mM NaCl, pH 7.70 ± 0.20.

[00140] The preparation derived from the recombinant host cells and containing impurities and/or contaminants is loaded on the equilibrated solid phase using a loading buffer which may be the same as the equilibration buffer. As the preparation containing impurities/contaminants flows through the solid phase, the protein is adsorbed to the immobilized Protein A and other impurities/contaminants (such as Chinese Hamster Ovary Proteins, CHOP, where the protein is produced in a CHO cell) may bind nonspecifically to the solid phase.

[00141] The next step performed sequentially entails removing the impurities/contaminants bound to the solid phase, antibody and/or Protein A, by washing the solid phase in an intermediate wash step. After loading, the solid phase may be equilibrated with equilibration buffer before beginning the intermediate wash step.

[00142] The intermediate wash buffer may comprise salt and optionally a further compound, such as (a) detergent (for example, polysorbate, e.g. polysorbate 20 or polysorbate 80); (b) solvent (such as hexylene glycol); and (c) polymer (such as polyethylene glycol {PEG]).

[00143] The salt employed may be selected based on the protein of interest. Exemplary salts include, but are not limited to, sodium acetate, sodium citrate, and potassium phosphate.

[00144] The amounts of the salt and further compound (if any) in the composition are such that the combined amount elutes the impurity(ies)/contaminant(s), without substantially removing the protein of interest. Exemplary salt concentrations in such wash buffers are from about 0.1 to about 2M, or from about 0.2M to about 0.6M. Useful detergent concentrations are from about 0.01 to about 5%, or from about 0.1 to 1%, or about 0.5%, e.g. where the detergent is polysorbate. Exemplary solvent concentrations are from about 1% to 40%, or from about 5 to about 25%. Where the further compound is a polymer (e.g. PEG 400 or PEG 8000), the concentration thereof may, for example, be from about 1% to about 20%, or from about 5% to about 15%.

[00145] The pH of the intermediate wash buffer is typically from about 4 to about 8, or from about 4.5 to about 5.5, or about 5.0. In one embodiment, the pH is 7.00 ± 0.10.

[00146] Following the intermediate wash step described above, the protein of interest is recovered from the column. This is typically achieved using a suitable elution buffer. The protein may, for example, be eluted from the column using an elution buffer having a low pH (also referred to as acidic conditions), e.g. in the range from about 2 to about 5, or in the range

acetate buffers.

[00147] The eluted protein preparation may be subjected to additional purification steps either prior to, or after, the Protein A chromatography step. Exemplary further purification steps include hydroxyapatite chromatography; dialysis; affinity chromatography using an antibody to capture the protein; hydrophobic interaction chromatography (HIC); ammonium sulphate precipitation; anion or cation exchange chromatography; ethanol precipitation; reverse phase HPLC; chromatography on silica; chromatofocusing; ultrafiltration-diafiltration (UFDF), and gel filtration. In the examples herein, the Protein A chromatography step is followed by downstream anion exchange (e.g., Q-Sepharose-Fast Flow) or multimodal (e.g. mixed-mode) ion exchange (e.g., CAPTO™ Adhere) and HIC (e.g., PHENYL SEPHAROSE™ 6 fast flow - high sub) purification steps.

[00148] The protein thus recovered may be formulated in a pharmaceutically acceptable carrier and is used for various diagnostic, therapeutic or other uses known for such molecules.

[00149] In some embodiments of any of the methods described herein, the chromatography material is an ion exchange chromatography material; for example, an anion exchange chromatography material. In some embodiments, the anion exchange chromatography material is a solid phase that is positively charged and has free anions for exchange with anions in an aqueous solution passed over or through the solid phase. In some embodiments of any of the methods described herein, the anion exchange material may be a membrane, a monolith, or resin. In an embodiment, the anion exchange material may be a resin. In some embodiments, the anion exchange material may comprise a primary amine, a secondary amine, a tertiary amine or a quarternary ammonium ion functional group, a polyamine functional group, or a diethylaminoaethyl functional group. In some embodiments of the above, the anion exchange chromatography material is an anion exchange chromatography column. In some embodiments of the above, the anion exchange chromatography material is an anion exchange chromatography membrane.

[00150] In some embodiments of any of the methods described herein, the ion exchange material may utilize a conventional chromatography material or a convective chromatography material. The conventional chromatography materials include, for example, perfusive materials (*e.g.*, poly(styrene-divinylbenzene) resin) and diffusive materials (*e.g.*, cross-linked agarose resin). In some embodiments, the poly(styrene-divinylbenzene) resin can be POROS® resin. In some embodiments, the cross-linked agarose resin may be sulphopropyl-Sepharose Fast Flow ("SPSFF") resin. The convective chromatography material may be a membrane (*e.g.*,

membrane may be Mustang. The cross-linked polymer monolith material may be cross-linked poly(glycidyl methacrylate-co-ethylene dimethacrylate).

[00151] Examples of anion exchange materials include, but are not limited to, POROS® HQ 50, POROS® PI 50, POROS® D, Mustang Q, Q SEPHAROSE™ FF, and DEAE Sepharose.

[00152] In some aspects, the chromatography material is a hydrophobic interaction chromatography material. Hydrophobic interaction chromatography (HIC) is a liquid chromatography technique that separates biomolecules according to hydrophobicity. Examples of HIC chromatography materials include, but are not limited to, Toyopearl hexyl 650, Toyopearl butyl 650, Toyopearl phenyl 650, Toyopearl ether 650, Source, Resource, Sepharose Hi-Trap, Octyl sepharose, PHENYL SEPHAROSE™ high performance, PHENYL SEPHAROSE™ 6 fast flow (low sub) and PHENYL SEPHAROSE™ 6 fast flow (high sub). In some embodiments of the above, the HIC chromatography material is a HIC chromatography column. In some embodiments of the above, the HIC chromatography material is a HIC chromatography membrane.

[00153] In some aspects, the chromatography material is an affinity chromatography material. Examples of affinity chromatography materials include, but are not limited to chromatography materials derivatized with protein A or protein G. Examples of affinity chromatography material include, but are not limited to, Prosep-VA, Prosep-VA Ultra Plus, Protein A sepharose fast flow, Tyopearl Protein A, MAbSelect, MABSELECT SURE™ and MABSELECT SURE™ LX. In some embodiments of the above, the affinity chromatography material is an affinity chromatography column. In some embodiments of the above, the affinity chromatography material is an affinity chromatography membrane.

[00154] Various buffers which can be employed depending, for example, on the desired pH of the buffer, the desired conductivity of the buffer, the characteristics of the protein of interest, and the purification method. In some embodiments of any of the methods described herein, the methods comprise using a buffer. The buffer can be a loading buffer, an equilibration buffer, or a wash buffer. In some embodiments, one or more of the loading buffer, the equilibration buffer, and/or the wash buffer are the same. In some embodiments, the loading buffer, the equilibration buffer, and/or the wash buffer are different. In some embodiments of any of the methods described herein, the buffer comprises a salt. The loading buffer may comprise sodium chloride, sodium acetate, or a mixture thereof. In some embodiments, the loading buffer is a sodium chloride buffer. In some embodiments, the loading buffer is a sodium acetate buffer.

Loading buffer is the buffer used to load the composition comprising the product of interest onto a chromatography material. The chromatography material may be equilibrated with an equilibration buffer prior to loading the composition which is to be purified. In some examples, the wash buffer is used after loading the composition onto a chromatography material and before elution of the polypeptide of interest from the solid phase. However, some of the product of interest, *e.g.* a polypeptide, may be removed from the chromatography material by the wash buffer (*e.g.* flow-through mode).

[00156] Elution, as used herein, is the removal of the product, *e.g.* polypeptide, from the chromatography material. Elution buffer is the buffer used to elute the polypeptide or other product of interest from a chromatography material. In many cases, an elution buffer has a different physical characteristic than the load buffer. For example, the elution buffer may have a different conductivity than load buffer or a different pH than the load buffer. In some embodiments, the elution buffer has a lower conductivity than the load buffer. In some embodiments, the elution buffer has a higher conductivity than the load buffer. In some embodiments, the elution buffer has a lower pH than the load buffer. In some embodiments, the elution buffer has a higher pH than the load buffer. In some embodiments the elution buffer has a different conductivity and a different pH than the load buffer. The elution buffer can have any combination of higher or lower conductivity and higher or lower pH.

[00157] Conductivity refers to the ability of an aqueous solution to conduct an electric current between two electrodes. In solution, the current flows by ion transport. Therefore, with an increasing amount of ions present in the aqueous solution, the solution will have a higher conductivity. The basic unit of measure for conductivity is the Siemen (or mho), mho (mS/cm), and can be measured using a conductivity meter, such as various models of Orion conductivity meters. Since electrolytic conductivity is the capacity of ions in a solution to carry electrical current, the conductivity of a solution may be altered by changing the concentration of ions therein. For example, the concentration of a buffering agent and/or the concentration of a salt (*e.g.* sodium chloride, sodium acetate, or potassium chloride) in the solution may be altered in order to achieve the desired conductivity. Preferably, the salt concentration of the various buffers is modified to achieve the desired conductivity.

[00158] In some embodiments of any of the methods described herein, the flow rate is less than about any of 50 CV/hr, 40 CV/hr, or 30 CV/hr. The flow rate may be between about any of 5 CV/hr and 50 CV/hr, 10 CV/hr and 40 CV/hr, or 18 CV/hr and 36 CV/hr. In some embodiments, the flow rate is about any of 9 CV/hr, 18 CV/hr, 25 CV/hr, 30 CV/hr, 36 CV/hr, or 40 CV/hr. In

of 100 cm/hr, 75 cm/hr, or 50 cm/hr. The flow rate may be between about any of 25 cm/hr and 150 cm/hr, 25 cm/hr and 100 cm/hr, 50 cm/hr and 100 cm/hr, or 65 cm/hr and 85 cm/hr, or 50 cm/hr and 250 cm/hr, or 100 cm/hr and 250 cm/hr, or 150 cm/hr and 250 cm/hr.

[00159] Bed height is the height of chromatography material used. In some embodiments of any of the method described herein, the bed height is greater than about any of 3 cm, 10 cm, or 15 cm. The bed height may be between about any of 3 cm and 35 cm, 5 cm and 15 cm, 3 cm and 10 cm, or 5 cm and 8 cm. In some embodiments, the bed height is about any of 3 cm, 5 cm, 10 cm, or 15 cm. In some embodiments, bed height is determined based on the amount of polypeptide or contaminants in the load.

[00160] In some embodiments, the chromatography is in a column of vessel with a volume of greater than about 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, 6 mL, 7 mL, 8 mL, 9 mL, 10 mL, 15 mL, 20 mL, 25 mL, 30 mL, 40 mL, 50 mL, 75 mL, 100 mL, 200 mL, 300 mL, 400 mL, 500 mL, 600 mL, 700 mL, 800 mL, 900 mL, 1 L, 2 L, 3 L, 4 L, 5 L, 6 L, 7 L, 8 L, 9 L, 10 L, 25 L, 50 L, 100 L, 200L, 400L, or 450L.

[00161] In some embodiments, fractions are collected from the chromatography. In some embodiments, fractions collected are greater than about 0.01 CV, 0.02 CV, 0.03 CV, 0.04 CV, 0.05 CV, 0.06 CV, 0.07 CV, 0.08 CV, 0.09 CV, 0.1 CV, 0.2 CV, 0.3 CV, 0.4 CV, 0.5 CV, 0.6 CV, 0.7 CV, 0.8 CV, 0.9 CV, 1.0 CV, 2.0 CV, 3.0 CV, 4.0 CV, 5.0, CV. In some embodiments, fractions containing the product, *e.g.* polypeptide, are pooled. In some embodiments, fractions containing the polypeptide from the load fractions and from the elution fractions are pooled. The amount of polypeptide in a fraction can be determined by one skilled in the art; for example, the amount of polypeptide in a fraction can be determined by UV spectroscopy. In some embodiments, fractions containing detectable polypeptide fragment are pooled.

[00162] In some embodiments of any of the methods described herein, the at least one impurity or contaminant is any one or more of host cell materials, such as CHOP; leached Protein A; nucleic acid; a variant, fragment, aggregate or derivative of the desired polypeptide; another polypeptide; endotoxin; viral contaminant; cell culture media component, gentamicin, etc. In some examples, the impurity or contaminant may be a host cell protein (HCP) from, for example but not limited to, a bacterial cell such as an E. coli cell, an insect cell, a prokaryotic cell, a eukaryotic cell, a yeast cell, a mammalian cell, an avian cell, a fungal cell.

[00163] Host cell proteins (HCP) are proteins from the cells in which the polypeptide was produced. For example, CHOP are proteins from host cells, *i.e.*, Chinese Hamster Ovary Proteins. The amount of CHOP may be measured by enzyme-linked immunosorbent assay

the amount of HCP (*e.g.* CHOP) is reduced by greater than about any of 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 %, or 95 %. The amount of HCP may be reduced by between about any of 10 % and 99 %, 30% and 95%, 30 % and 99 %, 50% and 95%, 50 % and 99 %, 75 % and 99 %, or 85 % and 99 %. In some embodiments, the amount of HCP is reduced by about any of 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 85 %, 90 %, 95 %, or 98 %. In some embodiments, the reduction is determined by comparing the amount of HCP in the composition recovered from a purification step(s) to the amount of HCP in the composition before the purification step(s).

[00164] In some embodiments of any of the methods described herein, the methods further comprise recovering the purified polypeptide. In some embodiments, the purified polypeptide is recovered from any of the purification steps described herein. The chromatography step may be anion exchange chromatography, HIC, or Protein A chromatography. In some embodiments, the first chromatography step is protein A, followed by anion exchange or multimodal ion exchange, followed by HIC.

[00165] In some embodiments, the polypeptide is further purified following chromatography by viral filtration. Viral filtration is the removal of viral contaminants in a polypeptide purification feedstream. Examples of viral filtration include ultrafiltration and microfiltration. In some embodiments the polypeptide is purified using a parvovirus filter.

[00166] In some embodiments, the polypeptide is concentrated after chromatography. Examples of concentration methods are known in the art and include but are not limited to ultrafiltration and diafiltration.

[00167] In some embodiments of any of the methods described herein, the methods further comprise combining the purified polypeptide of the methods of purification with a pharmaceutically acceptable carrier.

Monoclonal antibodies

[00168] In some embodiments, the antibodies purified according to the methods of the invention are monoclonal antibodies. Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope except for possible variants that arise during production of the monoclonal antibody, such variants generally being present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete or polyclonal antibodies.

first described by Kohler *et al., Nature* 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

[00170] In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as herein described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the polypeptide used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp. 59-103 (Academic Press, 1986)).

[00171] The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

[00172] In some embodiments, the myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, in some embodiments, the myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.* 133:3001 (1984); Brodeur *et al., Monoclonal Antibody Production Techniques and Applications* pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

[00173] Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. In some embodiments, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

[00174] The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson *et al., Anal.* Biochem. 107:220 (1980).

[00175] After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown

(Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown in vivo as ascites tumors in an animal.

[00176] The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, polypeptide A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

[00177] DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). In some embodiments, the hybridoma cells serve as a source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as E. coli cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin polypeptide, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra *et al.*, *Curr. Opinion in Immunol.* 5:256-262 (1993) and Plückthun, *Immunol. Revs.*, 130:151-188 (1992).

[00178] In a further embodiment, antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty *et al.*, *Nature* 348:552-554 (1990). Clackson *et al.*, *Nature* 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks *et al.*, *Bio/Technology* 10:779-783 (1992)), as well as combinatorial infection and in vivo recombination as a strategy for constructing very large phage libraries (Waterhouse *et al.*, *Nuc. Acids. Res.* 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

[00179] The DNA also may be modified, for example, by substituting the coding sequence for human heavy- and light chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison *et al.*, P*roc. Natl Acad. Sci. USA* 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

[00181] In some embodiments of any of the methods described herein, the antibody is IgA, IgD, IgE, IgG, or IgM. In some embodiments, the antibody is an IgG monoclonal antibody.

Humanized antibodies

[00182] In some embodiments, the antibody is a humanized antibody. Methods for humanizing non-human antibodies have been described in the art. In some embodiments, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-327 (1988); Verhoeyen *et al.*, *Science* 239:1534-1536 (1988)), by substituting hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some hypervariable region residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[00183] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence that is closest to that of the rodent is then accepted as the human framework region (FR) for the humanized antibody (Sims *et al.*, *J. Immunol.* 151:2296 (1993); Chothia *et al.*, *J. Mol. Biol.* 196:901 (1987)). Another method uses a particular framework region derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chain variable regions. The same framework may be used for several different humanized antibodies (Carter *et al.*, *Proc. Natl. Acad. Sci. USA* 89:4285 (1992); Presta *et al.*, *J. Immunol.* 151:2623 (1993)).

[00184] It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, in some embodiments of the methods, humanized antibodies are prepared by a process of analysis of the parental

parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available that illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *i.e.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

Human antibodies

[00185] In some embodiments, the antibody is a human antibody. As an alternative to humanization, human antibodies can be generated. For example, it is now possible to produce transgenic animals *(e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. *See, e.g.*, Jakobovits *et al., Proc. Natl. Acad. Sci. USA* 90:2551 (1993); Jakobovits *et al., Nature* 362:255-258 (1993); Bruggermann *et al., Year in Immuno.* 7:33 (1993); and US Patent Nos. 5,591,669; 5,589,369; and 5,545,807.

[00186] Alternatively, phage display technology (McCafferty *et al., Nature* 348:552-553 (1990)) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat polypeptide gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B cell. Phage display can be performed in a variety of formats; for their review *see, e.g.*, Johnson, Kevin S. and Chiswell, David J., *Current Opinion in Structural Biology* 3:564-571 (1993). Several sources of V-gene

diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991), or Griffith *et al.*, *EMBO J.* 12:725-734 (1993). *See also*, US Patent Nos. 5,565,332 and 5,573,905.

[00187] Human antibodies may also be generated by *in vitro* activated B cells (*see* US Patents 5,567,610 and 5,229,275).

Antibody fragments

[00188] In some embodiments, the antibody is an antibody fragment. Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (*see, e.g.*, Morimoto *et al.*, *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992) and Brennan *et al.*, *Science* 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. For example, the antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments (Carter *et al.*, *Bio/Technology* 10:163-167 (1992)). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). *See* WO 93/16185; US Patent No. 5,571,894; and US Patent No. 5,587,458. The antibody fragment may also be a "linear antibody," *e.g.*, as described in US Patent 5,641,870 for example. Such linear antibody fragments may be monospecific or bispecific.

[00189] In some embodiments, fragments of the antibodies described herein are provided. In some embodiments, the antibody fragment is an antigen binding fragment. In some embodiments, the antigen binding fragment is selected from the group consisting of a Fab fragment, a Fab' fragment, a $F(ab')_2$ fragment, a scFv, a Fv, and a diabody.

Chimeric Polypeptides

[00190] The polypeptide described herein may be modified in a way to form chimeric molecules comprising the polypeptide fused to another, heterologous polypeptide or amino acid sequence. In some embodiments, a chimeric molecule comprises a fusion of the polypeptide with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino- or carboxyl-terminus of the polypeptide.

against the tag polypeptide. Also, provision of the epitope tag enables the polypeptide to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag.

Other

[00191] Another type of covalent modification of the polypeptide comprises linking the polypeptide to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. The polypeptide also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sc*iences*, 18th edition, Gennaro, A.R., Ed., (1990).

OBTAINING POLYPEPTIDES

[00192] The polypeptides used in the methods of purification described herein may be obtained using methods well-known in the art, including the recombination methods. The following sections provide guidance regarding these methods.

Polynucleotides

[00193] "Polynucleotide," or "nucleic acid," as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA.

[00194] Polynucleotides encoding polypeptides may be obtained from any source including, but not limited to, a cDNA library prepared from tissue believed to possess the polypeptide mRNA and to express it at a detectable level. Accordingly, polynucleotides encoding polypeptide can be conveniently obtained from a cDNA library prepared from human tissue. The polypeptide-encoding gene may also be obtained from a genomic library or by known synthetic procedures (*e.g.*, automated nucleic acid synthesis).

[00195] For example, the polynucleotide may encode an entire immunoglobulin molecule chain, such as a light chain or a heavy chain. A complete heavy chain includes not only a heavy chain variable region (V_H) but also a heavy chain constant region (C_H), which typically will comprise three constant domains: C_H1, C_H2 and C_H3; and a "hinge" region. In some situations, the presence of a constant region is desirable.

[00196] Other polypeptides which may be encoded by the polynucleotide include antigen-binding antibody fragments such as single domain antibodies ("dAbs"), Fv, scFv, Fab' and

C_H1 and C_K or C_L domain has been excised. As minibodies are smaller than conventional antibodies they should achieve better tissue penetration in clinical/diagnostic use, but being bivalent they should retain higher binding affinity than monovalent antibody fragments, such as dAbs. Accordingly, unless the context dictates otherwise, the term "antibody" as used herein encompasses not only whole antibody molecules but also antigen-binding antibody fragments of the type discussed above. Preferably each framework region present in the encoded polypeptide will comprise at least one amino acid substitution relative to the corresponding human acceptor framework. Thus, for example, the framework regions may comprise, in total, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or fifteen amino acid substitutions relative to the acceptor framework regions.

[00197] Suitably, the polynucleotides described herein may be isolated and/or purified. In some embodiments, the polynucleotides are isolated polynucleotides.

[00198] The term "isolated polynucleotide" is intended to indicate that the molecule is removed or separated from its normal or natural environment or has been produced in such a way that it is not present in its normal or natural environment. In some embodiments, the polynucleotides are purified polynucleotides. The term purified is intended to indicate that at least some contaminating molecules or substances have been removed.

[00199] Suitably, the polynucleotides are substantially purified, such that the relevant polynucleotides constitutes the dominant (*i.e.*, most abundant) polynucleotides present in a composition.

Expression of Polynucleotides

[00200] The description below relates primarily to production of polypeptides by culturing cells transformed or transfected with a vector containing polypeptide-encoding polynucleotides. It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare polypeptides. For instance, the appropriate amino acid sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques (*see, e.g.*, Stewart *et al.*, *Solid-Phase Peptide Synthesis* W.H. Freeman Co., San Francisco, Calif. (1969); Merrifield, *J. Am. Chem. Soc.* 85:2149-2154 (1963)). In vitro protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be accomplished, for instance, using an Applied Biosystems Peptide Synthesizer (Foster City, Calif.) using manufacturer's instructions. Various portions of the polypeptide may be chemically synthesized separately and combined using chemical or enzymatic methods to produce the desired polypeptide.

production of the polypeptides. The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences include, but are not limited to, promoters (*e.g.*, naturally-associated or heterologous promoters), signal sequences, enhancer elements, and transcription termination sequences.

[00202] A polynucleotide is "operably linked" when it is placed into a functional relationship with another polynucleotide sequence. For example, nucleic acids for a presequence or secretory leader is operably linked to nucleic acids for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the nucleic acid sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[00203] For antibodies, the light and heavy chains can be cloned in the same or different expression vectors. The nucleic acid segments encoding immunoglobulin chains are operably linked to control sequences in the expression vector(s) that ensure the expression of immunoglobulin polypeptides.

[00204] The vectors containing the polynucleotide sequences (*e.g.*, the variable heavy and/or variable light chain encoding sequences and optional expression control sequences) can be transferred into a host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment, electroporation, lipofection, biolistics or viral-based transfection may be used for other cellular hosts. (*See generally* Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Press, 2nd ed., 1989). Other methods used to transform mammalian cells include the use of polybrene, protoplast fusion, liposomes, electroporation, and microinjection. For production of transgenic animals, transgenes can be microinjected into fertilized oocytes, or can be incorporated into the genome of embryonic stem cells, and the nuclei of such cells transferred into enucleated oocytes.

[00205] The term "vector" includes expression vectors and transformation vectors and shuttle vectors.

[00206] The term "expression vector" means a construct capable of *in vivo* or *in vitro* expression.

[00207] The term "transformation vector" means a construct capable of being transferred from one entity to another entity - which may be of the species or may be of a different species. If the construct is capable of being transferred from one species to another - such as from an *Escherichia coli* plasmid to a bacterium, such as of the genus *Bacillus*, then the transformation vector is sometimes called a "shuttle vector". It may even be a construct capable of being transferred from an *E. coli* plasmid to an *Agrobacterium* to a plant.

[00208] Vectors may be transformed into a suitable host cell as described below to provide for expression of a polypeptide. Various vectors are publicly available. The vector may, for example, be in the form of a plasmid, cosmid, viral particle, or phage. The appropriate nucleic acid sequence may be inserted into the vector by a variety of procedures. In general, DNA is inserted into an appropriate restriction endonuclease site(s) using techniques known in the art. Construction of suitable vectors containing one or more of these components employs standard ligation techniques which are known to the skilled artisan.

[00209] The vectors may be for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of the said polynucleotide and optionally a regulator of the promoter. Vectors may contain one or more selectable marker genes which are well known in the art.

[00210] These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA.

Host Cells

[00211] The host cell may be a bacterium, a yeast or other fungal cell, insect cell, a plant cell, or a mammalian cell, for example.

[00212] A transgenic multicellular host organism which has been genetically manipulated may be used to produce a polypeptide. The organism may be, for example, a transgenic mammalian organism (*e.g.*, a transgenic goat or mouse line).

[00213] Suitable prokaryotes include but are not limited to eubacteria, such as Gram-negative or Gram-positive organisms, for example, *Enterobacteriaceae* such as *E. coli*. Various E. coli strains are publicly available, such as *E. coli* K12 strain MM294 (ATCC 31,446); *E. coli* X1776 (ATCC 31,537); *E. coli* strain W3110 (ATCC 27,325) and K5 772 (ATCC 53,635). Other

Enterobacter, Erwinia, Klebsiella, Proteus, Salmonella, e.g., Salmonella typhimurium, Serratia, e.g., Serratia marcescans, and *Shigella*, as well as *Bacilli* such as *B. subtilis and B. licheniformis* (*e.g., B. licheniformis* 41P), *Pseudomonas* such as *P. aeruginosa,* and *Streptomyces*. These examples are illustrative rather than limiting. Strain W3110 is one particularly preferred host or parent host because it is a common host strain for recombinant polynucleotide product fermentations. Preferably, the host cell secretes minimal amounts of proteolytic enzymes. For example, strain W3110 may be modified to effect a genetic mutation in the genes encoding polypeptides endogenous to the host, with examples of such hosts including *E. coli* W3110 strain 1A2, which has the complete genotype *tonA*; *E. coli* W3110 strain 9E4, which has the complete genotype *tonA ptr3*; *E. coli* W3110 strain 27C7 (ATCC 55,244), which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT kan'*; *E. coli* W3110 strain 37D6, which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT rbs7 ilvG kan'*; *E. coli* W3110 strain 40B4, which is strain 37D6 with a non-kanamycin resistant *degP* deletion mutation; and an *E. coli* strain having mutant periplasmic protease. Alternatively, in vitro methods of cloning, *e.g.*, PCR or other nucleic acid polymerase reactions, are suitable.

[00214] In these prokaryotic hosts, one can make expression vectors, which will typically contain expression control sequences compatible with the host cell (*e.g.*, an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation.

[00215] Eukaryotic microbes may be used for expression. Eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for polypeptide-encoding vectors. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism. Others include *Schizosaccharomyces pombe*; *Kluyveromyces hosts* such as, *e.g., K. lactis* (MW98-8C, CBS683, CBS4574), *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickeramii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilarum* (ATCC 36,906), *K. thermotolerans*, and *K. marxianus; yarrowia* (EP 402,226); *Pichia pastoris*; *Candida; Trichoderma reesia*; *Neurospora crassa*; *Schwanniomyces* such as *Schwanniomyces occidentalis*; and filamentous fungi such as, *e.g.*, *Neurospora, Penicillium, Tolypocladium*, and Aspergillus hosts such as *A. nidulans,* and *A. niger*. Methylotropic yeasts are suitable herein and include, but are not limited to, yeast capable of growth on methanol selected from the genera

Rhodotorula. *Saccharomyces* is a preferred yeast host, with suitable vectors having expression control sequences (*e.g.*, promoters), an origin of replication, termination sequences and the like as desired. Typical promoters include 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase, isocytochrome C, and enzymes responsible for maltose and galactose utilization.

[00216] In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce the polypeptides as described herein and in some instances are preferred (*See* Winnacker, *From Genes to Clones* VCH Publishers, N.Y., N.Y. (1987). For some embodiments, eukaryotic cells may be preferred, because a number of suitable host cell lines capable of secreting heterologous polypeptides (*e.g.*, intact immunoglobulins) have been developed in the art, and include CHO cell lines, various Cos cell lines, HeLa cells, preferably, myeloma cell lines, or transformed B-cells or hybridomas. In some embodiments, the mammalian host cell is a CHO cell.

[00217] In some embodiments, the host cell is a vertebrate host cell. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR(CHO or CHO-DP-12 line); mouse sertoli cells; monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells; MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

FORMULATIONS AND METHODS OF MAKING THE FORMULATION

[00218] Provided herein are also formulations and methods of making the formulation comprising the polypeptides (*e.g.*, antibodies) purified by the methods described herein. For example, the purified polypeptide may be combined with a pharmaceutically acceptable carrier.

[00219] The polypeptide formulations in some embodiments may be prepared for storage by mixing a polypeptide having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions.

[00220] "Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and

solution.

[00221] Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[00222] In some embodiments, the polypeptide in the polypeptide formulation maintains functional activity.

[00223] The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

[00224] The formulations herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, in addition to a polypeptide, it may be desirable to include in the one formulation, an additional polypeptide (*e.g.*, antibody). Alternatively, or additionally, the composition may further comprise a chemotherapeutic agent, cytotoxic agent, cytokine, growth inhibitory agent, anti-hormonal agent, and/or cardioprotectant. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[00225] Exemplary formulations of the anti-IL13 antibodies described herein are provided in International Patent Pub. No. WO 2013/066866.

ARTICLES OF MANUFACTURE

[00226] The polypeptides purified by the methods described herein and/or formulations comprising the polypeptides purified by the methods described herein may be contained within an article of manufacture. The article of manufacture may comprise a container containing the polypeptide and/or the polypeptide formulation. In certain embodiments, the article of manufacture comprises:(a) a container comprising a composition comprising the polypeptide

insert with instructions for administering the formulation to a subject.

[00227] The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds or contains a formulation and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is the polypeptide. The label or package insert indicates that the composition's use in a subject with specific guidance regarding dosing amounts and intervals of polypeptide and any other drug being provided. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes. In some embodiments, the container is a syringe. In some embodiments, the syringe is further contained within an injection device. In some embodiments, the injection device is an autoinjector.

[00228] A "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications, other therapeutic products to be combined with the packaged product, and/or warnings concerning the use of such therapeutic products.

[00229] Exemplary articles of manufacture containing formulations of the anti-IL13 antibodies described herein are provided in International Patent Pub. No. WO 2013/066866.

[00230] Further details of the invention are illustrated by the following non-limiting Examples. The disclosures of all references in the specification are expressly incorporated herein by reference.

EXAMPLES

[00231] As used in the Examples below and elsewhere herein, "PLB2" and "PLBL2" and "PLBD2" are used interchangeably and refer to the enzyme "phospholipase B-like 2" or its synonym, "phospholipase B-domain-like 2".

EXAMPLE 1 – General Methods

[00232] Materials and methods for all Examples were performed as indicated below unless otherwise noted in the Example.

MAb feedstocks

[00233] MAb feedstocks for all examples were selected from industrial, pilot or small scale cell culture batches at Genentech (South San Francisco, CA, U.S.A.). After a period of cell culture

culture fluid, HCCF) was purified by Protein A chromatography and one or more additional chromatography steps and filtration steps as indicated in the Examples below.

MAb quantification

[00234] The concentration of antibody was determined via absorbance at 280 and 320 nm using a UV-visible spectrophotometer (8453 model G1103A; Agilent Technologies; Santa Clara, CA, U.S.A.) or NanoDrop 1000 model ND-1000 (Thermo Fisher Scientific; Waltham, MA, U.S.A.). Species other than antibody (*i.e.* impurities) were too low in concentration to have an appreciable effect on UV absorbance. As needed, samples were diluted with an appropriate non-interfering diluent in the range of 0.1–1.0 absorbance unit. Sample preparation and UV measurements were performed in duplicate and the average value was recorded. The mAb absorption coefficients ranged from 1.42 to 1.645/mg·ml·cm.

Total CHO host cell protein (CHOP) quantification

[00235] An ELISA was used to quantify the levels of the total host cell proteins called CHOP. The ELISAs used to detect CHO proteins in products were based upon a sandwich ELISA format. Affinity-purified polyclonal antibody to CHOP was coated onto a 96-well microtiter plate. Standards, controls, and samples were then loaded in duplicate into separate wells. CHOP, if present in the sample, will bind to the coat antibody (polyclonal anti-CHOP). After an incubation step, anti-CHOP polyclonal antibody-conjugated to horseradish peroxidase (HRP) was added to the plate. After a final wash step, CHOP was quantified by adding a solution of tetramethyl benzidine (TMB), also available as SUREBLUE RESERVE™ from KPL, Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD, cat no. 53-00-03), which when acted on by the HRP enzyme produces a colorimetric signal. The optical density (OD) at 450 nm was measured in each well. A five-parameter curve-fitting program (SOFTMAX® Pro, Molecular Devices, Sunnyvale, CA) was used to generate a standard curve, and sample CHOP concentrations were computed from the standard curve. The assay range for the total CHOP ELISA was from 5 to 320 ng/ml. CHOP concentration, in ng/mL, refers to the amount of CHOP in a sample using the CHOP standard as a calibrator. CHOP ratio (in ng/mg or ppm) refers to the calculated ratio of CHOP concentration to product concentration and, in certain instances, was the reported value for the test methods. The Total CHOP ELISA may be used to quantify total CHOP levels in a sample but does not quantify the concentration of individual proteins.

Murine Monoclonal Anti-Hamster PLBL2 ELISA Assay

[00236] The generation of mouse anti-hamster PLBL2 monoclonal antibodies and development of an ELISA assay for the detection and quantification of PLBL2 in recombinant polypeptide

61/877,503 and 61/991,228. Briefly, the assay is carried out as follows.

[00237] Murine monoclonal antibody 19C10 was coated onto a half area 96-well microtiter plate at a concentration of 0.5 μg/mL in carbonate buffer (0.05M sodium carbonate, pH 9.6), overnight at 2-8°C. After coating, the plate was blocked with Blocking Buffer (0.15M NaCl, 0.1M sodium phosphate, 0.1% fish gelatin, 0.05% polysorbate 20, 0.05% Proclin® 300 [Sigma-Aldrich]; also referred to as Assay Diluent) to prevent non-specific sticking of proteins. Standards, controls, and samples were diluted in Assay Diluent (0.15M NaCl, 0.1M sodium phosphate, 0.1% fish gelatin, 0.05% polysorbate 20, 0.05% Proclin® 300 [Sigma-Aldrich]) then loaded in duplicate into separate wells and incubated for 2 hrs at room temperature (22-27°C). PLBL2, if present in the sample, would bind to the coat (also referred to herein as capture) antibody. After the incubation step described above, unbound materials were washed away using Wash Buffer (0.05% polysorbate 20/PBS [Corning cellgro Cat. No. 99-717-CM]) and the 15G11 anti-PLBL2 murine monoclonal antibody conjugated to biotin was diluted in Assay Diluent to a concentration of 0.03125 μg/mL and added to the wells of the microtiter plate.

[00238] Biotin conjugation was carried out as follows. A biotinylation kit was purchased from Pierce Thermo Scientific, (P/N 20217, E-Z Link NHS-Biotin), and streptavidin-HRP (SA-HRP) from Jackson Immuno Cat. No. 016-030-084. Instructions in the Pierce Kit were followed. Briefly, IgG was dialyzed into PBS, pH 7.4, and biotin was added to the protein and mixed at room temperature for 1 hr. The labeled antibody was then dialized against PBS, pH 7.4 to remove excess biotin, filtered, and protein concentration determined by A280.

After a 2 hr. incubation step with biotinylated 15G11 at room temperature, Streptavidin HRP (1:200,000 dilution in Assay Diluent) was added to the microtiter plate wells. After a final wash step with Wash Buffer (described above), color was developed (for PLBL2 quantification) by adding a solution of TMB (50 μl/well) (SUREBLUE RESERVE™ from KPL, Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD, cat no. 53-00-03) followed by incubation at room temperature for 10-20 minutes. Detection was carried out by assessing optical density (OD) at 450 nm in each well using a Molecular Devices SpectraMax M5e. A four-parameter curve-fitting program (SoftMax Pro v5.2 rev C) was used to generate a standard curve, and sample PLBL2 concentrations were computed from the linear range of the standard curve. Values in the linear range of the standard curve were used to calculate nominal PLBL2 (ng/mg or ppm). The linear range was approximately EC_{10} – EC_{85} or 1.5 – 40 ng/mL as the range varied slightly from plate to plate. Values obtained for PLBL2 using this ELISA were comparable to estimates made

diluted to the LOQ of the assay

Rabbit Polyclonal Anti-Hamster PLBL2 ELISA Assay

[00239] The generation of rabbit anti-hamster PLBL2 polyclonal antibodies and development of an ELISA assay for the detection and quantification of PLBL2 in recombinant polypeptide preparations using such antibodies is described in US Provisional Patent Application Nos. 61/877,503 and 61/991,228. Briefly, the assay is carried out as follows.

[00240] Affinity purified rabbit polyclonal antibody was coated onto a half area 96-well microtiter plate at a concentration of 0.5 ug/mL in carbonate buffer (0.05M sodium carbonate, pH 9.6), overnight at 2-8°C. After coating, the plate was blocked with Blocking Buffer (0.15M NaCl, 0.1M sodium phosphate, 0.1% fish gelatin, 0.05% Polysorbate 20, 0.05% Proclin® 300 [Sigma-Aldrich]) to prevent non-specific sticking of proteins. Standards, controls, and samples were diluted in Assay Diluent (0.15M NaCl, 0.1M sodium phosphate, 0.1% fish gelatin, 0.05% Polysorbate 20, 0.05% Proclin® 300 [Sigma-Aldrich]) then loaded in duplicate into separate wells and incubated for 2 hr at room temperature (22-27°C). PLBL2, if present in the sample, would bind to the coat (also referred to herein as capture) antibody. After the incubation step described above, unbound materials were washed away using Wash Buffer (0.05% Polysorbate 20/PBS [Corning Cellgro Cat. No. 99-717-CM]) and the affinity purified rabbit polyclonal antibody conjugated to horseradish peroxidase (HRP) was diluted in Assay Diluent to a concentration of 40 ng/mL and added to the wells of the microtiter plate.

[00241] HRP conjugation was carried out as follows. A HRP conjugation kit was purchased from Pierce Thermo Scientific, (P/N 31489, E-Z Link Plus Activated Peroxidase and Kit). Instructions in the Pierce Kit were followed. Briefly, IgG was dialyzed into Carbonate-Bicarbonate buffer, pH 9.4, and EZ-Link Plus Activated Peroxidase was added to the protein and mixed at room temperature for 1 hr. Sodium cyanoborohydride and Quenching buffer were added subsequently to stabilize the conjugation and quench the reaction. The labeled antibody was then dialyzed against PBS, pH 7.4, filtered, and protein concentration determined by A280.

[00242] After a 2 hr. incubation step with HRP conjugated rabbit polyclonal antibody at room temperature, a final wash step with Wash Buffer (described above) was performed. Afterwards, color was developed (for PLBL2 quantification) by adding a solution of TMB (50 ul/well) (SUREBLUE RESERVE™ from KPL, Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD, cat no. 53-00-03) followed by incubation at room temperature for 10-20 minutes. Detection was carried out by assessing optical density (OD) at 450 nm in each well using a Molecular Devices SpectraMax M5e. A five-parameter curve-fitting program (SoftMax Pro

computed from the linear range of the standard curve. Values in the linear range of the standard curve were used to calculate nominal PLBL2 (ng/mg or ppm). The quantitative range of the assay was 0.5 -50 ng/mL. Values obtained for PLBL2 using this ELISA were comparable to estimates made by other methods (e.g., murine monoclonal PLBL2 ELISA, LC-MS/MS or total CHOP ELISA when diluted to the LOQ of the assay).

LC-MS/MS Assay

[00243] For quantification of PLBL2 by LC-MS/MS, a Waters Acquity H-Class Bio UPLC and AB Sciex TripleTOF 5600+ mass spectrometer were used. Samples and calibration standards (recombinant PLBL2 spiked into a recombinant humanized monoclonal antibody preparation obtained from a mouse NS0 cell line [the NS0 cell line does not contain hamster PLBL2]) were reduced and digested by trypsin. A total of 40 μg digested sample was injected onto the UPLC, using a Waters BEH300 C18 column, particle size 1.7 μm. A linear gradient of acetonitrile was used to elute the peptides, at a flow rate of 300 μl/min and a column temperature of 60°C.

[00244] Peptides eluting from the UPLC were introduced to the mass spectrometer by electrospray ionization in positive ionization mode. Ion source temperature was set at 400°C, with an IonSpray voltage of 5500 v. and declustering potential of 76 v. A collision energy setting of 32 was used for the fragmentation of selected peptide ions. The mass spectrometer was operated in multiple reaction monitoring high resolution (MRM^{HR}) mode, using four specific PLBL2 peptides and their fragment ion transitions. The parent ions were selected by the quadrupole mass spectrometer with a mass to charge (m/z) selection window of 1.2 amu. Fragment ions of each parent ion were separated by the time-of-flight mass spectrometer and selected for quantification post data acquisition with a selection window of 0.025 amu.
The concentration of PLBL2 in samples was determined by measuring the specific signal responses of the four transitions, calibrated by those from the standards in the range of 2-500 ppm using a linear fit. Table 2 below shows the list of PLBL2 peptides monitored by LC-MS/MS.

TripleTOF 5600+ Scan Cycle					
Scan #	Scan Type	Peptide	Fragment Ion of Interest	Parent m/z	Fragment m/z
1	TOF MS	N/A	N/A	N/A	N/A
2	Product Ion	SVLLDAASGQLR (SEQ ID NO: 31)	+2y8	615.3461	817.4163
3	Product Ion	GLEDSYEGR (SEQ ID NO: 32)	+2y7	513.2304	855.3479
4	Product Ion	AFIPNGPSPGSR (SEQ ID NO: 33)	+2y9	600.3120	868.4272
5	Product Ion	VTSFSLAK (SEQ ID NO: 34)	+2y6	426.7449	652.3665

EXAMPLE 2 – Improved Purification Process to Reduce Hamster PLBL2

[00245] A purification process for CHO-produced anti-IL13 MAb (lebrikizumab) was established to support early stage clinical trials and is referred to herein as the "Initial Process." The Initial Process employed the following chromatographic steps in order: Protein A affinity chromatography (MABSELECT SURE™) followed by cation exchange (POROS® HS) followed by anion exchange (Q SEPHAROSE™ Fast Flow). Additional virus inactivation and filtration steps were included and a final ultrafiltration-diafiltration (UFDF) step. The final product (drug substance) was formulated at a concentration of 125 mg/mL in 20 mM histidine acetate, 6% sucrose, 0.03% polysorbate 20, pH 5.7.

[00246] Using the Total CHOP ELISA Assay (described in Example 1 above), we observed that in-process intermediates and drug substance purified according to the Initial Process demonstrated atypical dilution-dependent behavior resulting in a > 20% coefficient of variation across a normalized series of sample dilutions. This dilution-dependent behavior is exemplified by the data presented in Table 3 in which each successive two-fold dilution of anti-IL13 MAb product resulted in higher levels of CHOP (expressed in ppm) as determined using the Total CHOP ELISA. Using sensitive analytical methods, such as LC-MS/MS, we determined that a single CHOP species, or HCP, was the cause of this atypical dilution-dependent behavior. In particular, we established that the dilution-dependent behavior on the Total CHOP ELISA was due to antigen excess. Further investigation enabled us to identify the single HCP as an enzyme, hamster phospholipase B-like 2 (PLBL2). By diluting the product samples to the limit of assay quantitation (LOQ), we were able to estimate the level of PLBL2 in clinical lots of lebrikizumab purified using the Initial Process and determined that levels as high as 300 ppm (300 ng/mg) and above were present.

Fold Dilution	Total CHOP (ppm)
2	0.58
4	1
8	2
16	4
32	7
64	14
128	26
256	49
512	97
1024	147
2048	228
4096	314
8192	346

[00247] This level of impurity (> 300 ppm) of a single CHOP species such as we observed, is considered undesirable in MAb products intended for human clinical and/or therapeutic use, particularly late stage clinical trials and beyond. For example, such levels may be immunogenic when administered to human subjects as described in Example 3.

[00248] Accordingly, we investigated various modifications to the Initial Process as briefly outlined below. Based on the results of these investigations, we developed an improved purification process, described in detail below, and referred to herein as "Improved Process." Use of the Improved Process resulted in purified anti-IL13 MAb (lebrikizumab) product containing substantially reduced levels of PLBL2.

[00249] Efforts for modifying the purification process to reduce PLBL2 included methods orthogonal to the Initial Process including: precipitation, testing various additives to HCCF, additional column washes, hydrophobic interaction and mixed mode chromatography. These efforts were informed by use of one or more of the assays described in Example 1 to monitor the effectiveness of each of the modifications investigated for reduction in total CHOP and/or PLBL2 levels. The various modifications explored are described below.

Precipitation of CHOP in HCCF and Protein A Pool with Caprylic Acid

[00250] Caprylic acid precipitation has been described previously, including use in the monoclonal antibody industry (Wang et al., BioPharm International; Downstream Processing 2010, p4-10, Oct2009; Brodsky et al., Biotechnology and Bioengineering, 109(10):2589, 2012) to selectively precipitate impurities from target proteins of interest. Caprylic acid, also known as octanoic acid, is a saturated fatty acid with eight carbons (formula $CH_3(CH_2)_6COOH$). Studies were done with anti-IL13 MAb to determine whether precipitation of the harvested cell

reduction of dilution-dependent behavior in the Total CHOP ELISA.

[00251] The anti-IL13 MAb starting material for these studies was HCCF and Protein A pools from a 1kL harvest. 1% (v/v) caprylic acid was added to the HCCF and varying concentrations of caprylic acid (0% - 3% v/v) were added to Protein A pools at pH 4.5 or pH 5.0. Samples were mixed for ≥ 5 hours at ambient temperature, 0.2 μm filtered, and diluted with Total CHOP ELISA diluent for detection and quantification using the Total CHOP ELISA. Titer of anti-IL13 MAb in HCCF before and after caprylic acid treatment was determined using an HPLC titer assay performed according to standard methods known in the art.

[00252] Treatment of HCCF with 1% v/v caprylic acid reduced CHOP by approximately 5-fold and resulted in a yield of anti-IL13 MAb of 91%. When Protein A pools were treated with various concentrations of caprylic acid, ranging from 0 – 3% v/v, we observed a loss in yield of > 20% at pH 5.0 and no loss in yield at pH 4.5. When we assessed total CHOP in these caprylic acid-treated Protein A pools, we found a 2-fold to 3-fold reduction of CHOP (Figs. 1A and B). However, as also shown in Figs. 1A and B, dilution-dependence was still present under each of the conditions tested indicating that caprylic acid precipitation was not effective for addressing the dilution-dependent behavior observed in the Total CHOP ELISA and would thus not be effective for reducing PLBL2 levels in this product.

Additives to HCCF

[00253] Previous work by Sisodiya et al., Biotech J. 7:1233 (2012) has demonstrated that additives such as guanidine or sodium chloride to HCCF can reduce the CHOP in the subsequently purified Protein A pools. As arginine has also been shown to reduce CHOP when utilized as a wash on Protein A columns (Millipore Technical Bulletin, Lit. No. TB1024EN00, Rev. A, December, 2005; Millipore Technical Bulletin, Lit. No. 1026EN00, July, 2006, *available at* www(dot)Millipore(dot)com), we included it as an additive to HCCF. Various salts, chaotropes, and caprylic acid were added to the anti-IL13 MAb HCCF to assess the effectiveness of each for reducing the product and CHOP interaction during capture of product on MABSELECT SURE™ (MSS) protein A chromatography. The additives to HCCF tested were: 0.6M guanidine, 0.6M arginine, 0.6M NaCl, phosphate-buffered saline, and 1% caprylic acid.

[00254] Samples that had been treated with each of the HCCF additives were subjected to Protein A chromatography on MSS. Protein A pools were adjusted to pH 4.9 and further purified on the POROS® HS cation exchange chromatography step using the Initial Process conditions. Protein A pools and POROS® HS pools were diluted and submitted to the Total

known in the art for the assessment of %aggregate, %variant species and the like.

[00255] Yields on MABSELECT SURE™ were slightly lower for the runs where guanidine or arginine was added to HCCF. Of all the additives to HCCF tested, guanidine and arginine were the most effective for reducing CHOP levels substantially (see Table 4) and appeared to reduce dilution-dependence on the Protein A pools (data not shown). Further downstream processing of the Protein A pools on POROS® HS, however, showed CHOP ELISA dilution-dependence remaining in the corresponding POROS® pools as shown in Fig. 2. Accordingly, the data demonstrate that addition of guanidine or arginine to HCCF would not be effective for reducing PLBL2 levels in this product.

Table 4. HCCF Additives and effect on CHOP.

Additive	Load pH	Yield (%)	Total CHOP (ppm)
Control (no additive)	7.4	101	3417
0.6M guanidine	7.6	90	892
0.6M arginine	7.1	88	1237
0.6M NaCl	7.7	99	2619
PBS	7.4	98	2773
1% caprylic acid	6	93	3173

Washing of Protein A Column (MABSELECT SURE™)

[00256] It was observed that the more dilution-dependent CHOP eluted in early product-containing fractions on MABSELECT SURE™ (MSS) Protein A chromatography. This suggested that an additional wash step on MSS before elution might further reduce CHOP/PLBL2. Several washes on MSS were tested for their ability to reduce CHOP/PLBL2 in the Protein A pools. For this study, purified anti-IL13 MAb UFDF pool was used as the load material. The UFDF pool was diluted to 1.7 mg/mL (approximate HCCF titer) and loaded onto MSS at 29 g/Lresin. Various washes were tested, for example; 0.5M arginine pH 8.5, 0.5M arginine pH 9.5 with and without 1% polysorbate 20, 0.5M TMAC pH 7.1, 25 mM MOPS pH 7.1, and compared with a high salt wash pH 7.0. Product was eluted under acidic conditions (pH 2.8) and pooled beginning at 0.5 OD (A280) and continuing for a total volume of 2.4 column volumes. Each adjusted pool was diluted and assayed using the Total CHOP ELISA. The summary of these results is that none of the washes adequately reduced CHOP/PLBL2 or dilution-dependence in the Total CHOP ELISA. It thus appeared unlikely we would find protein A wash conditions that would be effective for reducing PLBL2 levels in this anti-IL13 MAb product and we did not investigate these further.

[00257] Based on theoretical calculations using the amino acid sequences of anti-IL13 MAb and the PLBL2 impurity, we estimated that the pI of PLBL2 is approximately 6.0 and similar to anti-IL13 MAb (pI 6.1). We also estimated that there would be a significant difference in net charge between anti-IL13 MAb and PLBL2 at ≤ pH 4 and ≥ pH 10. As such, we tested various low pH washes on the Initial Process POROS® HS cation exchange step to assess whether these would be effective for selectively reducing total CHOP and/or PLBL2 and dilution-dependence behavior. The following washes were tested at pH 4: (i) acetate gradient, 300 mM – 1,000 mM over 20 column volumes (CV); (ii) citrate gradient, 100 mM – 500 mM over 20 CV; (iii) citrate wash step at 260 mM; and (iv) arginine gradient to 15 mS/cm (conductivity measurement) over 20 CV.

[00258] The results showed that anti-IL13 MAb and CHOP did not elute with the pH 4 acetate gradient up to the tested salt concentration of 1M. Increasing amounts of citrate or acetate resulted in product insolubility and precipitation. All of the pH 4 washes resulted in low yield on the POROS® HS step and none of the washes significantly reduced CHOP dilution-dependence. Accordingly, inclusion of a low pH wash of the cation exchange column was not effective for reducing PLBL2 levels in this product.

Hydroxyapatite Resin and CAPTO™ Adhere Resin

[00259] Ceramic hydroxyapatite (CHT) macroporous resin Type I, 40 um (BioRad) is comprised of calcium phosphate $(Ca_5(PO_4)_3OH)_2$ in repeating hexagonal structures. There are two distinct binding sites; C-sites with sets of 5 calcium ion doublets and P-sites containing pairs of –OH containing phosphate triplets. This resin has mixed mode properties and has been shown to separate challenging impurities such as aggregates (P. Gagnon, New Biotechnology 25(5):287 (2009)).

[00260] To identify initial conditions for running a CHT column, we performed high throughput robot screening of CHT resin Type I, 40 um testing a pH range of 6.5 – 8.0 and varying concentrations of sodium chloride and sodium phosphate for elution. Such high throughput robot screenings have been previously described, for example, in Wensel et al., Biotechnol. Bioeng. 100:839 (2008). Samples from these screenings were tested in the Total CHOP ELISA.

[00261] CAPTO™ Adhere (GE Healthcare) is a mixed mode resin that exhibits both ionic and hydrophobic properties. The base matrix is a rigid agarose, and the ligand is N-benzyl-N-methylethanolamine. The ability of this resin to reduce total CHOP and/or PLBL2 was assessed first with a high-throughput screening study and then with subsequent column conditions.

done using a high-throughput robot screening method similar to that described above to test binding of anti-IL13 MAb to CAPTO™ Adhere at two load densities (5 g/Lresin and 40 g/Lresin). Salt and pH ranges were also tested; from 25 mM – 200 mM sodium acetate and pH 4.0 – 6.5. The load material was the Initial Process UFDF pool that contained approximately 200 ppm of total CHOP at LOQ by the Total CHOP ELISA. Samples of the unbound (flow-through) on CAPTO™ Adhere were diluted and assayed using the Total CHOP ELISA.

[00263] The results were as follows. For CHT chromatography, none of the tested conditions substantially reduced total CHOP or PLBL2 or affected assay dilution-dependence behavior. In addition, yields were poor and no clearance of high molecular weight species was achieved. For CAPTO™ Adhere chromatography, yields were poor and the assayed material showed substantial dilution-dependence behavior in the Total CHOP ELISA. Accordingly, the use of CHT and CAPTO™ Adhere resins were not explored further as it was clear that we would be unlikely to find conditions using these resins that would be effective for reducing PLBL2 levels in this anti-IL13 MAb product.

Hydrophobic Interaction Chromatography Resins and Membranes

[00264] We initially tested HIC membrane adsorber referred to as Sartobind and manufactured by Sartorius. Sartobind is made with a base matrix of regenerated cellulose and covalently linked hydrophobic phenyl ligand groups.

[00265] The membrane tested was Sartobind HIC 3 mL device (8mm bed height). We adjusted the pool from the Initial Process POROS® HS pool to 0.55M potassium phosphate pH 7.0 and used a flow rate of 10 mL/min. Product was eluted in 0.55M potassium phosphate pH 7.0 (collected in the unbound fractions in 3 mL fractions).

[00266] We observed that the anti-IL13 MAb became hazy and turbid upon conditioning to 0.55M potassium phosphate and required an additional 0.2 um filtration step. The results showed a reduction in total CHOP, however, the remaining CHOP still demonstrated dilution dependent behavior in the Total CHOP ELISA. Use of this membrane was not evaluated further as it seemed unlikely that effective conditions would be identified for reducing PLBL2 levels in this product.

[00267] Next, we employed a high throughput screen to evaluate several different HIC resins. OCTYL-SEPHAROSE® Fast Flow (FF), BUTYL-SEPHAROSE® 4 Fast Flow, PHENYL SEPHAROSE™ 6 Fast Flow (high sub) and PHENYL SEPHAROSE™ 6 Fast Flow (low sub) were obtained from GE Healthcare. These four resins were chosen because they represent a wide range of varying hydrophobicity (OCTYL-SEPHAROSE® Fast Flow is the least

SEPHAROSE® 4 Fast Flow, with PHENYL SEPHAROSE™ 6 Fast Flow (high sub) the most hydrophobic. We tested several combinations of pH and salt concentrations for their effectiveness at reducing PLBL2 in anti-IL13 MAb preparations. The anti-IL13 MAb preparation employed for the HIC resin experiments was a UFDF pool from a run using the Initial Process. The anti-IL13 MAb concentration was 180 mg/mL and the load density was 40 mg antibody/mL resin. We tested pH 5.5 (25 mM sodium acetate), pH 6.0 (25 mM MES), pH 7.0 (25 mM MOPS), and pH 8.0 (25 mM Tris) and sodium sulfate concentrations between 0 mM and 400 mM. For each condition tested, flow-through samples were collected, diluted and tested using the Total CHOP ELISA assay.

[00268] The results are shown in Figs. 3A-D. With increasing salt, we observed less total CHOP in the flow-through for each resin. The OCTYL-SEPHAROSE® Fast Flow resin (Fig. 3A) showed the highest level of total CHOP while the PHENYL SEPHAROSE™ 6 Fast Flow (high sub) resin reduced total CHOP to very low levels, even with lower amounts of salt (Fig. 3D) and the PHENYL SEPHAROSE™ 6 Fast Flow (low sub) and BUTYL-SEPHAROSE® Fast Flow resins showed intermediate levels of total CHOP. Interestingly, there was also minimal effect of pH on CHOP removal using each of the resins except for PHENYL SEPHAROSE™ 6 Fast Flow (high sub) in low salt conditions (Fig. 3D). For this resin, at low salt conditions, higher pH resulted in higher CHOP in the flow-through fraction (Fig. 3D). Based on these results, PHENYL SEPHAROSE™ 6 Fast Flow (high sub) appeared promising and was chosen for further studies which included running the column in either bind-elute or flow-through mode.

[00269] Operation of HIC using the PHENYL SEPHAROSE™ 6 Fast Flow (high sub) resin in the bind-elute mode required conditioning of the anti-IL13 MAb load with salt to enable binding of the antibody to the resin. Increasing salt increased the dynamic binding capacity (mg of anti-IL13/mL resin) for loading product to the resin. But with increasing salt concentration in the product, we observed increased turbidity and formation of high molecular weight species (HMWs), in particular in combination with lower pH.

[00270] As mentioned above, PHENYL SEPHAROSE™ 6 Fast Flow (high sub) may also be operated in flow-through mode and such operation would require less salt conditioning of the load. From a product quality and product stability viewpoint, for example, product with less turbidity and less HMWs, less salt conditioning would be desirable. Accordingly, we proceeded with process optimization using PHENYL SEPHAROSE™ 6 Fast Flow (high sub) resin in flow-through mode.

column including load concentration, load pH, load salt molarity, load density on the resin, bed height, flowrate, temperature, equilibration buffer pH and molarity. For these experiments, we monitored total CHOP using the Total CHOP ELISA and also PLBL2 by LC-MS/MS. Certain exemplary data is shown in Table 5. The data in Table 5 shows that the HIC column run under the indicated conditions in flow-through mode was effective for substantially reducing PLBL2 levels from the high levels detected in the Protein A pool. The PLBL2 levels after HIC were reduced by several hundred fold compared to the levels in the Protein A pool.

Table 5. Total CHOP and PLBL2 Levels under Varying HIC Column Conditions.

Sample (bed height, flow rate)	% Yield	Total CHOP (ppm by ELISA at LOQ)	PLBL2 (ppm by LC-MS/MS)
Protein A pool (Load for HIC Column)		3324	957
15 cm, 150 cm/hr	88	43	4
25 cm, 150 cm/hr	92	44	2
15 cm, 100 cm/hr	90	67	5
25 cm, 100 cm/hr	92	63	3
15 cm, 200 cm/hr	93	62	6
25 cm, 200 cm/hr	90	72	4
15 cm, 150 cm/hr	54	76	2

[00272] Using the PLBL2 LC-MS/MS assay and other typical product quality assays (e.g., SE-HPLC, CE-SDS, iCIEF) to guide process parameter selections, we identified the following conditions as desirable for running of the HIC column as assessed by product quality attributes and reduction of PLBL2: equilibration and wash buffer: 50 mM sodium acetate, pH 5.0; target load density: 100 g/L, flow rate: 150 cm/hr, 22°C ± 3°C. Certain small variations of these conditions may also be desirable, for example, 25°C ± 3°C or 27°C ± 3°C. Optical density (OD) was monitored by absorbance at 280 nm (A280) and the pool (i.e. the flow-through) was collected between 0.5 OD to 1.5 OD or after 8 column volumes of wash.

[00273] As mentioned above, the Initial Process was: Protein A affinity chromatography (MABSELECT SURE™) followed by cation exchange (POROS® HS) followed by anion exchange (Q SEPHAROSE™ Fast Flow). After developing processes to reduce PLBL2 levels as described above, we next sought to implement process changes in a convenient manner. Accordingly, we explored adding the HIC column to the Initial Process thereby creating a four-column process as well as substituting the HIC column for either the CEX column or the AEX column and finally we explored the order of the columns. We found that a three column process, Protein A affinity chromatography (MABSELECT SURE™), followed by anion

(PHENYL SEPHAROSE™ 6 Fast Flow (high sub)) provided the most convenient process and was the most effective for reducing PLBL2 in the final drug substance. This three-column process is described in detail below.

[00274] The first affinity chromatography step was a bind-and-elute process using MABSELECT SURE™ resin. After column equilibration (25 mM sodium chloride, 25 mM Tris pH 7.7), the HCCF was loaded on the column and washed with the equilibration buffer and a high salt pH 7.0 wash buffer. Anti-IL13 MAb was eluted from the column under acidic conditions (pH 2.8).

[00275] The second anion-exchange chromatography step was operated in a bind-and-elute mode using Q SEPHAROSE™ Fast Flow (QSFF) resin. After column equilibration (50 mM Tris, pH 8.0), the anti-IL13 pool from the MABSELECT SURE™ column was adjusted to pH 8.05 and loaded onto the column. The column was washed (50 mM Tris, pH 8.0) and anti-IL13 MAb eluted from the column with 85 mM sodium chloride, 50 mM Tris pH 8.0.

[00276] The third and final hydrophobic interaction chromatography step was operated in a flow-through mode using PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin. After column equilibration (50 mM sodium acetate pH 5.0), the anti-IL13 pool from the QSFF column was adjusted to pH 5.0 and loaded on the column. The anti-IL13 MAb flowed through and the column was also washed with equilibration buffer (50 mM sodium acetate pH 5.0). The anti-IL13 MAb pool was initiated and terminated based on A280 with pooling occurring between 0.5 to 1.5 OD or a maximum of 8 column volumes.

[00277] As with the Initial Process, additional virus inactivation and filtration steps were included and a final ultrafiltration-diafiltration (UFDF) step. The final product (drug substance) was formulated at a concentration of 125 mg/mL in 20 mM histidine acetate, 6% sucrose, 0.03% polysorbate 20, pH 5.7.

[00278] A comparison of the Initial Process to the Improved Process with respect to total CHOP and PLBL2, as measured by the Total CHOP ELISA and the monoclonal PLBL2 ELISA, respectively, is provided in Tables 6 (Initial Process) and 7 (Improved Process). The data in Table 6 clearly shows that the Initial Process resulted in purified product (UFDF pool) containing high levels of total CHOP (179, 310, and 189 ng/mg in three different runs) and high levels of PLBL2 (242, 328, and 273 ng/mg in three different runs) while the data in Table 7 clearly shows that the Improved Process was quite effective for producing purified product with substantially reduced levels of total CHOP (1.1, < 0.9, 2.8, and 3.4 ng/mg in four different runs) and substantially reduced levels of PLBL2 (0.21, 0.42, 0.35, and 0.24 ng/mg in four different

run under the conditions described above was particularly effective for reducing total CHOP and PLBL2 levels in anti-IL13 MAb preparations.

Table 6. Total CHOP and PLBL2 Levels at Various Stages of Purification of Anti-IL13 MAb Using the Initial Process.

In-process sample	Total CHOP (ng/mg at LOQ by ELISA)			PLBL2 (ng/mg by ELISA)		
Run No.	1	2	3	1	2	3
HCCF	620920	541072	608789	1895	3669	2535
ProA Pool	2892	2855	3505	587	769	478
CEX Pool	136	310	138	345	439	287
AEX Pool	104	163	93	291	304	261
UFDF Pool	179	310	189	242	328	273

Table 7. Total CHOP and PLBL2 Levels at Various Stages of Purification of Anti-IL13 MAb Using the Improved Process.

In-process sample	Total CHOP (ng/mg at LOQ by ELISA)				PLBL2 (ng/mg by ELISA)			
Run No.	1	2	3	4	1	2	3	4
HCCF	332132	399157	540134	644549	4084	3770	3077	2986
ProA Pool	2318	2768	3552	3797	1354	1995	1027	975
AEX Pool	495	653	414	377	723	933	677	616
HIC Pool	< 2.1	< 1.9	5.0	7.7	< 0.6	< 0.6	< 0.6	< 0.6
UFDF Pool	1.1	< 0.9	2.8	3.4	0.21	0.42	0.35	0.24

[00279] In summary, faced with the problem of assay non-linear dilution behavior attributable to high levels of a single CHOP species in purified anti-IL13 MAb preparations, we first identified the CHOP species as hamster PLBL2, an impurity which has not been previously described in recombinant protein preparations produced from CHO cells. We next identified purification conditions to effectively reduce the levels of PLBL2 in the anti-IL13 MAb preparations. Finally, we integrated these purification conditions into the overall purification process resulting in an improvement to the prior anti-IL13 MAb purification process. This Improved Process employs a HIC column run in flow-through mode to reduce PLBL2 levels, which is run in combination with an affinity chromatography step and an anion exchange chromatography step. We showed that the Improved Process is robust and effective for substantially reducing hamster PLBL2 levels in anti-IL13 MAb preparations. We showed that the Improved Process reproducibly reduced PLBL2 levels by approximately 1000 fold compared to the Initial Process. Such reduction in PLBL2 levels was important for producing a purified

beyond.

Purification Process to Reduce Hamster PLBL2 in Anti-Abeta Antibody Preparations

[00280] We next sought to assess whether the purification methods described above, particularly use of a HIC column for a final chromatography step, would similarly be effective for reducing PLBL2 levels in other antibody preparations. For this experiment, we chose an anti-Abeta antibody, which was produced in CHO cells. Exemplary anti-Abeta antibodies and methods of producing such antibodies have been described previously, for example, in WO2008011348, WO2007068429, WO2001062801, and WO2004071408. These particular experiments used the anti-Abeta antibody known as crenezumab. As described for the anti-IL13 MAb, we explored various resins and buffers for the second column after the Protein A affinity column and we explored various buffers and run conditions for the HIC column to identify those that were optimal for anti-Abeta for product quality and stability attributes as well as for removal of hamster PLBL2.

[00281] We found that a three column process, Protein A affinity chromatography (MABSELECT SURE™), followed by use of a mixed mode resin (CAPTO™ Adhere), followed by HIC operated flow-through mode (PHENYL SEPHAROSE™ 6 Fast Flow (high sub)) was convenient and effective for reducing PLBL2 in the final drug substance. This three-column process is described in detail below.

[00282] The first affinity chromatography step was a bind-and-elute process using MABSELECT SURE™ resin similar to that described above for the anti-IL13 MAb.

[00283] The second mixed mode chromatography step was operated in a flow-through mode using CAPTO™ Adhere resin. After column equilibration (20 mM MES, 150 mM sodium acetate, pH 6.25), the anti-Abeta pool from the MABSELECT SURE™ column was adjusted to pH 6.25 and loaded onto the column. Pooling began at 0.5 OD during the load phase. After completing the load, the column was washed with 5 column volumes (CVs) of equilibration buffer (20 mM MES, 150 mM sodium acetate, pH 6.25) and the entire 5 CVs were also collected.

[00284] The third and final hydrophobic interaction chromatography step was operated in a flow-through mode using PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin. After column equilibration (150 mM sodium acetate pH 5.0), the anti-Abeta pool from the CAPTO™ Adhere column was adjusted to pH 5.0 and loaded on the column. The anti-Abeta MAb flowed through and the column was also washed with equilibration buffer (150 mM sodium acetate pH 5.0). The anti-Abeta MAb pool was initiated during the load phase based on A280 with pooling

sodium acetate pH 5.0) and the entire 10 CVs were also collected. As with the anti-IL13 MAb, additional virus inactivation and filtration steps were included and a final ultrafiltration-diafiltration (UFDF) step.

[00285] The results of using the above process during four different purification runs are shown in Table 8 below.

Table 8. PLBL2 Levels at Various Stages of Purification of Anti-Abeta MAb Using HIC.

In-process sample	PLBL2 (ng/mg by ELISA)			
Run No.	1	2	3	4
HCCF	622	564	1264	553
CpA Pool	7	8	9	2.5
HIC Pool (300 g/L Load density)	0.7	0.6	0.3	0.3
HIC Pool (100 g/L Load density)	< 0.2	< 0.2	< 0.2	Not tested

[00286] The results shown in Table 8 demonstrate that use of a HIC resin as a final chromatography step effectively reduced residual PLBL2 levels in the anti-Abeta MAb preparation to an amount similar to that seen for the anti-IL13 MAb. While a load density of 300 g/L produced desirable results from the viewpoint of both product recovery and reduction in PLBL2, further reduction in residual PLBL2 was seen by reducing the load density for the HIC column from 300 g/L to 100 g/L.

[00287] We also investigated two other conditions for the HIC chromatography step, load pH and load sulfate molarity. For these experiments, we started with a CAPTO^{TM} Adhere pool containing 51 ng/mg PLBL2 (as measured by ELISA), 15 mM sodium acetate pH 5.5. We adjusted the load pH and the load sulfate molarity to the values shown in Table 9 below using 0 mM sodium sulfate or 800 mM sodium sulfate stock solutions at varying pH. We tested each load pH indicated in Table 9 under low sulfate molarity conditions (0 mM) and high sulfate molarity conditions (240 mM). Each condition was tested at a load density of 60 g/L. As shown by the results presented in Table 9, decreasing the load pH to pH 4 or pH 5 or increasing the load sulfate molarity (to 240 mM sulfate) were each effective for reducing the levels of PLBL2 in the final HIC pool. The combination of pH 4.0 and 240 mM sulfate in the load was particularly effective for minimizing the amount of residual PLBL2 in the HIC pool.

Load pH	PLBL2 (ng/mg by ELISA)	
	Low Sulfate Molarity (0 mM)	High Sulfate Molarity (240 mM)
4	4	1
5	10	3
6	27	5
7	64	6

[00288] Accordingly, use of a HIC resin as a final chromatography step in the purification of CHO-produced polypeptides, such as the anti-IL13 MAb and the anti-Abeta MAb described herein, effectively reduced the residual amount of hamster PLBL2 to very low levels, e.g., 1 ng/mg or less in the HIC pool.

Purification Process to Reduce Hamster PLBL2 in IgG1 Antibody Preparations

[00289] Next, we assessed whether the purification methods described for the anti-IL13 and anti-Abeta IgG4 antibody preparations, particularly use of a HIC column for a final chromatography step, would similarly be effective for reducing PLBL2 levels in IgG1 antibody preparations. For these experiments, we first chose an anti-IL17 A/F antibody, which is an IgG1 antibody and which was produced in CHO cells. Exemplary anti-IL17 A/F antibodies and methods of producing such antibodies have been described previously, for example, in WO 2009136286 and U.S. Patent No. 8,715,669. As described for the anti-IL13 and anti-Abeta MAbs, we explored various resin (in particular, PHENYL SEPHAROSE™ FF [low sub] and PHENYL SEPHAROSE™ FF [high sub] and buffer conditions (in particular, 50 mM sodium acetate, pH 5.5 and 50 mM Tris, 85 mM sodium acetate, pH 8.0) for the HIC column to identify those that were optimal for anti-IL17 A/F for product quality and stability attributes as well as for removal of hamster PLBL2.

[00290] We found that a three column process, Protein A affinity chromatography (MABSELECT SURE™), followed by cation exchange chromatography (POROS® 50HS) operated in bind-and-elute mode, and HIC (PHENYL SEPHAROSE™ 6 Fast Flow (high sub)) operated in flow-through mode was convenient and effective for reducing PLBL2 in the final drug substance. This three-column process is described in detail below.

[00291] The first affinity chromatography step was a bind-and-elute process using MABSELECT SURE™ resin similar to that described above for the anti-IL13 and anti-Abeta MAbs. The second cation exchange chromatography step used POROS® 50HS resin and was operated in bind-and-elute mode. After column equilibration (40 mM sodium acetate, pH 5.5), the pH-adjusted anti-IL17 A/F MABSELECT SURE™ pool (pH 5.0) was loaded onto the

antibody was eluted from the column with a conductivity gradient created with 40 and 400 mM sodium acetate, pH 5.5. Pooling was based on A280 and was initiated at ≥ 0.5 OD and ended at ≤ 2.0 OD during the gradient elution phase.

[00292] The third and final hydrophobic interaction chromatography step used PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin and was operated in a flow-through mode. After column equilibration (50 mM sodium acetate pH 5.5), the anti-IL17 A/F pool from the POROS® 50HS column was loaded directly on the column without pH adjustment. The anti-IL17 A/F MAb flowed through. Anti-IL17 A/F MAb pooling was based on A280 and was initiated during the load phase at ≥ 0.5 OD. The column was washed with 10 CVs of equilibration buffer (50 mM sodium acetate, pH 5.5) and pooling ended during this wash phase at ≤ 1.0 OD.

[00293] The results of using the above process during one purification run are shown in Table 10 below.

Table 10. PLBL2 Levels at Various Stages of Purification of Anti-IL17 A/F MAb Using HIC.

In-Process Sample	PLBL2 (ng/mg by ELISA)
Run No.	1
HCCF	713
MABSELECT SURE™ Pool	151
POROS® 50HS Pool	47
HIC Pool (100 g/L load density)	< 0.7

[00294] The results shown in Table 10 demonstrate that use of a HIC resin as a final chromatography step effectively reduced residual PLBL2 levels in the anti-IL17 A/F MAb (IgG1) preparation to an amount similar to that seen for the anti-IL13 and anti-ABeta MAbs (IgG4).

Anti-CMV Antibody

[00295] In addition to testing anti-IL17 A/F, we tested another IgG1 MAb, anti-CMV-MSL antibody, which is also produced in CHO cells. Exemplary anti-CMV antibodies, including anti-CMV-MSL, and methods of producing such antibodies have been described previously, for example, in WO 2012047732.

(MABSELECT SURE™), followed by cation exchange chromatography (POROS® 50HS) operated in bind-and-elute mode, and HIC (PHENYL SEPHAROSE™ 6 Fast Flow (high sub)) operated in flow-through mode was convenient and effective for reducing PLBL2 in the final drug substance. This three-column process is described in detail below.

[00297] The first affinity chromatography step was a bind-and-elute process using MABSELECT SURE™ resin similar to that described above for the anti-IL13, anti-Abeta and anti-IL17 A/F MAbs. The second cation exchange chromatography step that used POROS® 50HS resin and was operated in bind-and-elute mode. After column equilibration (40 mM sodium acetate, pH 5.5), the pH-adjusted aCMV-MSL MABSELECT SURE™ pool (pH 5.0) was loaded onto the column. The column was washed (40 mM sodium acetate, pH 5.5), and then the aCMV-MSL antibody was eluted from the column with a conductivity gradient created with 40 and 400 mM sodium acetate, pH 5.5. Pooling was based on A280 and was initiated at ≥ 0.5 OD and ended at ≤ 1.0 OD during the gradient elution phase.

[00298] In this particular run, a viral filtration step was performed in between the cation exchange and hydrophobic interaction chromatography steps using Viresolve Pro as the virus filter and Fluorodyne UEDF filter as the pre-filter.

[00299] The third and final hydrophobic interaction chromatography step used PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin and was operated in a flow-through mode. After column equilibration (50 mM sodium acetate pH 5.5), the anti-CMV-MSL pool from the POROS® 50HS column was loaded directly on the column without pH adjustment. The anti-CMV-MSL MAb flowed through. Anti-CMV-MSL MAb pooling was based on A280 and was initiated during the load phase at ≥ 0.5 OD. The column was washed with 10 CVs of equilibration buffer (50 mM sodium acetate, pH 5.5) and pooling ended during this wash phase at ≤ 0.5 OD.

[00300] The results of using the above process during one purification run is shown in Table 11 below.

Table 11. PLBL2 Levels at Various Stages of Purification of Anti-CMV-MSL MAb Using HIC.

In-Process Sample	PLBL2 (ng/mg by ELISA)
Run No.	1
HCCF	2608
MABSELECT	319

POROS® 50HS Pool	33
Viresolve Pro Pool	32
HIC Pool (60 g/L load density)	< 0.6

[00301] The results shown in Table 11 demonstrate that use of a HIC resin as a final chromatography step effectively reduced residual PLBL2 levels in the anti-CMV-MSL MAb preparation to an amount similar to that seen for the anti-IL13, anti-ABeta, and anti-IL17 A/F MAbs. Accordingly, use of a HIC resin as a final chromatography step in the purification of CHO-produced polypeptides, such as the anti-IL13 MAb and other MAbs described herein, effectively reduced the residual amount of hamster PLBL2 to very low levels, e.g., less than 1 ng/mg in the HIC pool. Thus, we showed that use of the HIC chromatography step as described herein for reducing PLBL2 levels was as effective for IgG1 MAbs as for IgG4 MAbs, illustrating the general applicability of this method for reducing hamster PLBL2 levels in recombinant polypeptide preparations.

EXAMPLE 3 – Assessment of Human Anti-Hamster PLBL2 Response in Patients Administered Anti-IL13 MAb Compositions Containing Varying Amounts of Hamster PLBL2

[00302] To assess the potential impact of the CHO PLBL2 impurity, we developed an ELISA assay (a bridging ELISA assay) to detect antibodies to hamster PLBL2 in human subjects who had received the anti-IL13 MAb, lebrikizumab. Serum samples from patients who participated in various clinical studies of lebrikizumab were analyzed for evidence of anti-hamster PLBL2 antibodies pre-dose and post-dose as well as in subjects who received placebo. The details of the clinical studies have been described previously (WO 2012/083132, Corren et al., N Engl J Med 365:1088-98 (2011)) and only the most relevant details of these studies are provided below.

[00303] The antibody bridging ELISA assay that was developed and validated to detect antibodies to hamster PLBL2 in human serum used two conjugated reagents to capture all isotypes of antibodies directed against hamster PLBL2: purified hamster PLBL2 conjugated to biotin (Biotin-PLBL2) and purified hamster PLBL2 conjugated to digoxigenin (DIG-PLBL2). Production and purification of hamster PLBL2 was carried out using standard methods known to one skilled in the art is also described in U.S. Provisional Application Nos. 61/877,503 and 61/991,228 and conjugation to biotin or DIG were carried out using standard methods known to one skilled in the art. In this semi-homogenous antibody bridging ELISA assay, 75 µL/well of conjugated solution in assay diluent (PBS/0.5% BSA/0.05% Polysorbate 20/0.05% ProClin 300,

overnight (16 – 24 hours) at ambient temperature with 75 μL/well of 1:20 diluted serum samples and controls in assay diluent in polypropylene micronic tubes (National Scientific Supply Co.; Claremont, CA). After incubation, 100 μL/well of mixture from the micronic tubes were transferred to a streptavidin-coated 96-well microplate (StreptaWell™ High Bind; Roche Diagnostics; Indianapolis, IN) that was washed 3 times with 400 μL/well of wash buffer (PBS/0.05% Polysorbate 20) in an automatic plate washer (BioTek ELx405) and incubated at ambient temperature for 2 hours ± 10 minutes. The plate was washed 4 times with 400 μL/well of wash buffer in the plate washer, Subsequently, 100 μL/well of 400 ng/mL mouse anti-digoxin antibody conjugated with horseradish peroxidase (HRP) (Jackson ImmunoResearch Cat.200-032-156) was added and incubated at ambient temperature for 2 hours ± 10 minutes for detection. After the plate was washed 4 times with 400 μL/well of wash buffer in the plate washer, 100 μL/well of equal mixture solution of peroxidase substrate (tetramethyl benzidine) (0.4 g/L TMB) and Peroxidase Solution B (0.02% hydrogen peroxide) (KPL Cat. 50-76-03) was added and incubated at ambient temperature for 18 – 28 minutes for color development and the reaction was stopped by adding 100 μL/well of 1 M phosphoric acid. The plates were read at 450 nm for detection absorbance and 630 nm for reference absorbance. The positive control for the assay was a monoclonal antibody construct consisting of a murine anti–hamster PLBL2-specific complementarity determining region (CDR) on a human IgG1 framework. The relative sensitivity of the assay using this antibody was determined to be 25 ng/mL. Assay drug tolerance experiments using this antibody demonstrated that up to 50 μg/mL of lebrikizumab or 1 μg/mL of hamster PLBL2 in serum did not cause interference or cross-reactivity in the assay.

[00304] To carry out the assay, serum samples were first screened in the assay at a minimum dilution of 1/20. Samples that screened positive were then confirmed for hamster PLBL2 specificity using a competition confirmatory assay. If the sample was confirmed as positive, it was serially diluted to obtain a titer value. Positive responses were reported in titer units, which is the log10 of the dilution factor at which the sample signal was equal to the signal of the assay cutpoint (threshold for determining positivity).

[00305] The four clinical studies in which patient samples were analyzed using the anti-hamster PLBL2 ELISA described above are briefly described as follows. Study 1 was a Phase II randomized, double-blind, placebo-controlled, proof-of-concept study to evaluate the effects of lebrikizumab in patients with asthma whose disease was inadequately controlled during chronic therapy with inhaled corticosteriods (ICS). A total of 219 patients were randomized, with 106

monthly doses.

[00306] Study 2 was a Phase II randomized, double-blind, placebo-controlled, dose-ranging study in patients with asthma who were not on ICS therapy. Patients received one of three doses (500, 250, or 125 mg) of lebrikizumab or placebo via SC administration. Study drug was administered four times during the 12-week treatment period. A total of 158 patients were exposed to at least one dose of lebrikizumab, and 145 patients received all four doses.

[00307] Study 3 was a Phase I PK study of lebrikizumab in healthy Japanese and Caucasian volunteers. Three discrete cohorts of 20 healthy Japanese and Caucasian subjects (10 subjects in each racial group) were randomized between lebrikizumab (125, 250, and 375 mg SC) and placebo in a 7:3 ratio. Subjects were dosed once on Day 1and were subsequently monitored for 120 days. A total of 42 subjects each received one dose of lebrikizumab.

[00308] In Studies 1-3, a total of 306 subjects, 264 of which were asthma patients, each received at least one dose of material containing hamster PLBL2. Exposure to hamster PLBL2 was variable, depending on the dose of lebrikizumab received.

[00309] Study 4 was a Phase IIb randomized, double-blind, placebo-controlled studies to assess the efficacy and safety of lebrikizumab in patients with uncontrolled asthma who were using ICS and a second controller medication. Patients received one of three doses (250, 125, or 37.5 mg) of lebrikizumab or placebo via SC administration monthly. In Study 4, a total of 463 patients were randomized, with 347 receiving at least one dose of lebrikizumab. Exposure to hamster PLBL2 was variable, depending on the dose of lebrikizumab received.

[00310] Table 12 below provides a summary of each of the Studies 1-4 showing the range of hamster PLBL2 levels the subjects were exposed to and the dose of lebrikizumab.

Table 12. Hamster PLBL2 Exposure in Lebrikizumab Clinical Trials.

Study	Drug Substance PLBL2 (ng/mg)	Lebrikizumab Dose (mg/month)	PLBL2 (µg/dose)
1	34-137[a]	250	9-34
2	34-137[a]	125	4-17
		250	9-34
		500	17-69
3	34	125	4
		250	9
		375	13
4	242	37.5	9
	328	125	41
	328	250	82

[a]Range from four different lots of clinical material.

anti-hamster PLBL2 antibody assay described above to detect antibodies to hamster PLBL2. Samples from both placebo and dosed subjects were analyzed to determine the level of pre-existing response as well as the development of antibodies in response to lebrikizumab dosing. There were 113 placebo subjects and 106 dosed subjects who received at least one dose of lebrikizumab. Timepoints selected for analysis were Days 0, 29, 85, 141, 225, and early termination. Samples were taken prior to the next dose; therefore, Day 29 samples were taken prior to the administration of the second dose. The percentage of anti–hamster PLBL2 antibody-positive subjects at each timepoint was calculated by taking the number of positive subjects at each timepoint and dividing by the total number of subjects tested at each timepoint. The data is shown in Table 13.

Table 13. Anti-Hamster PLBL2 Antibody Results for Study 1.

	% Positive at Each Timepoint (no. positive subjects/total no. subjects evaluable)					
Study Day:	0	29	85	141	225	Early Termination
Placebo	6 (7/110)	7 (8/107)	9 (9/104)	8 (8/99)	5 (5/97)	25 (2/8)
250 mg dose	5 (5/102)	6 (6/100)	89 (90/101)	98 (92/94)	98 (91/93)	100 (8/8)[a]

[a] Of the 8 lebrikizumab subjects who discontinued study drug early, only 3 reported adverse events as the reason for study drug discontinuation.

[00312] The 6 Study 1 placebo subjects who were positive pre-dose on Day 0 continued to be positive throughout the study. Samples from these subjects were confirmed as positive in a confirmatory competition assay and had titers on Day 0 that ranged from 1.6 to 2.9 titer units. Titers obtained on subsequent visits were similar to those obtained on Day 0. A few additional placebo subjects had low-level positive responses during the Study.

[00313] Among the Study 1 subjects that received lebrikizumab, 98% (104/106) had a positive antibody response after dosing and remained positive through the end of the study, with most subjects becoming positive after receiving at least two doses of lebrikizumab. Titers after dosing ranged from 1.35 to 4.76 titer units, with titers generally increasing over time. The clinical significance of the development of anti–hamster PLBL2 antibodies is not known. No clinically important safety signals were identified in this study and, given the high incidence of antibodies to hamster PLBL2, no correlation with safety events could be made.

both placebo and dosed subjects were analyzed to determine the level of pre-existing response as well as the development of anti-hamster PLBL2 antibodies in response to lebrikizumab dosing. There were 116 placebo subjects and 347 dosed subjects who received at least one dose of lebrikizumab. Samples from 92 placebo subjects and 268 dosed subjects are represented in this data set. The results are shown in Table 14.

Table 14. Anti-Hamster PLBL2 Antibody Results for Study 4 for Subjects not Previously Exposed to Lebrikizumab.

	% Positive at Each Timepoint (no. positive subjects/total no. subjects evaluable)					
Study Day:	0	29	85	169	253	Early Termination
Placebo	4 (4/89)	4 (3/78)	4 (2/48)	0 (0/13)	NA	0 (0/5)
37.5 mg dose	9 (8/88)	9 (7/82)	55 (35/64)	79 (27/34)	66 (2/3)	43 (3/7)[a]
125 mg dose	4 (3/81)	11 (8/73)	87 (48/55)	100 (9/9)	NA	0 (0/2)[a]
250 mg dose	5 (4/88)	10 (7/72)	96 (49/51)	100 (13/13)	NA	67 (2/3)[a]

[a] Of the 12 lebrikizumab subjects who discontinued study drug early, only 4 reported adverse events as the reason for study drug discontinuation.

[00315] The four Study 4 placebo subjects that were positive pre-dose on Day 0 had low-level positive responses that were just above the detection limit of the assay. The low-level responses were detectable at some, but not all, subsequent timepoints.

[00316] The 15 Study 4 subjects receiving lebrikizumab that were positive pre-dose on Day 0 continued to be positive at subsequent timepoints, with increasing titers after multiple doses. In addition, there were 10 subjects in Study 4 who previously received lebrikizumab in Study 1. Nine of these subjects were subsequently re-dosed with lebrikizumab in Study 4 while 1 subject received placebo. All 10 subjects were pre-dose positive on Day 0 for Study 4 and continued to be positive at subsequent timepoints. The data from these 10 subjects were excluded from Table 14 due to their previous lebrikizumab exposure.

[00317] Among the Study 4 subjects receiving lebrikizumab, there appear to be differences in positivity rates between dose groups. However, as these data are incomplete, conclusions regarding the significance of these differences cannot be made at this time. Similar to the data from Study 1, the majority of subjects become positive after receiving at least two doses of lebrikizumab. Titers after dosing ranged from 1.68 to 4.55 titer units, with titers generally

may change as additional data are accumulated.

[00318] An interim safety assessment of Study 4 showed a safety profile similar those of the earlier completed studies with no clinically significant safety signals, including no reports of anaphylaxis or serious hypersensitivity reactions. Of note, 6 of the 9 patients who received lebrikizumab in Study 1 and were subsequently re-dosed with lebrikizumab in Study 4 had not reported any adverse events at the time of the interim analysis and only 1 patient reported any local injection-site reactions. No clinical sequelae of this anti-hamster PLBL2 antibody response have been identified in the clinical trials to date.

[00319] We also performed an assessment on the 125-mg dose group from Study 2 and those results are shown in Table 15.

Table 15. Anti-Hamster PLBL2 Antibody Results for Study 2.

	% Positive at Each Timepoint (no. positive subjects/total no. subjects evaluable)					
Study Day:	0	29	57	85	141	Early Termination
125 mg dose	4 (2/51)	21 (11/53)	70 (35/50)	88 (45/51)	86 (43/50)	100 (2/2)[a]

[a] Of the 2 subjects who discontinued study drug early, neither reported adverse events as the reason for study drug discontinuation.

[00320] The two Study 2 subjects that were positive pre-dose on Day 0 continued to be positive at all subsequent timepoints, with increasing titers after multiple doses. Among the Study 2 subjects that received 125 mg of lebrikizumab, 87% (46/53) had a positive antibody response after dosing and remained positive through the end of the study, with most subjects becoming positive after receiving at least two doses of lebrikizumab. Titers after dosing ranged from 1.51 to 4.09 titer units, with titers generally increasing over time.

Conclusions

[00321] To assess the potential impact of the CHO PLBL2 impurity, an assay was developed to detect antibodies to hamster PLBL2 in subjects who had received lebrikizumab preparations that contained significant levels of hamster PLBL2. On the basis of the completed data sets from Study 1 and the 125 mg dose group of Study 2 and on the partial data set from Study 4, the presence of hamster PLBL2 in lebrikizumab preparations produced immune responses in most subjects exposed to hamster PLBL2.

[00322] A number of subjects in both the placebo and lebrikizumab dose groups had pre-existing immunoreactivity in the anti–hamster PLBL2 antibody assay. The cause of this pre-

characterized and confirmed in normal human serum samples with no known prior exposure to CHO-derived biological products (Xue et al., The AAPS Journal 12(1):98–106 (2010)). However, there are no published data specific to the single species of CHOP, PLBL2.

[00323] For subjects with pre-existing immunoreactivity in the anti–hamster PLBL2 antibody assay at the start of the study, there was a sustained rise in antibody titers after repeat administration with lebrikizumab. For subjects that were antibody negative at the start of the study, the majority of subjects across all four studies became positive after at least two administrations of lebrikizumab and remained positive through all subsequent timepoints.

[00324] The clinical significance of the development of anti–hamster PLBL2 antibodies is not known . Although there was a high incidence of antibodies to hamster PLBL2 in the study subjects, no correlation between safety events could be made. Importantly, there were no safety signals identified in these completed or interim studies and in particular, no reported events of anaphylaxis, anaphylactoid, or serious hypersensitivity reactions. Nevertheless, there remains a concern that long term exposure with repeat dosing could increase the potential for undesirable effects such as anaphylaxis, hypersensitivity, and immune complex deposition, particularly in asthma patient populations and other allergic or hypersensitive patient populations. Accordingly, it is important to dose patients in late stage clinical studies and beyond, where there may be such repeat dosing over a long period of time, with anti-IL13 MAb (e.g., lebrikizumab) preparations containing substantially reduced levels of hamster PLBL2 so as to minimize immunogenicity as much as possible.

Table 16. Anti-IL17 A/F antibody amino acid sequences (SEQ ID NOS.: 15-22) and anti-Abeta antibody amino acid sequences (SEQ ID NOS.: 23-30).

CDR-H1 (SEQ ID NO.:15)	Asp Tyr Ala Met His
CDR-H2 (SEQ ID NO.:16)	Gly Ile Asn Trp Ser Ser Gly Gly Ile Gly Tyr Ala Asp Ser Val Lys Gly
CDR-H3 (SEQ ID NO.:17)	Asp Ile Gly Gly Phe Gly Glu Phe Tyr Trp Asn Phe Gly Leu
CDR-L1 (SEQ ID NO.:18)	Arg Ala Ser Gln Ser Val Arg Ser Tyr Leu Ala
CDR-L2 (SEQ ID NO.:19)	Asp Ala Ser Asn Arg Ala Thr
CDR-L3 (SEQ ID NO.:20)	Gln Gln Arg Ser Asn Trp Pro Pro Ala Thr
VH (SEQ ID NO.:21)	Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Gly Ile Asn Trp Ser Ser Gly Gly Ile Gly Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg Asp Ile Gly Gly Phe Gly Glu Phe Tyr Trp Asn Phe Gly Leu Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser
VL (SEQ ID NO.:22)	Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Arg Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Pro Ala Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
CDR-H1 (SEQ ID NO.:23)	GFTFSSYGMS
CDR-H2 SEQ ID NO.:24)	SINSNGGSTY YPDSVK
CDR-H3 SEQ ID NO.: 25)	GDY
CDR-L1 (SEQ ID NO.:26)	RSSQSLVYSN GDTYLH

(SEQ ID NO.: 27)	
CDR-L3 (SEQ ID NO.: 28)	SQSTHVPWT
VH (SEQ ID NO.: 29)	EVQLVESGGG LVQPGGSLRL SCAASGFTFS SYGMSWVRQA PGKGLELVAS INSNGGSTYY PDSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCASGD YWGQGTTVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT KTYTCNVDHK PSNTKVDKRV ESKYGPPCPP CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQ KSLSLSLG
VL (SEQ ID NO.: 30)	DIVMTQSPLS LPVTPGEPAS ISCRSSQSLV YSNGDTYLHW YLQKPGQSPQ LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCSQSTHVP WTFGQGTKVE IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK VQWKVDNALQ SGNSQESVTE QDSKDSTYSL SSTLTLSKAD YEKHKVYACE VTHQGLSSPV TKSFNRGEC

1. A composition comprising an anti-IL13 monoclonal antibody purified from Chinese hamster ovary host cells, wherein the composition comprises the anti-IL13 antibody and a residual amount of hamster PLBL2, wherein the amount of hamster PLBL2 is less than 20 ng/mg, or less than 15 ng/mg, or less than 10 ng/mg, or less than 8 ng/mg, or less than 5 ng/mg, or less than 3 ng/mg, or less than 2 ng/mg, or less than 1 ng/mg, or less than 0.5 ng/mg.

2. The composition of claim 1, wherein the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6.

3. The composition of claim 2, wherein the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7.

4. The composition of claim 2, wherein the anti-IL13 antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 9.

5. The composition of claim 3, wherein the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10.

6. The composition of claim 4, wherein the anti-IL13 antibody comprises a light chain having the amino acid sequence of SEQ ID NO.: 14.

7. The composition of claim 2, wherein the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7 and a light chain variable region having the amino acid sequence of SEQ ID NO.: 9.

8. The composition of claim 7, wherein the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10 and a light chain having the amino acid sequence of SEQ ID NO.: 14.

9. The composition of claim 1, wherein the amount of hamster PLBL2 was quantified using an immunoassay or a mass spectrometry assay.

10. The composition of claim 9, wherein the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA.

11. The composition of claim 9, wherein the mass spectrometry assay is LC-MS/MS.

12. A purified anti-IL13 monoclonal antibody preparation isolated from Chinese hamster ovary host cells, wherein the preparation is purified by a process comprising a hydrophobic interaction chromatography (HIC) step thereby producing a purified preparation, wherein the

wherein the amount of hamster PLBL2 is less than 20 ng/mg, or less than 15 ng/mg, or less than 10 ng/mg, or less than 8 ng/mg, or less than 5 ng/mg, or less than 3 ng/mg, or less than 2 ng/mg, or less than 1 ng/mg, or less than 0.5 ng/mg.

13. The anti-IL13 antibody preparation of claim 12, wherein the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin.

14. The anti-IL13 antibody preparation of claim 13, wherein the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode.

15. The anti-IL13 antibody preparation of claim 14, wherein the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 50 mM sodium acetate pH 5.0.

16. The anti-IL13 antibody preparation of claim 15, wherein the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected between 0.5 OD to 1.5 OD.

17. The anti-IL13 antibody preparation of claim 15, wherein the flow-through is collected for a maximum of 8 column volumes.

18. The anti-IL13 antibody preparation of claim 12, wherein the process further comprises an affinity chromatography step.

19. The anti-IL13 antibody preparation of claim 18, wherein the affinity chromatography is protein A chromatography.

20. The anti-IL13 antibody preparation of claim 12, wherein the process further comprises an ion exchange chromatography step.

21. The anti-IL13 antibody preparation of claim 20, wherein the ion exchange chromatography is anion exchange chromatography.

22. A purified anti-IL13 monoclonal antibody preparation isolated from Chinese hamster ovary cells, wherein the antibody preparation is purified by a process comprising a first Protein A affinity chromatography step, a second anion exchange chromatography step, and a third hydrophobic interaction chromatography (HIC) step thereby producing a purified preparation, wherein the purified preparation comprises the anti-IL13 antibody and a residual amount of hamster PLBL2, wherein the amount of hamster PLBL2 is less than 20 ng/mg, or less than 15 ng/mg, or less than 10 ng/mg, or less than 8 ng/mg, or less than 5 ng/mg, or less than 3 ng/mg, or less than 2 ng/mg, or less than 1 ng/mg, or less than 0.5 ng/mg.

23. The anti-IL13 antibody preparation of claim 22, wherein the affinity chromatography step comprises MABSELECT SURE™ resin, the anion exchange chromatography step

SEPHAROSE™ 6 Fast Flow (high sub) resin.

24. The anti-IL13 antibody preparation of claim 23, wherein:

the affinity chromatography step comprises operating a MABSELECT SURE™ resin-containing column in bind-elute mode;

the anion exchange chromatography step comprises operating a Q SEPHAROSE™ Fast Flow resin-containing column in bind-elute mode; and

the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode.

25. The anti-IL13 antibody preparation of claim 12 or claim 22, wherein the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6.

26. The anti-IL13 antibody preparation of claim 25, wherein the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7.

27. The anti-IL13 antibody preparation of claim 25, wherein the anti-IL13 antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 9.

28. The anti-IL13 antibody preparation of claim 26, wherein the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10.

29. The anti-IL13 antibody preparation of claim 27, wherein the anti-IL13 antibody comprises a light chain having the amino acid sequence of SEQ ID NO.: 14.

30. The anti-IL13 antibody preparation of claim 25, wherein the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7 and a light chain variable region having the amino acid sequence of SEQ ID NO.: 9.

31. The anti-IL13 antibody preparation of claim 30, wherein the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10 and a light chain having the amino acid sequence of SEQ ID NO.: 14.

32. The anti-IL13 antibody preparation of claim 12 or claim 22, wherein the amount of hamster PLBL2 was quantified using an immunoassay or a mass spectrometry assay.

33. The anti-IL13 antibody preparation of claim 32, wherein the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA.

LC-MS/MS.

35. A method of purifying a recombinant polypeptide produced in Chinese hamster ovary host cells, wherein the method provides a purified preparation comprising the recombinant polypeptide and a residual amount of hamster PLBL2.

36. The method of claim 35, wherein the amount of hamster PLBL2 is less than 20 ng/mg, or less than 15 ng/mg, or less than 10 ng/mg, or less than 8 ng/mg, or less than 5 ng/mg, or less than 3 ng/mg, or less than 2 ng/mg, or less than 1 ng/mg, or less than 0.5 ng/mg.

37. The method of claim 36 comprising a hydrophobic interaction chromatography (HIC) step.

38. The method of claim 37, wherein the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin.

39. The method of claim 38, wherein the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode.

40. The method of claim 37, wherein the recombinant polypeptide is selected from a growth factor, a cytokine, an antibody, an antibody fragment, and an immunoadhesin.

41. The method of claim 40, wherein the recombinant polypeptide is an antibody.

42. The method of claim 41, wherein the antibody is a humanized monoclonal antibody.

43. The method of claim 42, wherein the antibody is IgG1, or IgG2, or IgG3, or IgG4.

44. The method of claim 43, wherein the antibody is IgG4.

45. The method of claim 41, wherein the antibody is anti-IL13.

46. The method of claim 44, wherein the antibody is lebrikizumab.

47. The method of claim 45, wherein the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6.

48. The method of any one of claims 45, 46, or 47, wherein the HIC step comprises operating a resin-containing column in flow-through mode and an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 50 mM sodium acetate pH 5.0.

49. The method of claim 48, wherein the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected between 0.5 OD to 1.5 OD.

column volumes.

51. The method of claim 48, further comprising an affinity chromatography step.

52. The method of claim 51, wherein the affinity chromatography is protein A chromatography.

53. The method of claim 48, further comprising an ion exchange chromatography step.

54. The method of claim 53, wherein the ion exchange chromatography is anion exchange chromatography.

55. The method of claim 48 comprising a first Protein A affinity chromatography step and a second anion exchange chromatography step prior to the hydrophobic interaction chromatography (HIC) step.

56. The method of claim 55, wherein the affinity chromatography step comprises MABSELECT SURE™ resin, the anion exchange chromatography step comprises Q SEPHAROSE™ Fast Flow, and the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (high sub).

57. The method of claim 56, wherein:

the affinity chromatography step comprises operating a MABSELECT SURE™ resin-containing column in bind-elute mode;

the anion exchange chromatography step comprises operating a Q SEPHAROSE™ Fast Flow resin-containing column in bind-elute mode, and

the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode.

58. The method of claim 41, wherein the antibody is anti-Abeta.

59. The method of claim 44, wherein the antibody is crenezumab.

60. The method of claim 58, wherein the anti-Abeta antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.:23, CDR-H2 having the amino acid sequence of SEQ ID NO.:24, and CDR-H3 having the amino acid sequence of SEQ ID NO.:25, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.:26, CDR-L2 having the amino acid sequence of SEQ ID NO.:27, and CDR-L3 having the amino acid sequence of SEQ ID NO.:28.

61. The method of any one of claims 58, 59, or 60, wherein the HIC step comprises operating a resin-containing column in flow-through mode and an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 150 mM sodium acetate pH 5.0.

nanometers and the flow-through is collected beginning at 0.5 OD and for 10 column volumes.

63. The method of claim 61, further comprising an affinity chromatography step.

64. The method of claim 63, wherein the affinity chromatography is protein A chromatography.

65. The method of claim 61, further comprising a mixed mode chromatography step.

66. The method of claim 61 comprising a first Protein A affinity chromatography step and a second mixed mode chromatography step prior to the hydrophobic interaction chromatography (HIC) step.

67. The method of claim 66, wherein the affinity chromatography step comprises MABSELECT SURE™ resin, the mixed mode chromatography step comprises CAPTO™ Adhere, and the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (high sub).

68. The method of claim 67, wherein:

the affinity chromatography step comprises operating a MABSELECT SURE™ resin-containing column in bind-elute mode;

the mixed mode chromatography step comprises operating a CAPTO™ Adhere resin-containing column in flow-through mode, and

the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode.

69. The method of claim 36, wherein the amount of hamster PLBL2 is quantified using an immunoassay or a mass spectrometry assay.

70. The method of claim 69, wherein the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA.

71. The method of claim 69, wherein the mass spectrometry assay is LC-MS/MS.

72. A composition comprising an anti-Abeta monoclonal antibody purified from Chinese hamster ovary host cells, wherein the composition comprises the anti-Abeta antibody and a residual amount of hamster PLBL2, wherein the amount of hamster PLBL2 is less than 20 ng/mg, or less than 15 ng/mg, or less than 10 ng/mg, or less than 8 ng/mg, or less than 5 ng/mg, or less than 3 ng/mg, or less than 2 ng/mg, or less than 1 ng/mg, or less than 0.5 ng/mg.

73. The composition of claim 72, wherein the anti-Abeta antibody is crenezumab.

74. The composition of claim 72, wherein the anti-Abeta antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.:23, CDR-H2 having the amino acid sequence of SEQ ID NO.:24, and CDR-H3 having the amino acid sequence of SEQ ID NO.:25, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID

amino acid sequence of SEQ ID NO.:28.

75. The composition of claim 74, wherein the anti-Abeta antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:29.

76. The composition of claim 74, wherein the anti-Abeta antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.:30.

77. The composition of claim 74, wherein the anti-Abeta antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:29 and a light chain variable region having the amino acid sequence of SEQ ID NO.:30.

78. The method of claim 43, wherein the antibody is IgG1.

79. The method of claim 41, wherein the antibody is anti-IL17 A/F.

80. The method of claim 79, wherein the antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.:15, CDR-H2 having the amino acid sequence of SEQ ID NO.:16, and CDR-H3 having the amino acid sequence of SEQ ID NO.:17, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.:18, CDR-L2 having the amino acid sequence of SEQ ID NO.:19 and CDR-L3 having the amino acid sequence of SEQ ID NO.:20.

81. The method of claim 79 or claim 80, wherein the HIC step comprises operating a resin-containing column in flow-through mode and an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 50 mM sodium acetate pH 5.5.

82. The method of claim 81, wherein the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected beginning at 0.5 OD and for 10 column volumes.

83. The method of claim 81, further comprising an affinity chromatography step.

84. The method of claim 83, wherein the affinity chromatography is protein A chromatography.

85. The method of claim 81, further comprising a cation exchange chromatography step.

86. The method of claim 81 comprising a first Protein A affinity chromatography step and a second cation exchange chromatography step prior to the hydrophobic interaction chromatography (HIC) step.

87. The method of claim 86, wherein the affinity chromatography step comprises MABSELECT SURE™ resin, the cation exchange chromatography step comprises POROS® 50HS resin, and the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (high sub) resin.

88. The method of claim 87, wherein:

resin-containing column in bind-elute mode;

the cation exchange chromatography step comprises operating a POROS® 50HS resin-containing column in bind-elute mode, and

the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode.

89. A composition comprising an anti-IL17 A/F monoclonal antibody purified from Chinese hamster ovary host cells, wherein the composition comprises the anti-IL17 A/F antibody and a residual amount of hamster PLBL2, wherein the amount of hamster PLBL2 is less than 20 ng/mg, or less than 15 ng/mg, or less than 10 ng/mg, or less than 8 ng/mg, or less than 5 ng/mg, or less than 3 ng/mg, or less than 2 ng/mg, or less than 1 ng/mg, or less than 0.5 ng/mg.

90. The composition of claim 89, wherein the anti-IL17 A/F antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.:15, CDR-H2 having the amino acid sequence of SEQ ID NO.:16, and CDR-H3 having the amino acid sequence of SEQ ID NO.:17, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.:18, CDR-L2 having the amino acid sequence of SEQ ID NO.:19, and CDR-L3 having the amino acid sequence of SEQ ID NO.:20.

91. The composition of claim 90, wherein the anti-IL17 A/F antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:21.

92. The composition of claim 90, wherein the anti-IL17 A/F antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.:22.

93. The composition of claim 90, wherein the anti-IL17 A/F antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:21 and a light chain variable region having the amino acid sequence of SEQ ID NO.:22.

94. A method of treating an IL-13-mediated disorder in a patient comprising administering a treatment composition to the patient, wherein the treatment composition comprises the composition of any one of claims 1-8.

95. The method of claim 94, wherein administration of the treatment composition is less immunogenic for hamster PLBL2 compared to administration of a reference composition, wherein the reference composition comprises an anti-IL13 monoclonal antibody purified from Chinese hamster ovary host cells and a residual amount of hamster PLBL2 of greater than 30 ng/mg, or greater than 50 ng/mg, or greater than 100 ng/mg, or greater than 200 ng/mg, or greater than 300 ng/mg.

once every four weeks, or once every eight weeks, or once every 12 weeks.

97. The method of claim 96, wherein the patient is treated once every four weeks for at least one month, or at least three months, or at least six months, or at least nine months, or at least twelve months, or at least 18 months, or at least two years, or more than two years.

98. The method of claim 94, wherein the IL-13-mediated disorder is selected from asthma, idiopathic pulmonary fibrosis and atopic dermatitis.

99. The method of claim 94, wherein the IL-13-mediated disorder is selected from allergic asthma, non-allergic asthma, allergic rhinitis, allergic conjunctivitis, eczema, urticaria, food allergies, chronic obstructive pulmonary disease, ulcerative colitis, RSV infection, uveitis, scleroderma, and osteoporosis.

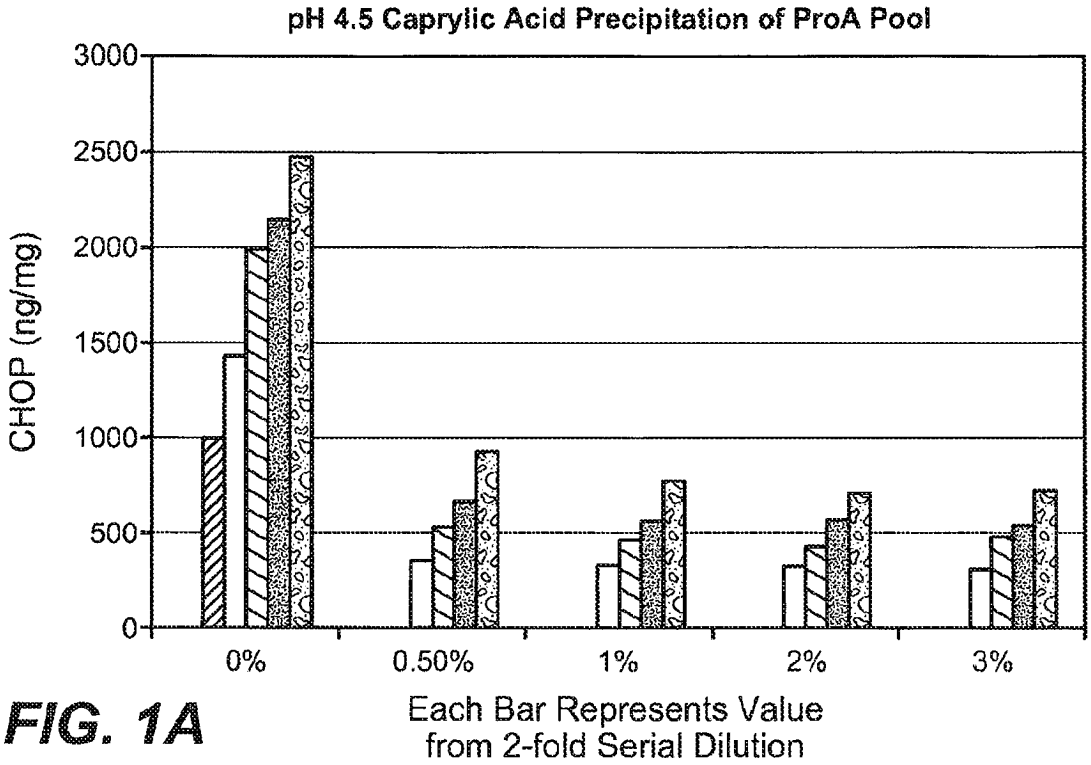
pH 4.5 Caprylic Acid Precipitation of ProA Pool
CHOP (ng/mg)
3000
2500
2000
1500
1000
500
0
0%
0.50%
1%
2%
3%
Each Bar Represents Value
from 2-fold Serial Dilution

FIG. 1A

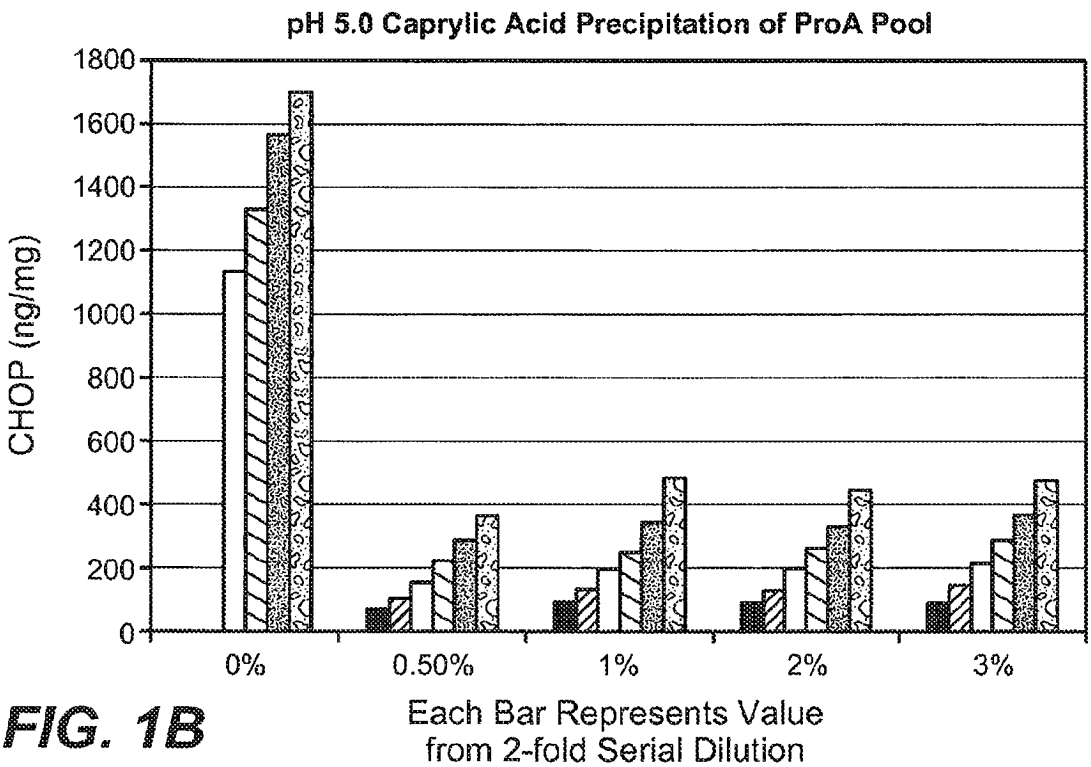
pH 5.0 Caprylic Acid Precipitation of ProA Pool
CHOP (ng/mg)
1800
1600
1400
1200
1000
800
600
400
200
0
0%
0.50%
1%
2%
3%
Each Bar Represents Value
from 2-fold Serial Dilution

FIG. 1B

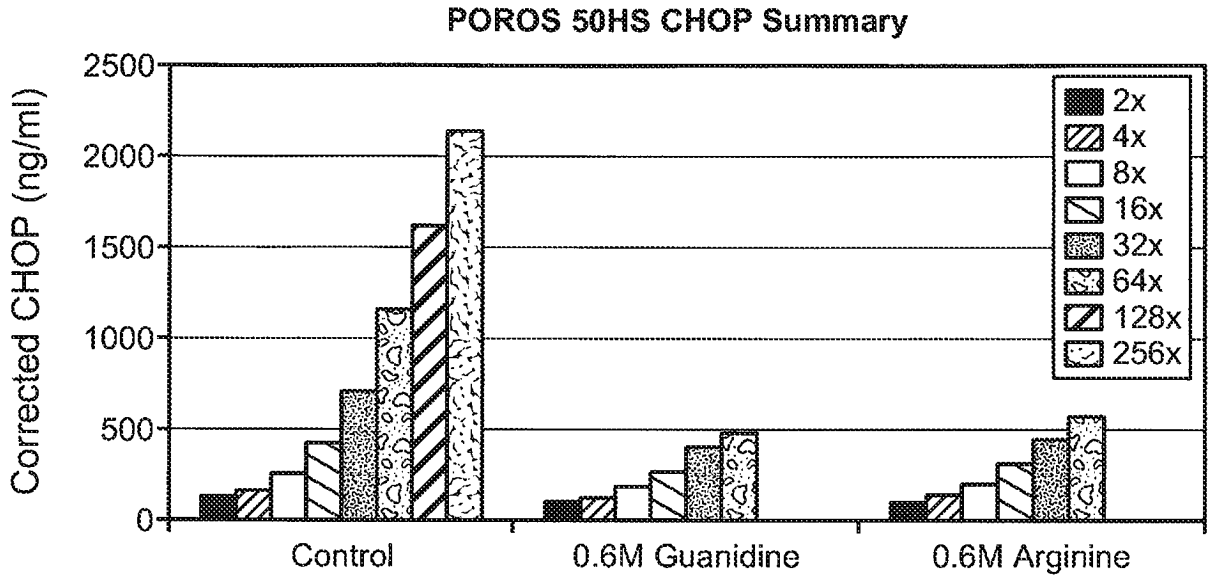
POROS 50HS CHOP Summary
Corrected CHOP (ng/ml)
2500
2000
1500
1000
500
0
Control
0.6M Guanidine
0.6M Arginine
2x
4x
8x
16x
32x
64x
128x
256x

FIG. 2

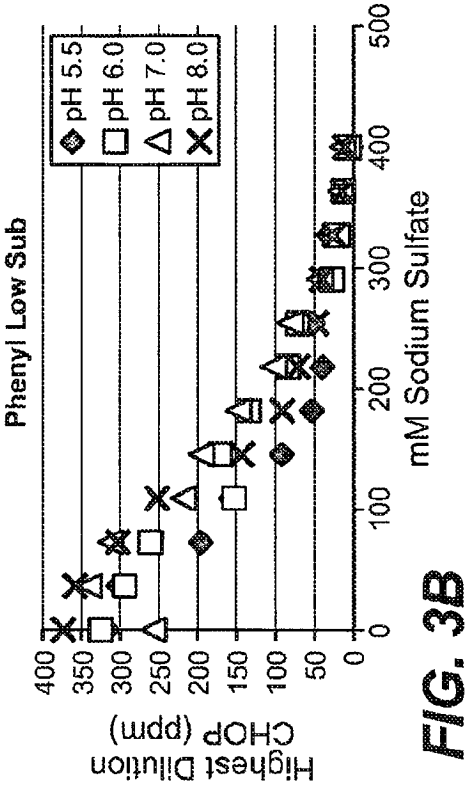

FIG. 3B

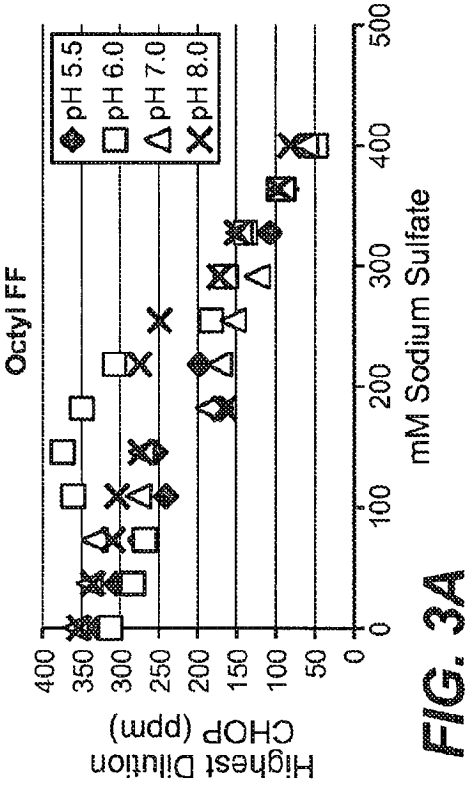

FIG. 3A

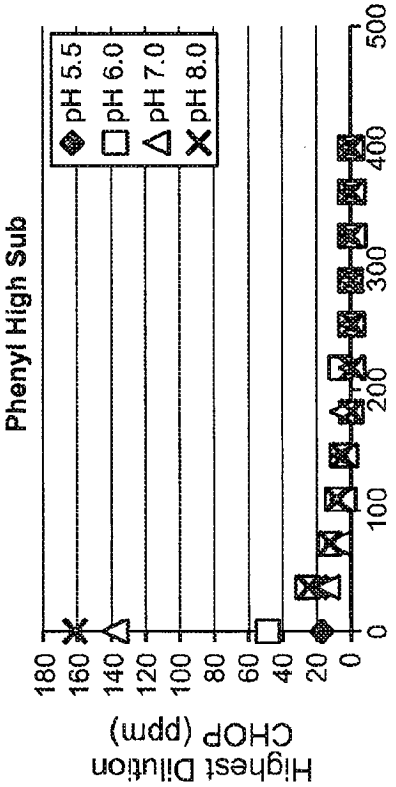

FIG. 3D

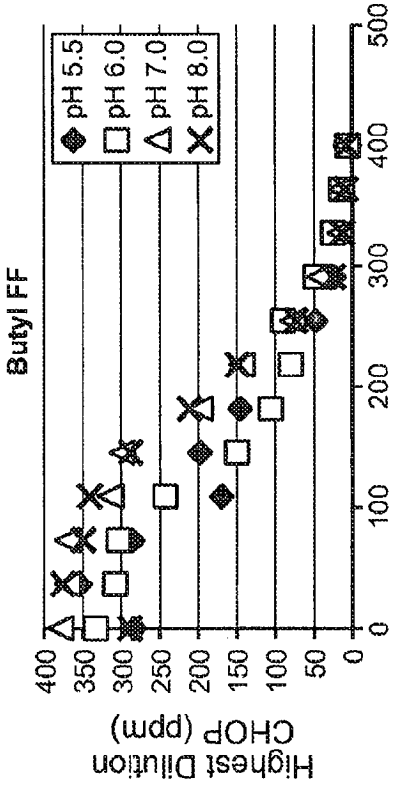

FIG. 3C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/055387

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/395 (2014.01)
CPC - A61K 2039/505 (2014.12)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 39/395, 49/00; A61P 17/00, 25/28 (2014.01)
USPC - 424/130.1, 141.1; 530/389.2;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - A61K 2039/505; C07K 16/18, 16/244; (2014.12) (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar, Google, PubMed

Search terms used: IL-13; IL-17A/F; Abeta; PLBL2, PLBD2, p76, LAMA-like; CHO; monoclonal antibody

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2005/062967 A2 (TANOX, INC) 14 July 2005 (14.07.2005), entire document	1-99
A	WO 2012/075037 A1 (GENENTECH, INC) 07 JUNE 2012 (07.06.2012), entire document	1-99
A	US 2010/0055103 A1 (CHEN et al) 04 March 2010 (04.03.2010), entire document	1-99
A	BECKER et al. "Unraveling the Chinese hamster ovary cell line transcriptome by next-generation sequencing," Journal of Biotechnology, 17 September 2011 (17.09.2011), Vol. 156, Pgs. 227- 235. entire document	1-99

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 December 2014	20 JAN 2015
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs1-6 and 8-10 were searched.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
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19 March 2015 (19.03.2015)

WIPO | PCT

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(25) Filing Language: English

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(30) Priority Data:
61/877,517 13 September 2013 (13.09.2013) US

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(74) Agents: **DAVIS, Jennifer L.** et al.; Genentech, Inc., 1 DNA Way, Mail Stop 49, South San Francisco, California 94080 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,

[Continued on next page]

(54) Title: METHODS AND COMPOSITIONS COMPRISING PURIFIED RECOMBINANT POLYPEPTIDES

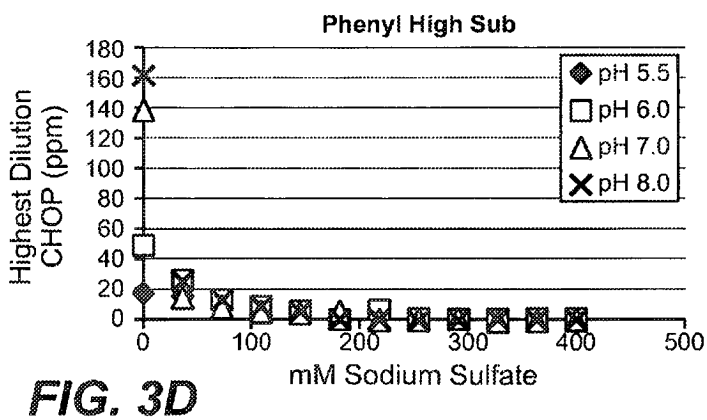

FIG. 3D

(57) Abstract: Purified recombinant polypeptides isolated from Chinese hamster ovary host cells, including antibodies, such as therapeutic antibodies, and methods of making and using such polypeptides are provided.

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PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

(48) **Date of publication of this corrected version**:
17 March 2016

(15) **Information about Correction**:
see Notice of 17 March 2016

METHODS AND COMPOSITIONS COMPRISING PURIFIED RECOMBINANT POLYPEPTIDES

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of priority of provisional U.S. Application No. 61/877,517 filed September 13, 2013, which is hereby incorporated by reference in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on August 28, 2014, is named 2014.AUG.28 P5704R1-WO Sequence Listing.txt and is 34,811 bytes in size.

FIELD

[0003] Purified recombinant polypeptides isolated from Chinese hamster ovary host cells, including antibodies, such as therapeutic antibodies, and methods of making and using such polypeptides are provided.

BACKGROUND

[0004] A number of drugs are on the market or in development for treating asthma and other respiratory disorders. One of the targets for asthma therapy is IL-13. IL-13 is a pleiotropic TH2 cytokine produced by activated T cells, NKT cells, basophils, eosinophils, and mast cells, and it has been strongly implicated in the pathogenesis of asthma in preclinical models. IL-13 antagonists, including anti-IL-13 antibodies, have previously been described. Certain such antibodies have also been developed as human therapeutics. Recently, several studies have shown clinical activity of monoclonal antibodies against IL-13 in the treatment of asthma (*See, e.g.,* Corren et al., 2011, *N. Engl. J. Med.* 365, 1088-1098; Gauvreau et al., 2011, *Am. J. Respir. Crit. Care Med.* 183, 1007-1014; Ingram and Kraft, 2012, *J. Allergy Clin. Immunol.* 130, 829-42; Webb, 2011, *Nat Biotechnol* 29, 860-863). Of these, lebrikizumab, a humanized IgG4 antibody that neutralizes IL-13 activity, improved lung function in asthmatics who were symptomatic despite treatment with, for the majority, inhaled corticosteroids and a long-acting beta2-adrenergic receptor agonist (Corren et al., 2011, *N. Engl. J. Med.* 365, 1088-1098).

[0005] In addition, IL-13 has been implicated in numerous other allergic and fibrotic disorders. For example, such diseases and/or conditions mediated by IL13 include, but are not limited to, allergic asthma, non-allergic (intrinsic) asthma, allergic rhinitis, atopic dermatitis, allergic

conjunctivitis, eczema, urticaria, food allergies, chronic obstructive pulmonary disease, ulcerative colitis, RSV infection, uveitis, scleroderma, and osteoporosis.

[0006] For recombinant biopharmaceutical proteins to be acceptable for administration to human patients, it is important that residual impurities resulting from the manufacture and purification process are removed from the final biological product. These process components include culture medium proteins, immunoglobulin affinity ligands, viruses, endotoxin, DNA, and host cell proteins. These host cell impurities include process-specific host cell proteins (HCPs), which are process-related impurities/contaminants in the biologics derived from recombinant DNA technology. While HCPs are typically present in the final drug substance in small quantities (in parts-per-million or nanograms per milligram of the intended recombinant protein), it is recognized that HCPs are undesirable and their quantities should be minimized. For example, the U.S. Food and Drug Administration (FDA) requires that biopharmaceuticals intended for in vivo human use should be as free as possible of extraneous impurities, and requires tests for detection and quantitation of potential contaminants/impurities, such as HCPs.

[0007] Procedures for purification of proteins from cell debris initially depend on the site of expression of the protein. Some proteins are secreted directly from the cell into the surrounding growth media; others are made intracellularly. For the latter proteins, the first step of a purification process involves lysis of the cell, which can be done by a variety of methods, including mechanical shear, osmotic shock, or enzymatic treatments. Such disruption releases the entire contents of the cell into the homogenate, and in addition produces subcellular fragments that are difficult to remove due to their small size. These are generally removed by centrifugation or by filtration. The same problem arises with directly secreted proteins due to the natural death of cells and release of intracellular host cell proteins in the course of the protein production run.

[0008] Once a solution containing the protein of interest is obtained, its separation from the other proteins produced by the cell is usually attempted using a combination of different chromatography techniques. Typically, these techniques separate mixtures of proteins on the basis of their charge, degree of hydrophobicity, or size. Several different chromatography resins are available for each of these techniques, allowing accurate tailoring of the purification scheme to the particular protein involved. The essence of each of these separation methods is that proteins can be caused either to move at different rates down a long column, achieving a physical separation that increases as they pass further down the column, or to adhere selectively to the separation medium, being then differentially eluted by different solvents. In some cases, the desired protein is separated from impurities when the impurities specifically adhere to the

column, and the protein of interest does not, that is, the protein of interest is present in the "flow-through."

[0009] Ion-exchange chromatography, named for the exchangeable counterion, is a procedure applicable to purification of ionizable molecules. Ionized molecules are separated on the basis of the non-specific electrostatic interaction of their charged groups with oppositely charged molecules attached to the solid phase support matrix, thereby retarding those ionized molecules that interact more strongly with solid phase. The net charge of each type of ionized molecule, and its affinity for the matrix, varies according to the number of charged groups, the charge of each group, and the nature of the molecules competing for interaction with the charged solid phase matrix. These differences result in resolution of various molecule types by ion-exchange chromatography. In typical protein purification using ion exchange chromatography, a mixture of many proteins derived from a host cell, such as in mammalian cell culture, is applied to an ion-exchange column. After non-binding molecules are washed away, conditions are adjusted, such as by changing pH, counter ion concentration and the like in step- or gradient-mode, to release from the solid phase a non-specifically retained or retarded ionized protein of interest and separating it from proteins having different charge characteristics. Anion exchange chromatography involves competition of an anionic molecule of interest with the negative counter ion for interaction with a positively charged molecule attached to the solid phase matrix at the pH and under the conditions of a particular separation process. By contrast, cation exchange chromatography involves competition of a cationic molecule of interest with the positive counter ion for a negatively charged molecule attached to the solid phase matrix at the pH and under the conditions of a particular separation process. Mixed mode ion exchange chromatography (also referred to as multimodal ion exchange chromatography) involves the use of a combination of cation and anion exchange chromatographic media in the same step. In particular, "mixed mode" refers to a solid phase support matrix to which is covalently attached a mixture of cation exchange, anion exchange, and hydrophobic interaction moieties.

[0010] Hydroxyapatite chromatography of proteins involves the non-specific interaction of the charged amino or carboxylate groups of a protein with oppositely charged groups on the hydroxyapatite, where the net charge of the hydroxyapatite and protein are controlled by the pH of the buffer. Elution is accomplished by displacing the non-specific protein-hydroxyapatite pairing with ions such as Ca2+ or Mg2+. Negatively charged protein groups are displaced by negatively charged compounds, such as phosphates, thereby eluting a net-negatively charged protein.

[0011] Hydrophobic interaction chromatography (HIC) is typically used for the purification and separation of molecules, such as proteins, based on differences in their surface hydrophobicity. Hydrophobic groups of a protein interact non-specifically with hydrophobic groups coupled to the chromatography matrix. Differences in the number and nature of protein surface hydrophobic groups results in differential retardation of proteins on a HIC column and, as a result, separation of proteins in a mixture of proteins.

[0012] Affinity chromatography, which exploits a specific structurally dependent (i.e., spatially complementary) interaction between the protein to be purified and an immobilized capture agent, is a standard purification option for some proteins, such as antibodies. Protein A, for example, is a useful adsorbent for affinity chromatography of proteins, such as antibodies, which contain an Fc region. Protein A is a 41kD cell wall protein from Staphylococcus aureas which binds with a high affinity (about 10^{-8}M to human IgG) to the Fc region of antibodies.

[0013] Purification of recombinant polypeptides is typically performed using bind and elute chromatography (B/E) or flow-through (F/T) chromatography. These are briefly described below.

[0014] Bind and Elute Chromatography (B/E): Under B/E chromatography the product is usually loaded to maximize dynamic binding capacity (DBC) to the chromatography material and then wash and elution conditions are identified such that maximum product purity is attained in the eluate.

[0015] Various B/E methods for use with protein A affinity chromatography, including various intermediate wash buffers, have been described. For example, US Patent Nos. 6,127,526 and 6,333,398 describe an intermediate wash step during Protein A chromatography using hydrophobic electrolytes, *e.g.*, tetramethylammonium chloride (TMAC) and tetraethylammonium chloride (TEAC), to remove the contaminants, but not the immobilized Protein A or the protein of interest, bound to the Protein A column. US Patent No. 6,870,034 describes additional methods and wash buffers for use with protein A affinity chromatography.

[0016] Flow Through Chromatography (F/T): Using F/T chromatography, load conditions are identified where impurities strongly bind to the chromatography material while the product flows through. F/T chromatography allows high load density for standard monoclonal antibody preparations (MAbs).

[0017] In recombinant anti-IL13 MAb preparations and certain other recombinant polypeptides produced in CHO cells, we identified an enzyme, phospholipase B-like 2, as a single CHOP species present in excess of available antibodies in a total CHOP ELISA assay. As used herein, "PLB2" and "PLBL2" and "PLBD2" are used interchangeably and refer to the enzyme

"phospholipase B-like 2" or its synonym, "phospholipase B-domain-like 2". Certain scientific publications on PLBL2 include Lakomek, K. et al., BMC Structural Biology 9:56 (2009); Deuschi, et al., FEBS Lett 580:5747-5752 (2006). PLBL2 is synthesized as a pre-pro-enzyme with parent MW of about 66,000. There is an initial leader sequence which is removed and potential 6 mannose-6-phosphate (M-6-P) groups are added during post-translational modification. M-6-P is a targeting modification that directs this enzyme to the lysosome via the M-6-P receptor. PLBL2 contains 6 cysteines, two of which have free sulfhydrals, and four form disulfide bonds. In acidic environments, PLBL2 is further clipped into the N- and C-terminal fragments having 32,000 and 45,000 MW, respectively. By analogy with other lysosomal enzymes, this cleavage is an activating step, allowing and access of the substrate to the active site.

[0018] There is about 80% PLBL2 amino acid sequence homology between hamster and human forms of the enzyme. The enzyme activity is thought to be to cleave either fatty acid chain from the phospholipids that make up cell membranes. There are other phospholipases with different substrate cleavage specificities. Similar enzymatic activities exist in microorganisms, where they are often a virulence factor. Although microorganisms have a similar enzymatic activity, the protein generating this activity is different, and there is low sequence homology between microbial and mammalian PLBL2 enzymes. Phospholipases produce free fatty acids (FFA) as one product of the substrate hydrolysis. Free fatty acids are themselves a potential immune-signaling factor. Dehydrogenation converts FFA to arachadonic acid which potentially participates in inflammation cascades involving eicosanoids.

[0019] Having identified PLBL2 as a single HCP (CHOP) in recombinant anti-IL13 MAb preparations and certain other recombinant polypeptides produced in CHO cells, we developed reagents, methods, and kits for the specific, sensitive, and quantitative determination of PLBL2 levels in anti-IL-13 Mab preparations (and other recombinant polypeptide products) and at various stages of purification. These are briefly described in the Examples below and also in US Provisional Patent Application Nos. 61/877,503 and 61/991,228. In addition, there was the formidable challenge of developing a large-scale, robust, and efficient process for the purification of anti-IL13 MAb (and other recombinant polypeptide products) resulting in MAb of sufficient purity (including removal of PLBL2) for human therapeutic use including late-stage clinical and commercial use. The invention described herein meets certain of the above-described needs and provides other benefits.

[0020] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

SUMMARY

[0021] The invention is based, at least in part, on the development of improved processes for the purification of recombinant polypeptides produced in Chinese hamster ovary (CHO) cells that provide purified product with substantially reduced levels of hamster PLBL2. Recombinant polypeptides purified according to the methods of the invention, including therapeutic antibodies such as an anti-IL13 antibody, may have reduced immunogenicity when administered to human subjects.

[0022] Accordingly, in one aspect, compositions comprising an anti-IL13 monoclonal antibody purified from CHO cells comprising the anti-IL13 antibody and a residual amount of hamster PLBL2 are provided. In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7. In certain embodiments, the anti-IL13 antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10. In certain embodiments, the anti-IL13 antibody comprises a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having

the amino acid sequence of SEQ ID NO.: 7 and a light chain variable region having the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10 and a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the amount of hamster PLBL2 in the composition is quantified using an immunoassay or a mass spectrometry assay. In certain embodiments, the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA. In certain embodiments, the mass spectrometry assay is LC-MS/MS.

[0023] In another aspect, anti-IL13 monoclonal antibody preparations isolated and purified from CHO cells by a process comprising a hydrophobic interaction chromatography (HIC) step are provided. In certain embodiments, the purified preparation comprises the anti-IL13 antibody and a residual amount of hamster PLBL2. In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin. In certain embodiments, the HIC step comprises operating a resin-containing column in flow-through mode. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 50 mM sodium acetate pH 5.0. In certain embodiments, the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected between 0.5 OD to 1.5 OD. In certain embodiments, the flow-through is collected for a maximum of 8 column volumes. In certain embodiments, the process further comprises an affinity chromatography step. In certain embodiments, the affinity chromatography is protein A chromatography. In certain embodiments, the process further comprises an ion exchange chromatography step. In certain embodiments, the ion exchange chromatography is anion exchange chromatography. In certain embodiments, the anti-IL13

antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7. In certain embodiments, the anti-IL13 antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10. In certain embodiments, the anti-IL13 antibody comprises a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7 and a light chain variable region having the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10 and a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the amount of hamster PLBL2 is quantified using an immunoassay or a mass spectrometry assay. In certain embodiments, the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA. In certain embodiments, the mass spectrometry assay is LC-MS/MS.

[0024] In yet another aspect, purified anti-IL13 monoclonal antibody preparations isolated from CHO cells are provided. In certain embodiments, the antibody preparation is purified by a process comprising a first Protein A affinity chromatography step, a second anion exchange chromatography step, and a third hydrophobic interaction chromatography (HIC) step thereby producing a purified preparation, In certain embodiments, the purified preparation comprises the anti-IL13 antibody and a residual amount of hamster PLBL2. In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between

0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the affinity chromatography step comprises MABSELECT SURE™ resin, the anion exchange chromatography step comprises Q SEPHAROSE™ Fast Flow, and the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (high sub). In certain embodiments, the affinity chromatography step comprises operating a MABSELECT SURE™ resin-containing column in bind-elute mode, the anion exchange chromatography step comprises operating a Q SEPHAROSE™ Fast Flow resin-containing column in bind-elute mode, and the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode. In certain embodiments, the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7. In certain embodiments, the anti-IL13 antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10. In certain embodiments, the anti-IL13 antibody comprises a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7 and a light chain variable region having the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10 and a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the amount of hamster PLBL2 is quantified using an immunoassay or a mass spectrometry assay. In certain embodiments, the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA. In certain embodiments, the mass spectrometry assay is LC-MS/MS.

[0025] In still yet another aspect, methods of purifying a recombinant polypeptide produced in CHO cells, wherein the method provides a purified preparation comprising the recombinant polypeptide and residual amount of hamster PLBL2 are provided. In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2

is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the recombinant polypeptide is selected from a growth factor, a cytokine, an antibody, an antibody fragment, and an immunoadhesin. In certain embodiments, the recombinant polypeptide is an antibody. In certain embodiments, the antibody is a humanized monoclonal antibody. In certain embodiments, the antibody is IgG1, or IgG2, or IgG3, or IgG4. In certain embodiments, the antibody is IgG1. In certain embodiments, the antibody is IgG2. In certain embodiments, the antibody is IgG3. In certain embodiments, the antibody is IgG4. In certain embodiments, the methods comprise a hydrophobic interaction chromatography (HIC) step. In certain embodiments, the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin.

[0026] In certain embodiments of the above purification methods, the purified antibody is anti-IL13. In certain embodiments, the antibody is lebrikizumab. In certain embodiments, the HIC step comprises operating a resin-containing column in flow-through mode. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 50 mM sodium acetate pH 5.0. In certain embodiments, the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected between 0.5 OD to 1.5 OD. In certain embodiments, the flow-through is collected for a maximum of 8 column volumes. In certain embodiments, the methods further comprise an affinity chromatography step. In certain embodiments, the affinity chromatography is protein A chromatography. In certain embodiments, the methods further comprise an ion exchange chromatography step. In certain embodiments, the ion exchange chromatography is anion exchange chromatography. In certain embodiments, the methods comprise a first Protein A affinity chromatography step, a second anion exchange chromatography step, and a third hydrophobic interaction chromatography (HIC) step. In certain embodiments, the affinity chromatography step comprises MABSELECT SURE™ resin, the anion exchange chromatography step comprises Q SEPHAROSE™ Fast Flow, and the HIC step comprises

PHENYL SEPHAROSE™ 6 Fast Flow (high sub). In certain embodiments, the affinity chromatography step comprises operating a MABSELECT SURE™ resin-containing column in bind-elute mode, the anion exchange chromatography step comprises operating a Q SEPHAROSE™ Fast Flow resin-containing column in bind-elute mode, and the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode. In certain embodiments, the amount of hamster PLBL2 is quantified using an immunoassay or a mass spectrometry assay. In certain embodiments, the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA. In certain embodiments, the mass spectrometry assay is LC-MS/MS.

[0027] In certain embodiments of the above purification methods, the purified antibody is anti-Abeta. In certain embodiments, the anti-Abeta antibody is crenezumab. In certain embodiments, the anti-Abeta antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.:23, CDR-H2 having the amino acid sequence of SEQ ID NO.:24, and CDR-H3 having the amino acid sequence of SEQ ID NO.:25, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.:26, CDR-L2 having the amino acid sequence of SEQ ID NO.:27, and CDR-L3 having the amino acid sequence of SEQ ID NO.:28. In certain embodiments, the anti-Abeta antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:29. In certain embodiments, the anti-Abeta antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.:30. In certain embodiments, the anti-Abeta antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:29 and a light chain variable region having the amino acid sequence of SEQ ID NO.:30. In certain embodiments, the HIC step comprises operating a resin-containing column in flow-through mode. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 150 mM sodium acetate pH 5.0. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 150 mM sodium acetate pH 4.0. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 150 mM sodium acetate, 240 mM sodium sulfate pH 4.0. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 150 mM sodium acetate, 240 mM sodium sulfate pH 5.0. In certain embodiments, the load density is 300 g/L. In certain embodiments, the load density is 100 g/L. In certain embodiments, the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected

beginning at 0.5 OD and collection continues for 10 column volumes. In certain embodiments, the methods further comprise an affinity chromatography step. In certain embodiments, the affinity chromatography is protein A chromatography. In certain embodiments, the methods further comprise a mixed mode chromatography step. In certain embodiments, the methods comprise a first Protein A affinity chromatography step, a second mixed mode chromatography step, and a third hydrophobic interaction chromatography (HIC) step. In certain embodiments, the affinity chromatography step comprises MABSELECT SURE™ resin, the mixed mode chromatography step comprises CAPTO™ Adhere, and the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (high sub). In certain embodiments, the affinity chromatography step comprises operating a MABSELECT SURE™ resin-containing column in bind-elute mode, the mixed mode chromatography step comprises operating a CAPTO™ Adhere resin-containing column in flow-through mode, and the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode. In certain embodiments, the amount of hamster PLBL2 is quantified using an immunoassay or a mass spectrometry assay. In certain embodiments, the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA. In certain embodiments, the mass spectrometry assay is LC-MS/MS.

[0028] In yet a further aspect of the above purification methods, the purified antibody is IgG1. In some embodiments, the antibody is anti-IL17 A/F. In some embodiments, the anti-IL17 A/F antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.:15, CDR-H2 having the amino acid sequence of SEQ ID NO.:16, and CDR-H3 having the amino acid sequence of SEQ ID NO.:17, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.:18, CDR-L2 having the amino acid sequence of SEQ ID NO.:19 and CDR-L3 having the amino acid sequence of SEQ ID NO.:20. In certain embodiments, the anti-IL17 A/F antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:21. In certain embodiments, the anti-IL17 A/F antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.:22. In certain embodiments, the anti-IL17 A/F antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:21 and a light chain variable region having the amino acid sequence of SEQ ID NO.:22. In certain embodiments, the HIC chromatography step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 50 mM sodium acetate pH 5.5. In certain embodiments, the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected beginning at 0.5 OD and for 10 column volumes. In certain embodiments, the methods further

comprise an affinity chromatography step. In certain embodiments, the affinity chromatography is protein A chromatography. In certain embodiments, the methods further comprise a cation exchange chromatography step. In some embodiments, the methods comprise a first Protein A affinity chromatography step and a second cation exchange chromatography step prior to the hydrophobic interaction chromatography (HIC) step. In some embodiments, the affinity chromatography step comprises MABSELECT SURE™ resin, the cation exchange chromatography step comprises POROS 50 HS resin, and the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (high sub) resin. In some embodiments, the affinity chromatography step comprises operating a MABSELECT SURE™ resin-containing column in bind-elute mode; the cation exchange chromatography step comprises operating a POROS 50 HS resin-containing column in bind-elute mode, and the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode.

[0029] In still yet another aspect, anti-Abeta monoclonal antibody preparations purified from CHO cells by a process comprising a hydrophobic interaction chromatography (HIC) step are provided. In certain embodiments, the purified preparation comprises the anti-Abeta antibody and a residual amount of hamster PLBL2. In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin. In certain embodiments, the HIC step comprises operating a resin-containing column in flow-through mode. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 150 mM sodium acetate pH 5.0. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of

the equilibration buffer and the wash buffer comprise 150 mM sodium acetate pH 4.0. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 150 mM sodium acetate, 240 mM sodium sulfate pH 4.0. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 150 mM sodium acetate, 240 mM sodium sulfate pH 5.0. In certain embodiments, the load density is 300 g/L. In certain embodiments, the load density is 100 g/L. In certain embodiments, the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected between 0.5 OD and for 10 column volumes. In certain embodiments, the process further comprises an affinity chromatography step. In certain embodiments, the affinity chromatography is protein A chromatography. In certain embodiments, the process further comprises a mixed mode chromatography step. In certain embodiments, the anti-Abeta antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 23, CDR-H2 having the amino acid sequence of SEQ ID NO.: 24, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 25, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 26, CDR-L2 having the amino acid sequence of SEQ ID NO.: 27, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 28. In certain embodiments, the anti-Abeta antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 29. In certain embodiments, the anti-Abeta antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 30. In certain embodiments, the anti-Abeta antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 29 and a light chain variable region having the amino acid sequence of SEQ ID NO.: 30. In certain embodiments, the amount of hamster PLBL2 is quantified using an immunoassay or a mass spectrometry assay. In certain embodiments, the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA. In certain embodiments, the mass spectrometry assay is LC-MS/MS.

[0030] In one aspect, anti-IL17 A/F monoclonal antibody preparations isolated and purified from CHO cells by a process comprising a hydrophobic interaction chromatography (HIC) step are provided. In certain embodiments, the purified preparation comprises the anti- IL17 A/F antibody and a residual amount of hamster PLBL2. In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the

amount of hamster PLBL2 is less than 3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin. In certain embodiments, the HIC step comprises operating a resin-containing column in flow-through mode. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 50 mM sodium acetate pH 5.5. In certain embodiments, the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected between 0.5 OD and for 10 column volumes. In certain embodiments, the process further comprises an affinity chromatography step. In certain embodiments, the affinity chromatography is protein A chromatography. In certain embodiments, the process further comprises a cation exchange chromatography step. In certain embodiments, the anti- IL17 A/F antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 15, CDR-H2 having the amino acid sequence of SEQ ID NO.: 16, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 17, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 18, CDR-L2 having the amino acid sequence of SEQ ID NO.: 19, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 20. In certain embodiments, the anti- IL17 A/F antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 21. In certain embodiments, the anti- IL17 A/F antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 22. In certain embodiments, the anti- IL17 A/F antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 21 and a light chain variable region having the amino acid sequence of SEQ ID NO.: 32. In certain embodiments, the amount of hamster PLBL2 is quantified using an immunoassay or a mass spectrometry assay. In certain embodiments, the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA. In certain embodiments, the mass spectrometry assay is LC-MS/MS.

[0031] In still another aspect, compositions comprising an anti-Abeta monoclonal antibody purified from CHO cells comprising the anti-Abeta antibody and a residual amount of hamster PLBL2 are provided. In certain embodiments, the amount of hamster PLBL2 is less than 20

ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the anti-Abeta antibody is crenezumab. In certain embodiments, the anti-Abeta antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.:23, CDR-H2 having the amino acid sequence of SEQ ID NO.:24, and CDR-H3 having the amino acid sequence of SEQ ID NO.:25, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.:26, CDR-L2 having the amino acid sequence of SEQ ID NO.:27, and CDR-L3 having the amino acid sequence of SEQ ID NO.:28. In certain embodiments, the anti-Abeta antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:29. In certain embodiments, the anti-Abeta antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.:30. In certain embodiments, the anti-Abeta antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:29 and a light chain variable region having the amino acid sequence of SEQ ID NO.:30.

[0032] In yet still another aspect, compositions comprising an anti-IL17 A/F monoclonal antibody purified from CHO cells comprising the anti-IL17 A/F antibody and a residual amount of hamster PLBL2 are provided. In certain embodiments, the composition comprises the anti-IL17 A/F antibody and a residual amount of hamster PLBL2, wherein the amount of hamster PLBL2 is less than 20 ng/mg, or less than 15 ng/mg, or less than 10 ng/mg, or less than 8 ng/mg, or less than 5 ng/mg, or less than 3 ng/mg, or less than 2 ng/mg, or less than 1 ng/mg, or less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than

3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the anti-IL17 A/F antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.:15, CDR-H2 having the amino acid sequence of SEQ ID NO.:16, and CDR-H3 having the amino acid sequence of SEQ ID NO.:17, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.:18, CDR-L2 having the amino acid sequence of SEQ ID NO.:19, and CDR-L3 having the amino acid sequence of SEQ ID NO.:20. In certain embodiments, the anti-IL17 A/F antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:21. In certain embodiments, the anti-IL17 A/F antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.:22. In certain embodiments, the anti-IL17 A/F antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:21 and a light chain variable region having the amino acid sequence of SEQ ID NO.:22.

[0033] In one aspect, methods of treating an IL-13-mediated disorder comprising administering a treatment composition comprising an anti-IL13 monoclonal antibody purified from CHO cells and a residual amount of hamster PLBL2 are provided. In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.:

1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7. In certain embodiments, the anti-IL13 antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10. In certain embodiments, the anti-IL13 antibody comprises a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7 and a light chain variable region having the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10 and a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the treatment composition is administered subcutaneously once every four weeks. In certain embodiments, the treatment composition is administered subcutaneously once every eight weeks. In certain embodiments, the treatment composition is administered subcutaneously once every 12 weeks. In certain embodiments, the patient is treated once every four weeks for at least one month. In certain embodiments, the patient is treated once every four weeks for at least three months. In certain embodiments, the patient is treated once every four weeks for at least six months. In certain embodiments, the patient is treated once every four weeks for at least nine months. In certain embodiments, the patient is treated once every four weeks for at least 12 months. In certain embodiments, the patient is treated once every four weeks for at least 18 months. In certain embodiments, the patient is treated once every four weeks for at least two years. In certain embodiments, the patient is treated once every four weeks for more than two years. In certain embodiments, the IL-13-mediated disorder is asthma. In certain embodiments, the IL-13-mediated disorder is idiopathic pulmonary fibrosis. In certain embodiments, the IL-13-mediated disorder is atopic dermatitis. In certain embodiments, the IL-13-mediated disorder is selected from allergic asthma, non-allergic asthma, allergic rhinitis, allergic conjunctivitis, eczema, urticaria, food allergies, chronic obstructive pulmonary disease, ulcerative colitis, RSV infection, uveitis, scleroderma, and osteoporosis.

[0034] In another aspect, administration of a treatment composition to a patient according to any of the methods described above is less immunogenic for hamster PLBL2 compared to administration of a reference composition, wherein the reference composition comprises an anti-

IL13 monoclonal antibody purified from Chinese hamster ovary host cells and a residual amount of hamster PLBL2 of greater than 30 ng/mg. In certain embodiments, the amount of hamster PLBL2 in the reference composition is greater than 50 ng/mg. In certain embodiments, the amount of hamster PLBL2 in the reference composition is greater than 100 ng/mg. In certain embodiments, the amount of hamster PLBL2 in the reference composition is greater than 200 ng/mg. In certain embodiments, the amount of hamster PLBL2 in the reference composition is greater than 300 ng/mg. In certain embodiments, the amount of hamster PLBL2 in the reference composition is between 30 ng/mg and 300 ng/mg, or between 30 ng/mg and 200 ng/mg, or between 30 ng/mg and 100 ng/mg, or between 30 ng/mg and 50 ng/mg.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] Figure 1 shows total CHOP levels in caprylic acid-treated Protein A pools of anti-IL13 MAb as described in Example 2. (A) Caprylic acid precipitation of Protein A pool at pH 4.5; (B) Caprylic acid precipitation of Protein A pool at pH 5.0. CHOP levels in ng/mg are indicated along the vertical axis; percentage of caprylic acid is shown along the horizontal axis, each bar represents the value from 2-fold serial dilution.

[0036] Figure 2 shows total CHOP levels in additive-treated HCCF anti-IL13 MAb following Protein A chromatography which was followed by cation exchange chromatography on POROS® 50HS as described in Example 2. Corrected CHOP levels in ng/ml are shown on the vertical axis; the additive (control, 0.6M guanidine, or 0.6M arginine) is indicated on the horizontal axis, each bar represents the value from 2-fold serial dilution as indicated.

[0037] Figure 3 shows total CHOP levels in UFDF pools of anti-IL13 MAb subjected to different HIC resins under varying salt and pH conditions as described in Example 2. (A) OCTYL-SEPHAROSE® Fast Flow resin; (B) PHENYL SEPHAROSE™ 6 Fast Flow (low sub) resin; (C) BUTYL-SEPHAROSE® 4 Fast Flow resin; (D) PHENYL SEPHAROSE™ 6 Fast Flow (high sub) resin; highest dilution CHOP (in ppm) is shown on the vertical axis and sodium sulfate concentration is shown on the horizontal axis; pH (5.5, 6.0, 7.0, or 8.0 is indicated by the legend.

DETAILED DESCRIPTION

[0038] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton et al., Dictionary of Microbiology and Molecular Biology 2nd ed., J. Wiley & Sons (New York, N.Y. 1994), and March, Advanced Organic Chemistry Reactions,

Mechanisms and Structure 4th ed., John Wiley & Sons (New York, N.Y. 1992), provide one skilled in the art with a general guide to many of the terms used in the present application.

CERTAIN DEFINITIONS

[0039] For purposes of interpreting this specification, the following definitions will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa. In the event that any definition set forth below conflicts with any document incorporated herein by reference, the definition set forth below shall control.

[0040] As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a protein" or an "antibody" includes a plurality of proteins or antibodies, respectively; reference to "a cell" includes mixtures of cells, and the like.

[0041] The term "detecting" is used herein in the broadest sense to include both qualitative and quantitative measurements of a target molecule. Detecting includes identifying the mere presence of the target molecule in a sample as well as determining whether the target molecule is present in the sample at detectable levels.

[0042] A "sample" refers to a small portion of a larger quantity of material. Generally, testing according to the methods described herein is performed on a sample. The sample is typically obtained from a recombinant polypeptide preparation obtained, for example, from cultured host cells. A sample may be obtained from, for example but not limited to, harvested cell culture fluid, from an in-process pool at a certain step in a purification process, or from the final purified product.

[0043] The term "product" as described herein is the substance to be purified by various chromatographic methods; for example, a polypeptide.

[0044] The term "polypeptide" or "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art. The terms "polypeptide" and "protein" as used herein specifically encompass antibodies.

[0045] "Purified" polypeptide (*e.g.*, antibody or immunoadhesin) means that the polypeptide has been increased in purity, such that it exists in a form that is more pure than it exists in its natural

environment and/or when initially synthesized and/or amplified under laboratory conditions. Purity is a relative term and does not necessarily mean absolute purity.

[0046] The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising a polypeptide fused to a "tag polypeptide." The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the polypeptide to which it is fused. The tag polypeptide preferably also is fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (in certain instances, between about 10 and 20 amino acid residues).

[0047] "Active" or "activity" for the purposes herein refers to form(s) of a polypeptide which retain a biological and/or an immunological activity of interest, wherein "biological" activity refers to a biological function (either inhibitory or stimulatory) caused by the polypeptide other than the ability to induce the production of an antibody against an antigenic epitope possessed by the polypeptide and an "immunological" activity refers to the ability to induce the production of an antibody against an antigenic epitope possessed by the polypeptide.

[0048] The term "antagonist" is used in the broadest sense, and includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of a native polypeptide, e.g., a cytokine. In a similar manner, the term "agonist" is used in the broadest sense and includes any molecule that mimics a biological activity of a native polypeptide. Suitable agonist or antagonist molecules specifically include agonist or antagonist antibodies or antibody fragments, fragments or amino acid sequence variants of native polypeptides, and the like. Methods for identifying agonists or antagonists of a polypeptide may comprise contacting a polypeptide with a candidate agonist or antagonist molecule and measuring a detectable change in one or more biological activities normally associated with the polypeptide.

[0049] A polypeptide "which binds" an antigen of interest, *e.g.* a tumor-associated polypeptide antigen target, is one that binds the antigen with sufficient affinity such that the polypeptide is useful as an assay reagent, a diagnostic and/or therapeutic agent in targeting a sample containing the antigen, a cell or tissue expressing the antigen, and does not significantly cross-react with other polypeptides.

[0050] With regard to the binding of a polypeptide to a target molecule, the term "specific binding" or "specifically binds to" or is "specific for" a particular polypeptide or an epitope on a particular polypeptide target means binding that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a

molecule compared to binding of a control molecule, which generally is a molecule of similar structure that does not have binding activity. For example, specific binding can be determined by competition with a control molecule that is similar to the target, for example, an excess of non-labeled target. In this case, specific binding is indicated if the binding of the labeled target to a probe is competitively inhibited by excess unlabeled target.

[0051] The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies *(e.g.* bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity. The term "immunoglobulin" (Ig) is used interchangeable with antibody herein.

[0052] Antibodies are naturally occurring immunoglobulin molecules which have varying structures, all based upon the immunoglobulin fold. For example, IgG antibodies have two "heavy" chains and two "light" chains that are disulphide-bonded to form a functional antibody. Each heavy and light chain itself comprises a "constant" (C) and a "variable" (V) region. The V regions determine the antigen binding specificity of the antibody, whilst the C regions provide structural support and function in non-antigen-specific interactions with immune effectors. The antigen binding specificity of an antibody or antigen-binding fragment of an antibody is the ability of an antibody to specifically bind to a particular antigen.

[0053] The antigen binding specificity of an antibody is determined by the structural characteristics of the V region. The variability is not evenly distributed across the 110-amino acid span of the variable domains. Instead, the V regions consist of relatively invariant stretches called framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-12 amino acids long. The variable domains of native heavy and light chains each comprise four FRs, largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (*see* Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

[0054] Each V region typically comprises three complementarity determining regions ("CDRs", each of which contains a "hypervariable loop"), and four framework regions. An antibody

binding site, the minimal structural unit required to bind with substantial affinity to a particular desired antigen, will therefore typically include the three CDRs, and at least three, preferably four, framework regions interspersed there between to hold and present the CDRs in the appropriate conformation. Classical four chain antibodies have antigen binding sites which are defined by V_H and V_L domains in cooperation. Certain antibodies, such as camel and shark antibodies, lack light chains and rely on binding sites formed by heavy chains only. Single domain engineered immunoglobulins can be prepared in which the binding sites are formed by heavy chains or light chains alone, in absence of cooperation between V_H and V_L.

[0055] The term "hypervariable region" when used herein refers to certain amino acid residues of an antibody that are responsible for antigen binding. The hypervariable region may comprise amino acid residues from a "complementarity determining region" or "CDR" as discussed above (*e.g.*, around about residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the V_L, and around about 31-35B (H1), 50-65 (H2) and 95-102 (H3) in the V_H (Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)) and/or those residues from a "hypervariable loop" (*e.g.* residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the V_L, and 26-32 (H1), 52A-55 (H2) and 96-101 (H3) in the V_H (Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)).

[0056] "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

[0057] "Antibody fragments" comprise a portion of an intact antibody, preferably comprising the antigen binding region thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; tandem diabodies (taDb), linear antibodies(*e.g.*, U.S. Patent No. 5,641,870, Example 2; Zapata *et al.*, *Protein Eng.* 8(10):1057-1062 (1995)); one-armed antibodies, single variable domain antibodies, minibodies, single-chain antibody molecules; multispecific antibodies formed from antibody fragments (*e.g.*, including but not limited to, Db-Fc, taDb-Fc, taDb-CH3, (scFV)4-Fc, di-scFv, bi-scFv, or tandem (di,tri)-scFv); and Bi-specific T-cell engagers (BiTEs).

[0058] Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-binding sites and is still capable of cross-linking antigen.

[0059] "Fv" is the minimum antibody fragment that contains a complete antigen-recognition and antigen-binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. It is in this configuration that the three

hypervariable regions of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six hypervariable regions confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0060] The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear at least one free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments that have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0061] The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

[0062] Depending on the amino acid sequence of the constant domain of their heavy chains, antibodies can be assigned to different classes. There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy chain constant domains that correspond to the different classes of antibodies are called α, δ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0063] "Single-chain Fv" or "scFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. In some embodiments, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains that enables the scFv to form the desired structure for antigen binding. For a review of scFv *see* Plückthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[0064] The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (V_H) connected to a light chain variable domain (V_L) in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are

described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al., Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993).

[0065] The term "multispecific antibody" is used in the broadest sense and specifically covers an antibody that has polyepitopic specificity. Such multispecific antibodies include, but are not limited to, an antibody comprising a heavy chain variable domain (V_H) and a light chain variable domain (V_L), where the V_HV_L unit has polyepitopic specificity, antibodies having two or more V_L and V_H domains with each V_HV_L unit binding to a different epitope, antibodies having two or more single variable domains with each single variable domain binding to a different epitope, full length antibodies, antibody fragments such as Fab, Fv, dsFv, scFv, diabodies, bispecific diabodies, triabodies, tri-functional antibodies, antibody fragments that have been linked covalently or non-covalently. "Polyepitopic specificity" refers to the ability to specifically bind to two or more different epitopes on the same or different target(s). "Monospecific" refers to the ability to bind only one epitope. According to one embodiment the multispecific antibody is an IgG antibody that binds to each epitope with an affinity of 5 μM to 0.001 pM, 3 μM to 0.001 pM, 1 μM to 0.001 pM, 0.5 μM to 0.001 pM, or 0.1 μM to 0.001 pM.

[0066] The expression "single domain antibodies" (sdAbs) or "single variable domain (SVD) antibodies" generally refers to antibodies in which a single variable domain (VH or VL) can confer antigen binding. In other words, the single variable domain does not need to interact with another variable domain in order to recognize the target antigen. Examples of single domain antibodies include those derived from camelids (lamas and camels) and cartilaginous fish (*e.g.*, nurse sharks) and those derived from recombinant methods from humans and mouse antibodies (Nature (1989) 341:544-546; Dev Comp Immunol (2006) 30:43-56; Trend Biochem Sci (2001) 26:230-235; Trends Biotechnol (2003):21:484-490; WO 2005/035572; WO 03/035694; Febs Lett (1994) 339:285-290; WO00/29004; WO 02/051870).

[0067] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variants that may arise during production of the monoclonal antibody, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed

as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the methods provided herein may be made by the hybridoma method first described by Kohler *et al., Nature* 256:495 (1975), or may be made by recombinant DNA methods (*see, e.g.*, U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al., Nature* 352:624-628 (1991) and Marks *et al., J. Mol. Biol.* 222:581-597 (1991), for example.

[0068] The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison *et al., Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984)). Chimeric antibodies of interest herein include "primatized" antibodies comprising variable domain antigen-binding sequences derived from a non-human primate *(e.g.* Old World Monkey, such as baboon, rhesus or cynomolgus monkey) and human constant region sequences (US Pat No. 5,693,780).

[0069] "Humanized" forms of non-human *(e.g.,* murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence, except for FR substitution(s) as noted above. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region, typically that of a human immunoglobulin. For further details, *see* Jones *et al., Nature* 321:522-525 (1986); Riechmann *et al., Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992).

[0070] For the purposes herein, an "intact antibody" is one comprising heavy and light variable domains as well as an Fc region. The constant domains may be native sequence constant domains (*e.g.* human native sequence constant domains) or amino acid sequence variant thereof. Preferably, the intact antibody has one or more effector functions.

[0071] "Native antibodies" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

[0072] "Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

[0073] In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

[0074] The terms "anti-IL-13 antibody" and "an antibody that binds to IL-13" refer to an antibody that is capable of binding IL-13 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting IL-13. In some embodiments, the extent of binding of an anti-IL-13 antibody to an unrelated, non-IL-13 protein is less than about 10% of the binding of the antibody to IL-13 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to IL-13 has a dissociation constant (Kd) of $\leq 1\mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g. 10-8 M or less, e.g. from 10-8 M to 10-13 M, e.g., from 10-9 M to 10-13 M). In certain embodiments, an anti-IL-13 antibody binds to an epitope of IL-13 that is conserved among IL-13 from different species.

[0075] "IL-13 mediated disorder" means a disorder associated with excess IL-13 levels or activity in which atypical symptoms may manifest due to the levels or activity of IL-13 locally and/or systemically in the body. Examples of IL-13 mediated disorders include: cancers (e.g., non-Hodgkin's lymphoma, glioblastoma), atopic dermatitis, allergic rhinitis, asthma, fibrosis, inflammatory bowel disease, Crohn's disease, lung inflammatory disorders (including pulmonary fibrosis such as IPF), COPD, and hepatic fibrosis.

[0076] The term "respiratory disorder" includes, but is not limited to, asthma (e.g., allergic and non-allergic asthma (e.g., due to infection, e.g., with respiratory syncytial virus (RSV), e.g., in younger children)); bronchitis (e.g., chronic bronchitis); chronic obstructive pulmonary disease (COPD) (e.g., emphysema (e.g., cigarette-induced emphysema); conditions involving airway inflammation, eosinophilia, fibrosis and excess mucus production, e.g., cystic fibrosis, pulmonary fibrosis, and allergic rhinitis. Examples of diseases that can be characterized by

airway inflammation, excessive airway secretion, and airway obstruction include asthma, chronic bronchitis, bronchiectasis, and cystic fibrosis.

[0077] The term "therapeutic agent" refers to any agent that is used to treat a disease. A therapeutic agent may be, for example, a polypeptide(s) (e.g., an antibody, an immunoadhesin or a peptibody), an aptamer or a small molecule that can bind to a protein or a nucleic acid molecule that can bind to a nucleic acid molecule encoding a target (i.e., siRNA), and the like.

[0078] A "naked antibody" is an antibody (as herein defined) that is not conjugated to a heterologous molecule, such as a cytotoxic moiety or radiolabel.

[0079] The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0080] The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

[0081] An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, *see, e.g.*, Flatman et al., *J. Chromatogr.* B 848:79-87 (2007).

[0082] The term "sequential" as used herein with regard to chromatography refers to having a first chromatography followed by a second chromatography. Additional steps may be included between the first chromatography and the second chromatography.

[0083] The term "continuous" as used herein with regard to chromatography refers to having a first chromatography material and a second chromatography material either directly connected or some other mechanism which allows for continuous flow between the two chromatography materials.

[0084] "Impurities" and "contaminants" refer to materials that are different from the desired polypeptide product. Impurities and contaminants include, without limitation: host cell materials, such as CHOP, including single CHOP species; leached Protein A; nucleic acid; a variant, fragment, aggregate or derivative of the desired polypeptide; another polypeptide; endotoxin; viral contaminant; cell culture media component, etc. In some examples, the contaminant may be a host cell protein (HCP) from, for example but not limited to, a bacterial cell such as an *E. coli* cell, an insect cell, a prokaryotic cell, a eukaryotic cell, a yeast cell, a mammalian cell, an avian cell, a fungal cell.

[0085] The terms "Chinese hamster ovary cell protein" and "CHOP" are used interchangeably to refer to a mixture of host cell proteins ("HCP") derived from a Chinese hamster ovary ("CHO") cell culture. The HCP or CHOP is generally present as an impurity in a cell culture medium or lysate (e.g., a harvested cell culture fluid ("HCCF")) comprising a protein of interest such as an antibody or immunoadhesin expressed in a CHO cell.) The amount of CHOP present in a mixture comprising a protein of interest provides a measure of the degree of purity for the protein of interest. HCP or CHOP includes, but is not limited to, a protein of interest expressed by the host cell, such as a CHO host cell. Typically, the amount of CHOP in a protein mixture is expressed in parts per million relative to the amount of the protein of interest in the mixture. It is understood that where the host cell is another mammalian cell type, an E. coli, a yeast, an insect cell, or a plant cell, HCP refers to the proteins, other than target protein, found in a lysate of the host cell.

[0086] The term "parts per million" or "ppm" are used interchangeably herein to refer to a measure of purity of the protein of interest purified by a method of the invention. The units ppm refer to the amount of HCP or CHOP in nanograms/milliliter per protein of interest in milligrams/milliliter (i.e., CHOP ppm = (CHOP ng/ml)/(protein of interest mg/ml), where the proteins are in solution). Where the proteins are dried (such as by lyophilization), ppm refers to (CHOP ng)/(protein of interest mg)). Impurities may also be expressed as "ng/mg" which is used interchangeably with ppm.

[0087] By "purifying" a polypeptide from a composition comprising the polypeptide and one or more impurities is meant increasing the degree of purity of the polypeptide in the composition by removing (completely or partially) at least one impurity from the composition.

[0088] A "purification step" may be part of an overall purification process resulting in a "homogeneous" composition, which is used herein to refer to a composition comprising less than 100ppm HCP (100 ng/mg) in a composition comprising the protein of interest, or less than 90ppm (90 ng/mg), or less than 80ppm (80 ng/mg), or less than 70ppm (70 ng/mg), or less than

60ppm (60 ng/mg), or less than 50ppm 50 ng/mg), or less than 40ppm (40 ng/mg), or less than 30ppm (30 ng/mg), or less than 20ppm (20 ng/mg), or less than 10ppm (10 ng/mg), or less than 5ppm (5 ng/mg), or less than 3ppm (3 ng/mg) or less than 1 ppm (1 ng/mg). In certain embodiments, the HCP is a single HCP species. In one embodiment, the single HCP species is hamster PLBL2.

[0089] The "composition" to be purified herein comprises the polypeptide of interest and one or more impurities or contaminants. The composition may be "partially purified" (i.e. having been subjected to one or more purification steps or may be obtained directly from a host cell or organism producing the polypeptide (e.g. the composition may comprise harvested cell culture fluid).

[0090] The terms "Protein A" and "ProA" are used interchangeably herein and encompasses Protein A recovered from a native source thereof, Protein A produced synthetically (e.g. by peptide synthesis or by recombinant techniques), and variants thereof which retain the ability to bind proteins which have a CH2/CH3 region, such as an Fc region. Protein A can be purchased commercially from various sources. Protein A is generally immobilized on a solid phase support material. The term "ProA" also refers to an affinity chromatography resin or column containing chromatographic solid support matrix to which is covalently attached Protein A.

[0091] The term "chromatography" refers to the process by which a solute of interest in a mixture is separated from other solutes in a mixture as a result of differences in rates at which the individual solutes of the mixture migrate through a stationary medium under the influence of a moving phase, or in bind and elute processes.

[0092] The term "affinity chromatography" and "protein affinity chromatography" are used interchangeably herein and refer to a protein separation technique in which a protein of interest or antibody of interest is reversibly and specifically bound to a biospecific ligand. Typically, the biospecific ligand is covalently attached to a chromatographic solid phase material and is accessible to the protein of interest in solution as the solution contacts the chromatographic solid phase material. The protein of interest (e.g., antibody, enzyme, or receptor protein) retains its specific binding affinity for the biospecific ligand (antigen, substrate, cofactor, or hormone, for example) during the chromatographic steps, while other solutes and/or proteins in the mixture do not bind appreciably or specifically to the ligand. Binding of the protein of interest to the immobilized ligand allows contaminating proteins or protein impurities to be passed through the chromatographic medium while the protein of interest remains specifically bound to the immobilized ligand on the solid phase material. The specifically bound protein of interest is then removed in active form from the immobilized ligand with low pH, high pH, high salt, competing

ligand, and the like, and passed through the chromatographic column with the elution buffer, free of the contaminating proteins or protein impurities that were earlier allowed to pass through the column. Any component can be used as a ligand for purifying its respective specific binding protein, e.g. antibody.

[0093] The terms "non-affinity chromatography" and "non-affinity purification" refer to a purification process in which affinity chromatography is not utilized. Non-affinity chromatography includes chromatographic techniques that rely on non-specific interactions between a molecule of interest (such as a protein, e.g. antibody) and a solid phase matrix.

[0094] The term "specific binding" as used herein in the context of chromatography, such as to describe interactions between a molecule of interest and a ligand bound to a solid phase matrix, refers to the generally reversible binding of a protein of interest to a ligand through the combined effects of spatial complementarity of protein and ligand structures at a binding site coupled with electrostatic forces, hydrogen bonding, hydrophobic forces, and/or van der Waals forces at the binding site. The greater the spatial complementarity and the stronger the other forces at the binding site, the greater will be the binding specificity of a protein for its respective ligand. Non-limiting examples of specific binding includes antibody-antigen binding, enzyme-substrate binding, enzyme-cofactor binding, metal ion chelation, DNA binding protein-DNA binding, regulatory protein-protein interactions, and the like. Typically, in affinity chromatography specific binding occurs with an affinity of about 10^{-4} to 10^{-8} M in free solution.

[0095] The term "non-specific binding" as used herein in the context of chromatography, such as to describe interactions between a molecule of interest and a ligand or other compound bound to a solid phase matrix, refers to binding of a protein of interest to the ligand or compound on a solid phase matrix through electrostatic forces, hydrogen bonding, hydrophobic forces, and/or van der Waals forces at an interaction site, but lacking structural complementarity that enhances the effects of the non-structural forces. Examples of non-specific interactions include, but are not limited to, electrostatic, hydrophobic, and van der Waals forces as well as hydrogen bonding.

[0096] A "salt" is a compound formed by the interaction of an acid and a base. Exemplary salts include, but are not limited to, acetate (e.g. sodium acetate), citrate (e.g. sodium citrate), chloride (e.g. sodium chloride), sulphate (e.g. sodium sulphate), or a potassium salt.

[0097] As used herein, "solvent" refers to a liquid substance capable of dissolving or dispersing one or more other substances to provide a solution. Solvents include aqueous and organic solvents, where certain organic solvents include a non-polar solvent, ethanol, methanol, isopropanol, acetonitrile, hexylene glycol, propylene glycol, and 2,2-thiodiglycol.

[0098] The term "detergent" refers to ionic and nonionic surfactants such as polysorbates (e.g. polysorbates 20 or 80); poloxamers (e.g. poloxamer 188); Triton; sodium dodecyl sulfate (SDS); sodium laurel sulfate; sodium octyl glycoside; lauryl-, myristyl-, linoleyl-, or stearyl-sulfobetaine; lauryl-, myristyl-, linoleyl- or stearyl-sarcosine; linoleyl-, myristyl-, or cetyl-betaine; lauroamidopropyl-, cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-betaine (e.g. lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl oleyl-taurate; and the MONAQUAT(tm) series (Mona Industries, Inc., Paterson, New Jersey), polysorbate, such as polysorbate 20 (TWEEN 20(r)) or polysorbate 80 (TWEEN 80(r)).

[0099] A "polymer" herein is a molecule formed by covalent linkage of two or more monomers, where the monomers are not amino acid residues. Examples of polymers include, but are not limited to, polyethyl glycol, polypropyl glycol, and copolymers (e.g. PLURONICS™, PF68 etc), polyethylene glycol (PEG), e.g. PEG 400 and PEG 8000.

[00100] The term "ion-exchange" and "ion-exchange chromatography" refers to the chromatographic process in which a solute of interest (such as a protein) in a mixture interacts with a charged compound linked (such as by covalent attachment) to a solid phase ion exchange material such that the solute of interest interacts non-specifically with the charged compound more or less than solute impurities or contaminants in the mixture. The contaminating solutes in the mixture elute from a column of the ion exchange material faster or slower than the solute of interest or are bound to or excluded from the resin relative to the solute of interest. "Ion-exchange chromatography" specifically includes cation exchange, anion exchange, and mixed mode chromatography.

[00101] The phrase "ion exchange material" refers to a solid phase that is negatively charged (i.e. a cation exchange resin) or positively charged (i.e. an anion exchange resin). The charge may be provided by attaching one or more charged ligands to the solid phase, e.g. by covalent linking. Alternatively, or in addition, the charge may be an inherent property of the solid phase (e.g. as is the case for silica, which has an overall negative charge).

[00102] By "solid phase" is meant a non-aqueous matrix to which one or more charged ligands can adhere. The solid phase may be a purification column, a discontinuous phase of discrete particles, a membrane, or filter etc. Examples of materials for forming the solid phase include polysaccharides (such as agarose and cellulose); and other mechanically stable matrices such as silica (e.g. controlled pore glass), poly(styrenedivinyl)benzene, polyacrylamide, ceramic particles and derivatives of any of the above.

[00103] A "cation exchange resin" refers to a solid phase which is negatively charged, and which thus has free cations for exchange with cations in an aqueous solution passed over or through the solid phase. A negatively charged ligand attached to the solid phase to form the cation exchange resin may, e.g., be a carboxylate or sulfonate. Commercially available cation exchange resins include, but are not limited to, carboxy-methyl-cellulose, sulphopropyl (SP) immobilized on agarose (e.g. SP-SEPHAROSE FAST FLOW(or SP-SEPHAROSE HIGH PERFORMANCE) and sulphonyl immobilized on agarose (e.g. S-SEPHAROSE FAST FLOW), and POROS®HS.

[00104] A "mixed mode ion exchange resin" refers to a solid phase which is covalently modified with cationic, anionic, and hydrophobic moieties. Mixed mode ion exchange is also referred to as "multimodal ion exchange." Commercially available mixed mode ion exchange resin are available, e.g., BAKERBOND ABX containing weak cation exchange groups, a low concentration of anion exchange groups, and hydrophobic ligands attached to a silica gel solid phase support matrix. Additional exemplary mixed mode ion exchange resins include, but are not limited to, CAPTO™ Adhere resin, QMA resin, CAPTO™ MMC resin, MEP HyperCel resin, HEA HyperCel resin, PPA HyperCel resin, or ChromaSorb membrane or Sartobind STIC. In some embodiments, the mixed mode material is CAPTO™ Adhere resin.

[00105] The term "anion exchange resin" is used herein to refer to a solid phase which is positively charged, e.g. having one or more positively charged ligands, such as quaternary amino groups, attached thereto. Commercially available anion exchange resins include DEAE cellulose, QAE SEPHADEX and FAST Q SEPHAROSE™ and Q SEPHAROSE™ FAST FLOW.

[00106] A "buffer" is a solution that resists changes in pH by the action of its acid-base conjugate components. Various buffers which can be employed depending, for example, on the desired pH of the buffer are described in Buffers. A Guide for the Preparation and Use of Buffers in Biological Systems, Gueffroy, D., ed. Calbiochem Corporation (1975). In certain instances, the buffer has a pH in the range from about 2 to about 9, alternatively from about 3 to about 8, alternatively from about 4 to about 7 alternatively from about 5 to about 7. Non-limiting examples of buffers that will control the pH in this range include MES, MOPS, MOPSO, Tris, HEPES, phosphate, acetate, citrate, succinate, and ammonium buffers, as well as combinations of these.

[00107] The term "hydrophobic interaction chromatography" or "HIC" is used herein to refer to a chromatographic process that separates molecule based on their hydrophobicity. Exemplary resins that can be used for HIC include, but are not limited to phenyl-, butyl-, octyl-

SEPHAROSE, BUTYL-SEPHAROSE® 4 Fast Flow, PHENYL SEPHAROSE™ High Performance, PHENYL SEPHAROSE™ 6 Fast Flow (low sub), and PHENYL SEPHAROSE™ 6 Fast Flow (high sub). Typically, sample molecules in a high salt buffer are loaded onto the HIC column. The salt in the buffer interacts with water molecules to reduce the solvation of the molecules in solution, thereby exposing hydrophobic regions in the sample molecules which are consequently adsorbed by the HIC column. The more hydrophobic the molecule, the less salt needed to promote binding. Typically, a decreasing salt gradient is used to elute samples from the column. As the ionic strength decreases, the exposure of the hydrophilic regions of the molecules increases and molecules elute from the column in order of increasing hydrophobicity. Sample elution may also be achieved by the addition of mild organic modifiers or detergents to the elution buffer.

[00108] The "loading buffer" is that which is used to load the composition comprising the polypeptide molecule of interest and one or more impurities onto the ion exchange resin. The loading buffer has a conductivity and/or pH such that the polypeptide molecule of interest (and generally one or more impurities) is/are bound to the ion exchange resin or such that the protein of interest flows through the column while the impurities bind to the resin.

[00109] The "intermediate buffer" is used to elute one or more impurities from the ion exchange resin, prior to eluting the polypeptide molecule of interest. The conductivity and/or pH of the intermediate buffer is/are such that one or more impurity is eluted from the ion exchange resin, but not significant amounts of the polypeptide of interest.

[00110] The term "wash buffer" when used herein refers to a buffer used to wash or re-equilibrate the ion exchange resin, prior to eluting the polypeptide molecule of interest. In certain instances, for convenience, the wash buffer and loading buffer may be the same, but this is not required.

[00111] The "elution buffer" is used to elute the polypeptide of interest from the solid phase. The conductivity and/or pH of the elution buffer is/are such that the polypeptide of interest is eluted from the ion exchange resin.

[00112] A "regeneration buffer" may be used to regenerate the ion exchange resin such that it can be re-used. The regeneration buffer has a conductivity and/or pH as required to remove substantially all impurities and the polypeptide of interest from the ion exchange resin.

[00113] The term "conductivity" refers to the ability of an aqueous solution to conduct an electric current between two electrodes. In solution, the current flows by ion transport. Therefore, with an increasing amount of ions present in the aqueous solution, the solution will have a higher conductivity. The unit of measurement for conductivity is milliSeimens per

centimeter (mS/cm), and can be measured using a conductivity meter sold, e.g., by Orion. The conductivity of a solution may be altered by changing the concentration of ions therein. For example, the concentration of a buffering agent and/or concentration of a salt (e.g. NaCl or KCl) in the solution may be altered in order to achieve the desired conductivity.

[00114] The "pI" or "isoelectric point" of a polypeptide refer to the pH at which the polypeptide's positive charge balances its negative charge. pI can be calculated from the net charge of the amino acid residues or sialic acid residues of attached carbohydrates of the polypeptide or can be determined by isoelectric focusing.

[00115] By "binding" a molecule to an ion exchange material is meant exposing the molecule to the ion exchange material under appropriate conditions (pH/conductivity) such that the molecule is reversibly immobilized in or on the ion exchange material by virtue of ionic interactions between the molecule and a charged group or charged groups of the ion exchange material.

[00116] By "washing" the ion exchange material is meant passing an appropriate buffer through or over the ion exchange material.

[00117] To "elute" a molecule (e.g. polypeptide or impurity) from an ion exchange material is meant to remove the molecule therefrom by altering the ionic strength of the buffer surrounding the ion exchange material such that the buffer competes with the molecule for the charged sites on the ion exchange material.

[00118] "Ultrafiltration" is a form of membrane filtration in which hydrostatic pressure forces a liquid against a semipermeable membrane. Suspended solids and solutes of high molecular weight are retained, while water and low molecular weight solutes pass through the membrane. In some examples, ultrafiltration membranes have pore sizes in the range of 1 to 100 nm. The terms "ultrafiltration membrane" and "ultrafiltration filter" may be used interchangeably.

[00119] "Diafiltration" is a method that incorporates ultrafiltration membranes to remove salts or other microsolutes from a solution. Small molecules are separated from a solution while retaining larger molecules in the retentate. The process selectively utilizes permeable (porous) membrane filters to separate the components of solutions and suspensions based on their molecular size.

[00120] As used herein, "filtrate" refers to that portion of a sample that passes through the filtration membrane.

[00121] As used herein, "retentate" refers to that portion of a sample that is substantially retained by the filtration membrane.

[00122] The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[00123] A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[00124] As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies are used to delay development of a disease or to slow the progression of a disease.

[00125] Reference to "about" a value or parameter herein includes (and describes) variations that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X".

ANTI-IL13 ANTIBODIES

[00126] In some embodiments, isolated and purified antibodies that bind IL-13 are provided. Exemplary anti-IL13 antibodies are known and include, for example, but not limited to, lebrikizumab, IMA-026, IMA-638 (also referred to as, anrukinzumab, INN No. 910649-32-0; QAX-576), tralokinumab (also referred to as CAT-354, CAS No. 1044515-88-9); AER-001, ABT-308 (also referred to as humanized 13C5.5 antibody. Examples of such anti-IL13 antibodies and other inhibitors of IL13 are disclosed, for example, in WO 2005/062967, WO2008/086395, WO2006/085938, US 7,615,213, US 7,501,121, WO2007/036745, WO2010/073119, WO2007/045477. In one embodiment, the anti-IL13 antibody is a humanized IgG4 antibody. In one embodiment, the anti-IL13 antibody is lebrikizumab. In one embodiment, the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 (SEQ ID NO.: 1), CDR-H2 (SEQ ID NO.: 2), and CDR-H3 (SEQ ID NO.: 3). In one embodiment, the anti-IL13 antibody comprises three light chain CDRS, CDR-L1 (SEQ ID NO.: 4), CDR-L2 (SEQ ID NO.: 5), and CDR-L3 (SEQ ID NO.: 6). In one embodiment, the anti-IL13 antibody comprises three heavy chain CDRs and three light chain CDRs, CDR-H1 (SEQ ID NO.: 1),

CDR-H2 (SEQ ID NO.: 2), CDR-H3 (SEQ ID NO.: 3), CDR-L1 (SEQ ID NO.: 4), CDR-L2 (SEQ ID NO.: 5), and CDR-L3 (SEQ ID NO.: 6). In one embodiment, the anti-IL13 antibody comprises a variable heavy chain region, VH, having an amino acid sequence selected from SEQ ID NOs. 7 and 8. In one embodiment, the anti-IL13 antibody comprises a variable light chain region, VL, having the amino acid sequence of SEQ ID NO.: 9. In one embodiment, the anti-IL13 antibody comprises a variable heavy chain region, VH, having an amino acid sequence selected from SEQ ID NOs. 7 and 8 and a variable light chain region, VL, having an amino acid sequence of SEQ ID NO.: 9. In one embodiment, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10 or SEQ ID NO.: 11 or SEQ ID NO.: 12 or SEQ ID NO.: 13. In one embodiment, the anti-IL13 antibody comprises a light chain having the amino acid sequence of SEQ ID NO.: 14. In one embodiment, the anti-IL13 antibody comprises a heavy chain having an amino acid sequence selected from SEQ ID NO.: 10, SEQ ID NO.: 11, SEQ ID NO.: 12, and SEQ ID NO.: 13 and a light chain having the amino acid sequence of SEQ ID NO.: 14.

[00127] In another aspect, an anti-IL-13 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO.: 8. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-IL-13 antibody comprising that sequence retains the ability to bind to human IL-13. In certain embodiments, a total of 1 to 10 amino acids have been substituted, altered inserted and/or deleted in SEQ ID NO.: 8. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the CDRs (i.e., in the FRs). Optionally, the anti-IL13 antibody comprises the VH sequence in SEQ ID NO.: 8, including post-translational modifications of that sequence.

[00128] In another aspect, an anti-IL-13 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-IL-13 antibody comprising that sequence retains the ability to bind to IL-13. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO.: 9. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the CDRs (i.e., in the FRs).

Optionally, the anti-IL-13 antibody comprises the VL sequence in SEQ ID NO.: 9, including post-translational modifications of that sequence.

[00129] In yet another embodiment, the anti-IL-13 antibody comprises a VL region having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO.: 9 and a VH region having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO.: 8.

[00130] The table below shows the amino acid sequences of the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3 regions of lebrikizumab, along with VH, VL, heavy chain sequences and light chain sequences. As indicated in Table 1 below, VH and the heavy chain may include an N-terminal glutamine and the heavy chain may also include a C-terminal lysine. As is well known in the art, N-terminal glutamine residues can form pyroglutamate and C-terminal lysine residues can be clipped during manufacturing processes.

Table 1. Anti-IL13 antibody (lebrikizumab) amino acid sequences.

CDR-H1 (SEQ ID NO.:1)	Ala Tyr Ser Val Asn
CDR-H2 (SEQ ID NO.:2)	Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys Ser
CDR-H3 (SEQ ID NO.:3)	Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn
CDR-L1 (SEQ ID NO.:4)	Arg Ala Ser Lys Ser Val Asp Ser Tyr Gly Asn Ser Phe Met His
CDR-L2 (SEQ ID NO.:5)	Leu Ala Ser Asn Leu Glu Ser
CDR-L3 (SEQ ID NO.:6)	Gln Gln Asn Asn Glu Asp Pro Arg Thr
VH (SEQ ID NO.:7)	Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ala Tyr Ser Val Asn Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu Ala Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala Gly Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser
VH (SEQ ID NO.:8)	Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ala Tyr Ser Val Asn Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu Ala Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys

	Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala Gly Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser
VL (SEQ ID NO.:9)	Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ser Val Ser Leu Gly Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Lys Ser Val Asp Ser Tyr Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Asn Asn Glu Asp Pro Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
H Chain (SEQ ID NO.:10)	VTLRESGPA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGDGY YPYAMDNWGQ GSLVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTKTYT CNVDHKPSNT KVDKRVESKY GPPCPPCPAP EFLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLG
H Chain (SEQ ID NO.:11)	QVTLRESGPA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGDGY YPYAMDNWGQ GSLVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTKTYT CNVDHKPSNT KVDKRVESKY GPPCPPCPAP EFLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLG
H Chain (SEQ ID NO.:12)	VTLRESGPA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGDGY YPYAMDNWGQ GSLVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTKTYT CNVDHKPSNT KVDKRVESKY GPPCPPCPAP EFLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLGK
H Chain (SEQ ID NO.:13)	QVTLRESGPA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGDGY YPYAMDNWGQ GSLVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTKTYT CNVDHKPSNT KVDKRVESKY GPPCPPCPAP EFLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLGK
L Chain (SEQ ID NO.: 14)	DIVMTQSPDS LSVSLGERAT INCRASKSVD SYGNSFMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQNNEDPR TFGGGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC

OTHER RECOMBINANT POLYPEPTIDES

[00131] Recombinant polypeptides produced in CHO cells may be purified according to the methods described herein to remove or reduce levels of hamster PLBL2 such that only residual

amounts or an undetectable amount remain. Such polypeptides include, without limitation, growth factors, cytokines, immunoglobulins, antibodies, peptibodies and the like.

[00132] Certain exemplary antibodies include antibodies to Abeta, antibodies to IL17A/F and antibodies to CMV. Exemplary anti-Abeta antibodies and methods of producing such antibodies have been described previously, for example, in WO2008011348, WO2007068429, WO2001062801, and WO2004071408. Exemplary anti-IL17 A/F antibodies and methods of producing such antibodies have been described previously, for example, in WO 2009136286 and U.S. Patent No. 8,715,669. Exemplary anti-CMV antibodies, including anti-CMV-MSL, and methods of producing such antibodies have been described previously, for example, in WO 2012047732.

[00133] Exemplary polypeptides include include mammalian proteins, such as, *e.g.,* CD4, integrins and their subunits, such as beta7, growth hormone, including human growth hormone and bovine growth hormone; growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; lipoproteins; ct-l-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor, and von Willebrands factor; anti-clotting factors such as Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator, such as urokinase or tissue-type plasminogen activator (t-PA, *e.g.,* Activase®, TNKase®, Retevase®); bombazine; thrombin; tumor necrosis factor-α and -β; enkephalinase; RANTES (regulated on activation normally T-cell expressed and secreted); human macrophage inflammatory protein (MIP-I-a); serum albumin such as human serum albumin; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; DNase; inhibin; activin; vascular endothelial growth factor (VEGF); IgE, receptors for hormones or growth factors; an integrin; protein A or D; rheumatoid factors; a neurotrophic factor such as bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6), or a nerve growth factor such as NGF-β; platelet-derived growth factor (PDGF); fibroblast growth factor such as aFGF and bFGF; epidermal growth factor (EGF); transforming growth factor (TGF) such as TGF–α and TGF-β including TGF-β1, TGF-β2, TGF-β3, TGF-β4, or TGF-β5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(1-3)-IGF-I (brain IGF-I); insulin-like growth factor binding proteins; other CD proteins such as CD3, CD8, CD19 and CD20; erythropoietin (EPO); thrombopoietin (TPO); osteoinductive factors; immunotoxins; a bone morphogenetic protein (BMP); an interferon such as interferon-α, -β, or –γ; colony stimulating factors (CSFs), *e.g.,* M-CSF, GM-CSF, and G-CSF; interleukins (ILs), *e.g.,* IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25,

IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33 and so on; superoxide dismutase; T-cell receptors; surface membrane proteins; decay accelerating factor (DAF); a viral antigen such as, for example, a portion of an HIV envelope; transport proteins; homing receptors; addressins; regulatory proteins; integrins such as CDlla, CDllb, CDllc, CD18, integrin subunits such alpha4, alphaE, beta7; cellular adhesion molecules such as an ICAM, VLA-4 and VCAM; a tumor associated antigen such as HER1, (EGFR), HER2, HER3 or HER4 receptor; Apo2L/TRAIL, and fragments of any of the above listed polypeptides; as well as immunoadhesins and antibodies binding to; and biologically active fragments or variants of any of the above-listed proteins.

[00134] Additional exemplary polypeptides include brain polypeptides, including but not limited to, beta-secretase 1 (BACE1), Abeta, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), tau, apolipoprotein E (ApoE), alpha-synuclein, CD20, huntingtin, prion protein (PrP), leucine rich repeat kinase 2 (LRRK2), parkin, presenilin 1, presenilin 2, gamma secretase, death receptor 6 (DR6), amyloid precursor protein (APP), p75 neurotrophin receptor (p75NTR), P-selectin, and caspase 6, and fragments of any of the above listed polypeptides; as well as immunoadhesins and antibodies binding to; and biologically active fragments or variants of any of the above-listed proteins.

[00135] Further exemplary polypeptides include therapeutic antibodies and immunoadhesins, including, without limitation, antibodies, including antibody fragments, to one or more of the following antigens: HER1 (EGFR), HER2 (e.g., trastuzumab, pertuzumab), HER3, HER4, VEGF (e.g., bevacizumab, ranibizumab), MET (e.g., onartuzumab), CD20 (e.g., rituximab, obinutuzumab, ocrelizumab), CD22, CD11a, CD11b, CDllc, CD18, an ICAM, VLA-4, VCAM, IL-17A and/or F, IgE (e.g., omalizumab), DR5, CD40, Apo2L/TRAIL, EGFL7 (e.g., parsatuzumab), NRP1, integrin beta7 (e.g., etrolizumab), IL-13 (e.g., lebrikizumab), Abeta (e.g., crenezumab, gantenerumab), P-selectin (e.g., inclacumab), IL-6R (e.g., tociluzumab), IFNα (e.g., rontalizumab), M1prime (e.g., quilizumab), mitogen activated protein kinase (MAPK), OX40L, TSLP, Factor D (e.g., lampalizumab) and receptors such as: IL-9 receptor, IL-5 receptor, IL-4receptor alpha, IL-13receptoralpha1 and IL-13receptoralpha2, OX40, TSLP-R, IL-7Ralpha (a co-receptor for TSLP), IL17RB (receptor for IL-25), ST2 (receptor for IL-33), CCR3, CCR4, CRTH2, FcepsilonRI and FcepsilonRII/CD23 (receptors for IgE). Other exemplary antibodies include those selected from, and without limitation, antiestrogen receptor antibody, anti-progesterone receptor antibody, anti-p53 antibody, anticathepsin D antibody, anti-Bcl-2 antibody, anti-E-cadherin antibody, anti-CA125 antibody, anti- CA15-3 antibody, anti-CA19-9 antibody, anti-c-erbB-2 antibody, anti-P-glycoprotein antibody, anti-CEA antibody,

anti-retinoblastoma protein antibody, anti-ras oncoprotein antibody, anti-Lewis X antibody, anti-Ki-67 antibody, anti-PCNA antibody, anti-CD3 antibody, anti-CD4 antibody, anti-CD5 antibody, anti-CD7 antibody, anti-CD8 antibody, anti-CD9/p24 antibody, anti-CD10 antibody, anti-CDllc antibody, anti-CD13 antibody, anti-CD14 antibody, anti-CD15 antibody, anti-CD19 antibody, anti-CD23 antibody, anti-CD30 antibody, anti-CD31 antibody, anti-CD33 antibody, anti-CD34 antibody, anti-CD35 antibody, anti-CD38 antibody, anti-CD41 antibody, anti-LCA/CD45 antibody, anti-CD45RO antibody, anti-CD45RA antibody, anti-CD39 antibody, anti-CD100 antibody, anti-CD95/Fas antibody, anti-CD99 antibody, anti-CD106 antibody, anti-ubiquitin antibody, anti-CD71 antibody, anti-c-myc antibody, anti-cytokeratins antibody, anti-vimentins antibody, anti-HPV proteins antibody, anti-kappa light chains antibody, anti-lambda light chains antibody, anti-melanosomes antibody, anti-prostate specific antigen antibody, anti-S-100 antibody, anti-tau antigen antibody, anti-fibrin antibody, anti-keratins antibody and anti-Tn-antigen antibody.

CERTAIN PURIFICATION METHODS

[00136] The protein to be purified using the methods described herein is generally produced using recombinant techniques. Methods for producing recombinant proteins are described, *e.g.*, in US Pat No's 5,534,615 and 4,816,567, specifically incorporated herein by reference. In certain embodiments, the protein of interest is produced in a CHO cell (see, *e.g.* WO 94/11026). Examples of proteins, including anti-IL13 monoclonal antibodies (anti-IL13 MAb), which can be purified using the processes described herein have been described above.

[00137] When using recombinant techniques, the protein can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the protein is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration. Where the protein is secreted into the medium, the recombinant host cells may be separated from the cell culture medium by tangential flow filtration, for example.

[00138] Protein A immobilized on a solid phase is used to purify the anti-IL13 MAb preparation. In certain embodiments, the solid phase is a column comprising a glass, silica, agarose or polystyrene surface for immobilizing the Protein A. In certain embodiments, the solid phase is a controlled pore glass column or a silicic acid column. Sometimes, the column has been coated with a reagent, such as glycerol, in an attempt to prevent nonspecific adherence to the column. The PROSEP A™ column, commercially available from Bioprocessing Limited, is an example of a Protein A controlled pore glass column which is coated with glycerol. Other examples of columns contemplated herein include the POROS® 50 ATM (polystyrene) column

or rProtein A SEPHAROSE FAST FLOW™ (agarose) column or MABSELECT SURE™ (agarose) column available from GE Healthcare Life Sciences (agarose).

[00139] The solid phase for the Protein A chromatography is equilibrated with a suitable buffer. For example, the equilibration buffer may be 25mM Tris, 25mM NaCl, pH 7.70 ± 0.20.

[00140] The preparation derived from the recombinant host cells and containing impurities and/or contaminants is loaded on the equilibrated solid phase using a loading buffer which may be the same as the equilibration buffer. As the preparation containing impurities/contaminants flows through the solid phase, the protein is adsorbed to the immobilized Protein A and other impurities/contaminants (such as Chinese Hamster Ovary Proteins, CHOP, where the protein is produced in a CHO cell) may bind nonspecifically to the solid phase.

[00141] The next step performed sequentially entails removing the impurities/contaminants bound to the solid phase, antibody and/or Protein A, by washing the solid phase in an intermediate wash step. After loading, the solid phase may be equilibrated with equilibration buffer before beginning the intermediate wash step.

[00142] The intermediate wash buffer may comprise salt and optionally a further compound, such as (a) detergent (for example, polysorbate, e.g. polysorbate 20 or polysorbate 80); (b) solvent (such as hexylene glycol); and (c) polymer (such as polyethylene glycol {PEG]).

[00143] The salt employed may be selected based on the protein of interest. Exemplary salts include, but are not limited to, sodium acetate, sodium citrate, and potassium phosphate.

[00144] The amounts of the salt and further compound (if any) in the composition are such that the combined amount elutes the impurity(ies)/contaminant(s), without substantially removing the protein of interest. Exemplary salt concentrations in such wash buffers are from about 0.1 to about 2M, or from about 0.2M to about 0.6M. Useful detergent concentrations are from about 0.01 to about 5%, or from about 0.1 to 1%, or about 0.5%, e.g. where the detergent is polysorbate. Exemplary solvent concentrations are from about 1% to 40%, or from about 5 to about 25%. Where the further compound is a polymer (e.g. PEG 400 or PEG 8000), the concentration thereof may, for example, be from about 1% to about 20%, or from about 5% to about 15%.

[00145] The pH of the intermediate wash buffer is typically from about 4 to about 8, or from about 4.5 to about 5.5, or about 5.0. In one embodiment, the pH is 7.00 ± 0.10.

[00146] Following the intermediate wash step described above, the protein of interest is recovered from the column. This is typically achieved using a suitable elution buffer. The protein may, for example, be eluted from the column using an elution buffer having a low pH (also referred to as acidic conditions), e.g. in the range from about 2 to about 5, or in the range

from about 2.5 to about 3.5. Examples of elution buffers for this purpose include citrate or acetate buffers.

[00147] The eluted protein preparation may be subjected to additional purification steps either prior to, or after, the Protein A chromatography step. Exemplary further purification steps include hydroxyapatite chromatography; dialysis; affinity chromatography using an antibody to capture the protein; hydrophobic interaction chromatography (HIC); ammonium sulphate precipitation; anion or cation exchange chromatography; ethanol precipitation; reverse phase HPLC; chromatography on silica; chromatofocusing; ultrafiltration-diafiltration (UFDF), and gel filtration. In the examples herein, the Protein A chromatography step is followed by downstream anion exchange (e.g., Q-Sepharose-Fast Flow) or multimodal (e.g. mixed-mode) ion exchange (e.g., CAPTO™ Adhere) and HIC (e.g., PHENYL SEPHAROSE™ 6 fast flow - high sub) purification steps.

[00148] The protein thus recovered may be formulated in a pharmaceutically acceptable carrier and is used for various diagnostic, therapeutic or other uses known for such molecules.

[00149] In some embodiments of any of the methods described herein, the chromatography material is an ion exchange chromatography material; for example, an anion exchange chromatography material. In some embodiments, the anion exchange chromatography material is a solid phase that is positively charged and has free anions for exchange with anions in an aqueous solution passed over or through the solid phase. In some embodiments of any of the methods described herein, the anion exchange material may be a membrane, a monolith, or resin. In an embodiment, the anion exchange material may be a resin. In some embodiments, the anion exchange material may comprise a primary amine, a secondary amine, a tertiary amine or a quarternary ammonium ion functional group, a polyamine functional group, or a diethylaminoaethyl functional group. In some embodiments of the above, the anion exchange chromatography material is an anion exchange chromatography column. In some embodiments of the above, the anion exchange chromatography material is an anion exchange chromatography membrane.

[00150] In some embodiments of any of the methods described herein, the ion exchange material may utilize a conventional chromatography material or a convective chromatography material. The conventional chromatography materials include, for example, perfusive materials (*e.g.*, poly(styrene-divinylbenzene) resin) and diffusive materials (*e.g.*, cross-linked agarose resin). In some embodiments, the poly(styrene-divinylbenzene) resin can be POROS® resin. In some embodiments, the cross-linked agarose resin may be sulphopropyl-Sepharose Fast Flow ("SPSFF") resin. The convective chromatography material may be a membrane (*e.g.*,

polyethersulfone) or monolith material (*e.g.* cross-linked polymer). The polyethersulfone membrane may be Mustang. The cross-linked polymer monolith material may be cross-linked poly(glycidyl methacrylate-co-ethylene dimethacrylate).

[00151] Examples of anion exchange materials include, but are not limited to, POROS® HQ 50, POROS® PI 50, POROS® D, Mustang Q, Q SEPHAROSE™ FF, and DEAE Sepharose.

[00152] In some aspects, the chromatography material is a hydrophobic interaction chromatography material. Hydrophobic interaction chromatography (HIC) is a liquid chromatography technique that separates biomolecules according to hydrophobicity. Examples of HIC chromatography materials include, but are not limited to, Toyopearl hexyl 650, Toyopearl butyl 650, Toyopearl phenyl 650, Toyopearl ether 650, Source, Resource, Sepharose Hi-Trap, Octyl sepharose, PHENYL SEPHAROSE™ high performance, PHENYL SEPHAROSE™ 6 fast flow (low sub) and PHENYL SEPHAROSE™ 6 fast flow (high sub). In some embodiments of the above, the HIC chromatography material is a HIC chromatography column. In some embodiments of the above, the HIC chromatography material is a HIC chromatography membrane.

[00153] In some aspects, the chromatography material is an affinity chromatography material. Examples of affinity chromatography materials include, but are not limited to chromatography materials derivatized with protein A or protein G. Examples of affinity chromatography material include, but are not limited to, Prosep-VA, Prosep-VA Ultra Plus, Protein A sepharose fast flow, Tyopearl Protein A, MAbSelect, MABSELECT SURE™ and MABSELECT SURE™ LX. In some embodiments of the above, the affinity chromatography material is an affinity chromatography column. In some embodiments of the above, the affinity chromatography material is an affinity chromatography membrane.

[00154] Various buffers which can be employed depending, for example, on the desired pH of the buffer, the desired conductivity of the buffer, the characteristics of the protein of interest, and the purification method. In some embodiments of any of the methods described herein, the methods comprise using a buffer. The buffer can be a loading buffer, an equilibration buffer, or a wash buffer. In some embodiments, one or more of the loading buffer, the equilibration buffer, and/or the wash buffer are the same. In some embodiments, the loading buffer, the equilibration buffer, and/or the wash buffer are different. In some embodiments of any of the methods described herein, the buffer comprises a salt. The loading buffer may comprise sodium chloride, sodium acetate, or a mixture thereof. In some embodiments, the loading buffer is a sodium chloride buffer. In some embodiments, the loading buffer is a sodium acetate buffer.

[00155] Load, as used herein, is the composition loaded onto a chromatography material. Loading buffer is the buffer used to load the composition comprising the product of interest onto a chromatography material. The chromatography material may be equilibrated with an equilibration buffer prior to loading the composition which is to be purified. In some examples, the wash buffer is used after loading the composition onto a chromatography material and before elution of the polypeptide of interest from the solid phase. However, some of the product of interest, *e.g.* a polypeptide, may be removed from the chromatography material by the wash buffer (*e.g.* flow-through mode).

[00156] Elution, as used herein, is the removal of the product, *e.g.* polypeptide, from the chromatography material. Elution buffer is the buffer used to elute the polypeptide or other product of interest from a chromatography material. In many cases, an elution buffer has a different physical characteristic than the load buffer. For example, the elution buffer may have a different conductivity than load buffer or a different pH than the load buffer. In some embodiments, the elution buffer has a lower conductivity than the load buffer. In some embodiments, the elution buffer has a higher conductivity than the load buffer. In some embodiments, the elution buffer has a lower pH than the load buffer. In some embodiments, the elution buffer has a higher pH than the load buffer. In some embodiments the elution buffer has a different conductivity and a different pH than the load buffer. The elution buffer can have any combination of higher or lower conductivity and higher or lower pH.

[00157] Conductivity refers to the ability of an aqueous solution to conduct an electric current between two electrodes. In solution, the current flows by ion transport. Therefore, with an increasing amount of ions present in the aqueous solution, the solution will have a higher conductivity. The basic unit of measure for conductivity is the Siemen (or mho), mho (mS/cm), and can be measured using a conductivity meter, such as various models of Orion conductivity meters. Since electrolytic conductivity is the capacity of ions in a solution to carry electrical current, the conductivity of a solution may be altered by changing the concentration of ions therein. For example, the concentration of a buffering agent and/or the concentration of a salt (*e.g.* sodium chloride, sodium acetate, or potassium chloride) in the solution may be altered in order to achieve the desired conductivity. Preferably, the salt concentration of the various buffers is modified to achieve the desired conductivity.

[00158] In some embodiments of any of the methods described herein, the flow rate is less than about any of 50 CV/hr, 40 CV/hr, or 30 CV/hr. The flow rate may be between about any of 5 CV/hr and 50 CV/hr, 10 CV/hr and 40 CV/hr, or 18 CV/hr and 36 CV/hr. In some embodiments, the flow rate is about any of 9 CV/hr, 18 CV/hr, 25 CV/hr, 30 CV/hr, 36 CV/hr, or 40 CV/hr. In

some embodiments of any of the methods described herein, the flow rate is less than about any of 100 cm/hr, 75 cm/hr, or 50 cm/hr. The flow rate may be between about any of 25 cm/hr and 150 cm/hr, 25 cm/hr and 100 cm/hr, 50 cm/hr and 100 cm/hr, or 65 cm/hr and 85 cm/hr, or 50 cm/hr and 250 cm/hr, or 100 cm/hr and 250 cm/hr, or 150 cm/hr and 250 cm/hr.

[00159] Bed height is the height of chromatography material used. In some embodiments of any of the method described herein, the bed height is greater than about any of 3 cm, 10 cm, or 15 cm. The bed height may be between about any of 3 cm and 35 cm, 5 cm and 15 cm, 3 cm and 10 cm, or 5 cm and 8 cm. In some embodiments, the bed height is about any of 3 cm, 5 cm, 10 cm, or 15 cm. In some embodiments, bed height is determined based on the amount of polypeptide or contaminants in the load.

[00160] In some embodiments, the chromatography is in a column of vessel with a volume of greater than about 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, 6 mL, 7 mL, 8 mL, 9 mL, 10 mL, 15 mL, 20 mL, 25 mL, 30 mL, 40 mL, 50 mL, 75 mL, 100 mL, 200 mL, 300 mL, 400 mL, 500 mL, 600 mL, 700 mL, 800 mL, 900 mL, 1 L, 2 L, 3 L, 4 L, 5 L, 6 L, 7 L, 8 L, 9 L, 10 L, 25 L, 50 L, 100 L, 200L, 400L, or 450L.

[00161] In some embodiments, fractions are collected from the chromatography. In some embodiments, fractions collected are greater than about 0.01 CV, 0.02 CV, 0.03 CV, 0.04 CV, 0.05 CV, 0.06 CV, 0.07 CV, 0.08 CV, 0.09 CV, 0.1 CV, 0.2 CV, 0.3 CV, 0.4 CV, 0.5 CV, 0.6 CV, 0.7 CV, 0.8 CV, 0.9 CV, 1.0 CV, 2.0 CV, 3.0 CV, 4.0 CV, 5.0, CV. In some embodiments, fractions containing the product, *e.g.* polypeptide, are pooled. In some embodiments, fractions containing the polypeptide from the load fractions and from the elution fractions are pooled. The amount of polypeptide in a fraction can be determined by one skilled in the art; for example, the amount of polypeptide in a fraction can be determined by UV spectroscopy. In some embodiments, fractions containing detectable polypeptide fragment are pooled.

[00162] In some embodiments of any of the methods described herein, the at least one impurity or contaminant is any one or more of host cell materials, such as CHOP; leached Protein A; nucleic acid; a variant, fragment, aggregate or derivative of the desired polypeptide; another polypeptide; endotoxin; viral contaminant; cell culture media component, gentamicin, etc. In some examples, the impurity or contaminant may be a host cell protein (HCP) from, for example but not limited to, a bacterial cell such as an E. coli cell, an insect cell, a prokaryotic cell, a eukaryotic cell, a yeast cell, a mammalian cell, an avian cell, a fungal cell.

[00163] Host cell proteins (HCP) are proteins from the cells in which the polypeptide was produced. For example, CHOP are proteins from host cells, *i.e.*, Chinese Hamster Ovary Proteins. The amount of CHOP may be measured by enzyme-linked immunosorbent assay

("ELISA") or mass spectrometry. In some embodiments of any of the methods described herein, the amount of HCP (*e.g.* CHOP) is reduced by greater than about any of 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 %, or 95 %. The amount of HCP may be reduced by between about any of 10 % and 99 %, 30% and 95%, 30 % and 99 %, 50% and 95%, 50 % and 99 %, 75 % and 99 %, or 85 % and 99 %. In some embodiments, the amount of HCP is reduced by about any of 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 85 %, 90 %, 95 %, or 98 %. In some embodiments, the reduction is determined by comparing the amount of HCP in the composition recovered from a purification step(s) to the amount of HCP in the composition before the purification step(s).

[00164] In some embodiments of any of the methods described herein, the methods further comprise recovering the purified polypeptide. In some embodiments, the purified polypeptide is recovered from any of the purification steps described herein. The chromatography step may be anion exchange chromatography, HIC, or Protein A chromatography. In some embodiments, the first chromatography step is protein A, followed by anion exchange or multimodal ion exchange, followed by HIC.

[00165] In some embodiments, the polypeptide is further purified following chromatography by viral filtration. Viral filtration is the removal of viral contaminants in a polypeptide purification feedstream. Examples of viral filtration include ultrafiltration and microfiltration. In some embodiments the polypeptide is purified using a parvovirus filter.

[00166] In some embodiments, the polypeptide is concentrated after chromatography. Examples of concentration methods are known in the art and include but are not limited to ultrafiltration and diafiltration.

[00167] In some embodiments of any of the methods described herein, the methods further comprise combining the purified polypeptide of the methods of purification with a pharmaceutically acceptable carrier.

Monoclonal antibodies

[00168] In some embodiments, the antibodies purified according to the methods of the invention are monoclonal antibodies. Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope except for possible variants that arise during production of the monoclonal antibody, such variants generally being present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete or polyclonal antibodies.

[00169] For example, the monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al., Nature* 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

[00170] In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as herein described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the polypeptide used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp. 59-103 (Academic Press, 1986)).

[00171] The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

[00172] In some embodiments, the myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, in some embodiments, the myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.* 133:3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications* pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

[00173] Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. In some embodiments, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

[00174] The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson *et al.*, *Anal.* Biochem. 107:220 (1980).

[00175] After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown

by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice* pp. 59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown in vivo as ascites tumors in an animal.

[00176] The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, polypeptide A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

[00177] DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). In some embodiments, the hybridoma cells serve as a source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as E. coli cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin polypeptide, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra *et al.*, *Curr. Opinion in Immunol.* 5:256-262 (1993) and Plückthun, *Immunol. Revs.*, 130:151-188 (1992).

[00178] In a further embodiment, antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty *et al.*, *Nature* 348:552-554 (1990). Clackson *et al.*, *Nature* 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks *et al.*, *Bio/Technology* 10:779-783 (1992)), as well as combinatorial infection and in vivo recombination as a strategy for constructing very large phage libraries (Waterhouse *et al.*, *Nuc. Acids. Res.* 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

[00179] The DNA also may be modified, for example, by substituting the coding sequence for human heavy- and light chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison *et al.*, P*roc. Natl Acad. Sci. USA* 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

[00180] Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

[00181] In some embodiments of any of the methods described herein, the antibody is IgA, IgD, IgE, IgG, or IgM. In some embodiments, the antibody is an IgG monoclonal antibody.

Humanized antibodies

[00182] In some embodiments, the antibody is a humanized antibody. Methods for humanizing non-human antibodies have been described in the art. In some embodiments, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-327 (1988); Verhoeyen *et al.*, *Science* 239:1534-1536 (1988)), by substituting hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some hypervariable region residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[00183] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence that is closest to that of the rodent is then accepted as the human framework region (FR) for the humanized antibody (Sims *et al.*, *J. Immunol.* 151:2296 (1993); Chothia *et al.*, *J. Mol. Biol.* 196:901 (1987)). Another method uses a particular framework region derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chain variable regions. The same framework may be used for several different humanized antibodies (Carter *et al.*, *Proc. Natl. Acad. Sci. USA* 89:4285 (1992); Presta *et al.*, *J. Immunol.* 151:2623 (1993)).

[00184] It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, in some embodiments of the methods, humanized antibodies are prepared by a process of analysis of the parental

sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available that illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *i.e.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

Human antibodies

[00185] In some embodiments, the antibody is a human antibody. As an alternative to humanization, human antibodies can be generated. For example, it is now possible to produce transgenic animals *(e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. *See, e.g.*, Jakobovits *et al., Proc. Natl. Acad. Sci. USA* 90:2551 (1993); Jakobovits *et al., Nature* 362:255-258 (1993); Bruggermann *et al., Year in Immuno.* 7:33 (1993); and US Patent Nos. 5,591,669; 5,589,369; and 5,545,807.

[00186] Alternatively, phage display technology (McCafferty *et al., Nature* 348:552-553 (1990)) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat polypeptide gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B cell. Phage display can be performed in a variety of formats; for their review *see, e.g.*, Johnson, Kevin S. and Chiswell, David J., *Current Opinion in Structural Biology* 3:564-571 (1993). Several sources of V-gene

segments can be used for phage display. Clackson *et al.*, *Nature* 352:624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991), or Griffith *et al.*, *EMBO J.* 12:725-734 (1993). *See also*, US Patent Nos. 5,565,332 and 5,573,905.

[00187] Human antibodies may also be generated by *in vitro* activated B cells (*see* US Patents 5,567,610 and 5,229,275).

Antibody fragments

[00188] In some embodiments, the antibody is an antibody fragment. Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (*see, e.g.*, Morimoto *et al.*, *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992) and Brennan *et al.*, *Science* 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. For example, the antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments (Carter *et al.*, *Bio/Technology* 10:163-167 (1992)). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). *See* WO 93/16185; US Patent No. 5,571,894; and US Patent No. 5,587,458. The antibody fragment may also be a "linear antibody," *e.g.*, as described in US Patent 5,641,870 for example. Such linear antibody fragments may be monospecific or bispecific.

[00189] In some embodiments, fragments of the antibodies described herein are provided. In some embodiments, the antibody fragment is an antigen binding fragment. In some embodiments, the antigen binding fragment is selected from the group consisting of a Fab fragment, a Fab' fragment, a $F(ab')_2$ fragment, a scFv, a Fv, and a diabody.

Chimeric Polypeptides

[00190] The polypeptide described herein may be modified in a way to form chimeric molecules comprising the polypeptide fused to another, heterologous polypeptide or amino acid sequence. In some embodiments, a chimeric molecule comprises a fusion of the polypeptide with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino- or carboxyl-terminus of the polypeptide.

The presence of such epitope-tagged forms of the polypeptide can be detected using an antibody against the tag polypeptide. Also, provision of the epitope tag enables the polypeptide to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag.

Other

[00191] Another type of covalent modification of the polypeptide comprises linking the polypeptide to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. The polypeptide also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sc*iences*, 18th edition, Gennaro, A.R., Ed., (1990).

OBTAINING POLYPEPTIDES

[00192] The polypeptides used in the methods of purification described herein may be obtained using methods well-known in the art, including the recombination methods. The following sections provide guidance regarding these methods.

Polynucleotides

[00193] "Polynucleotide," or "nucleic acid," as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA.

[00194] Polynucleotides encoding polypeptides may be obtained from any source including, but not limited to, a cDNA library prepared from tissue believed to possess the polypeptide mRNA and to express it at a detectable level. Accordingly, polynucleotides encoding polypeptide can be conveniently obtained from a cDNA library prepared from human tissue. The polypeptide-encoding gene may also be obtained from a genomic library or by known synthetic procedures (*e.g.*, automated nucleic acid synthesis).

[00195] For example, the polynucleotide may encode an entire immunoglobulin molecule chain, such as a light chain or a heavy chain. A complete heavy chain includes not only a heavy chain variable region (V_H) but also a heavy chain constant region (C_H), which typically will comprise three constant domains: C_H1, C_H2 and C_H3; and a "hinge" region. In some situations, the presence of a constant region is desirable.

[00196] Other polypeptides which may be encoded by the polynucleotide include antigen-binding antibody fragments such as single domain antibodies ("dAbs"), Fv, scFv, Fab' and

$F(ab')_2$ and "minibodies." Minibodies are (typically) bivalent antibody fragments from which the C_H1 and C_K or C_L domain has been excised. As minibodies are smaller than conventional antibodies they should achieve better tissue penetration in clinical/diagnostic use, but being bivalent they should retain higher binding affinity than monovalent antibody fragments, such as dAbs. Accordingly, unless the context dictates otherwise, the term "antibody" as used herein encompasses not only whole antibody molecules but also antigen-binding antibody fragments of the type discussed above. Preferably each framework region present in the encoded polypeptide will comprise at least one amino acid substitution relative to the corresponding human acceptor framework. Thus, for example, the framework regions may comprise, in total, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or fifteen amino acid substitutions relative to the acceptor framework regions.

[00197] Suitably, the polynucleotides described herein may be isolated and/or purified. In some embodiments, the polynucleotides are isolated polynucleotides.

[00198] The term "isolated polynucleotide" is intended to indicate that the molecule is removed or separated from its normal or natural environment or has been produced in such a way that it is not present in its normal or natural environment. In some embodiments, the polynucleotides are purified polynucleotides. The term purified is intended to indicate that at least some contaminating molecules or substances have been removed.

[00199] Suitably, the polynucleotides are substantially purified, such that the relevant polynucleotides constitutes the dominant (*i.e.*, most abundant) polynucleotides present in a composition.

Expression of Polynucleotides

[00200] The description below relates primarily to production of polypeptides by culturing cells transformed or transfected with a vector containing polypeptide-encoding polynucleotides. It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare polypeptides. For instance, the appropriate amino acid sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques (*see, e.g.*, Stewart *et al.*, *Solid-Phase Peptide Synthesis* W.H. Freeman Co., San Francisco, Calif. (1969); Merrifield, *J. Am. Chem. Soc.* 85:2149-2154 (1963)). In vitro protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be accomplished, for instance, using an Applied Biosystems Peptide Synthesizer (Foster City, Calif.) using manufacturer's instructions. Various portions of the polypeptide may be chemically synthesized separately and combined using chemical or enzymatic methods to produce the desired polypeptide.

[00201] Polynucleotides as described herein are inserted into an expression vector(s) for production of the polypeptides. The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences include, but are not limited to, promoters (*e.g.*, naturally-associated or heterologous promoters), signal sequences, enhancer elements, and transcription termination sequences.

[00202] A polynucleotide is "operably linked" when it is placed into a functional relationship with another polynucleotide sequence. For example, nucleic acids for a presequence or secretory leader is operably linked to nucleic acids for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the nucleic acid sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[00203] For antibodies, the light and heavy chains can be cloned in the same or different expression vectors. The nucleic acid segments encoding immunoglobulin chains are operably linked to control sequences in the expression vector(s) that ensure the expression of immunoglobulin polypeptides.

[00204] The vectors containing the polynucleotide sequences (*e.g.*, the variable heavy and/or variable light chain encoding sequences and optional expression control sequences) can be transferred into a host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment, electroporation, lipofection, biolistics or viral-based transfection may be used for other cellular hosts. (*See generally* Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Press, 2nd ed., 1989). Other methods used to transform mammalian cells include the use of polybrene, protoplast fusion, liposomes, electroporation, and microinjection. For production of transgenic animals, transgenes can be microinjected into fertilized oocytes, or can be incorporated into the genome of embryonic stem cells, and the nuclei of such cells transferred into enucleated oocytes.

Vectors

[00205] The term "vector" includes expression vectors and transformation vectors and shuttle vectors.

[00206] The term "expression vector" means a construct capable of *in vivo* or *in vitro* expression.

[00207] The term "transformation vector" means a construct capable of being transferred from one entity to another entity - which may be of the species or may be of a different species. If the construct is capable of being transferred from one species to another - such as from an *Escherichia coli* plasmid to a bacterium, such as of the genus *Bacillus*, then the transformation vector is sometimes called a "shuttle vector". It may even be a construct capable of being transferred from an *E. coli* plasmid to an *Agrobacterium* to a plant.

[00208] Vectors may be transformed into a suitable host cell as described below to provide for expression of a polypeptide. Various vectors are publicly available. The vector may, for example, be in the form of a plasmid, cosmid, viral particle, or phage. The appropriate nucleic acid sequence may be inserted into the vector by a variety of procedures. In general, DNA is inserted into an appropriate restriction endonuclease site(s) using techniques known in the art. Construction of suitable vectors containing one or more of these components employs standard ligation techniques which are known to the skilled artisan.

[00209] The vectors may be for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of the said polynucleotide and optionally a regulator of the promoter. Vectors may contain one or more selectable marker genes which are well known in the art.

[00210] These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA.

Host Cells

[00211] The host cell may be a bacterium, a yeast or other fungal cell, insect cell, a plant cell, or a mammalian cell, for example.

[00212] A transgenic multicellular host organism which has been genetically manipulated may be used to produce a polypeptide. The organism may be, for example, a transgenic mammalian organism (*e.g.*, a transgenic goat or mouse line).

[00213] Suitable prokaryotes include but are not limited to eubacteria, such as Gram-negative or Gram-positive organisms, for example, *Enterobacteriaceae* such as *E. coli*. Various E. coli strains are publicly available, such as *E. coli* K12 strain MM294 (ATCC 31,446); *E. coli* X1776 (ATCC 31,537); *E. coli* strain W3110 (ATCC 27,325) and K5 772 (ATCC 53,635). Other

suitable prokaryotic host cells include *Enterobacteriaceae* such as *Escherichia*, *e.g.*, *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, *e.g.*, *Salmonella typhimurium*, *Serratia*, *e.g.*, *Serratia marcescans*, and *Shigella*, as well as *Bacilli* such as *B. subtilis and B. licheniformis* (*e.g.*, *B. licheniformis* 41P), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. These examples are illustrative rather than limiting. Strain W3110 is one particularly preferred host or parent host because it is a common host strain for recombinant polynucleotide product fermentations. Preferably, the host cell secretes minimal amounts of proteolytic enzymes. For example, strain W3110 may be modified to effect a genetic mutation in the genes encoding polypeptides endogenous to the host, with examples of such hosts including *E. coli* W3110 strain 1A2, which has the complete genotype *tonA*; *E. coli* W3110 strain 9E4, which has the complete genotype *tonA ptr3*; *E. coli* W3110 strain 27C7 (ATCC 55,244), which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT kan'*; *E. coli* W3110 strain 37D6, which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT rbs7 ilvG kan'*; *E. coli* W3110 strain 40B4, which is strain 37D6 with a non-kanamycin resistant *degP* deletion mutation; and an *E. coli* strain having mutant periplasmic protease. Alternatively, in vitro methods of cloning, *e.g.*, PCR or other nucleic acid polymerase reactions, are suitable.

[00214] In these prokaryotic hosts, one can make expression vectors, which will typically contain expression control sequences compatible with the host cell (*e.g.*, an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation.

[00215] Eukaryotic microbes may be used for expression. Eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for polypeptide-encoding vectors. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism. Others include *Schizosaccharomyces pombe*; *Kluyveromyces hosts* such as, *e.g.*, *K. lactis* (MW98-8C, CBS683, CBS4574), *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickeramii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilarum* (ATCC 36,906), *K. thermotolerans*, and *K. marxianus; yarrowia* (EP 402,226); *Pichia pastoris*; *Candida; Trichoderma reesia*; *Neurospora crassa*; *Schwanniomyces* such as *Schwanniomyces occidentalis*; and filamentous fungi such as, *e.g.*, *Neurospora, Penicillium, Tolypocladium*, and Aspergillus hosts such as *A. nidulans*, and *A. niger*. Methylotropic yeasts are suitable herein and include, but are not limited to, yeast capable of growth on methanol selected from the genera

consisting of *Hansenula, Candida, Kloeckera, Pichia, Saccharomyces, Torulopsis*, and *Rhodotorula*. *Saccharomyces* is a preferred yeast host, with suitable vectors having expression control sequences (*e.g.*, promoters), an origin of replication, termination sequences and the like as desired. Typical promoters include 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase, isocytochrome C, and enzymes responsible for maltose and galactose utilization.

[00216] In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce the polypeptides as described herein and in some instances are preferred (*See* Winnacker, *From Genes to Clones* VCH Publishers, N.Y., N.Y. (1987). For some embodiments, eukaryotic cells may be preferred, because a number of suitable host cell lines capable of secreting heterologous polypeptides (*e.g.*, intact immunoglobulins) have been developed in the art, and include CHO cell lines, various Cos cell lines, HeLa cells, preferably, myeloma cell lines, or transformed B-cells or hybridomas. In some embodiments, the mammalian host cell is a CHO cell.

[00217] In some embodiments, the host cell is a vertebrate host cell. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR(CHO or CHO-DP-12 line); mouse sertoli cells; monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells; MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

FORMULATIONS AND METHODS OF MAKING THE FORMULATION

[00218] Provided herein are also formulations and methods of making the formulation comprising the polypeptides (*e.g.*, antibodies) purified by the methods described herein. For example, the purified polypeptide may be combined with a pharmaceutically acceptable carrier.

[00219] The polypeptide formulations in some embodiments may be prepared for storage by mixing a polypeptide having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions.

[00220] "Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and

concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution.

[00221] Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[00222] In some embodiments, the polypeptide in the polypeptide formulation maintains functional activity.

[00223] The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

[00224] The formulations herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, in addition to a polypeptide, it may be desirable to include in the one formulation, an additional polypeptide (*e.g.*, antibody). Alternatively, or additionally, the composition may further comprise a chemotherapeutic agent, cytotoxic agent, cytokine, growth inhibitory agent, anti-hormonal agent, and/or cardioprotectant. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[00225] Exemplary formulations of the anti-IL13 antibodies described herein are provided in International Patent Pub. No. WO 2013/066866.

ARTICLES OF MANUFACTURE

[00226] The polypeptides purified by the methods described herein and/or formulations comprising the polypeptides purified by the methods described herein may be contained within an article of manufacture. The article of manufacture may comprise a container containing the polypeptide and/or the polypeptide formulation. In certain embodiments, the article of manufacture comprises:(a) a container comprising a composition comprising the polypeptide

and/or the polypeptide formulation described herein within the container; and (b) a package insert with instructions for administering the formulation to a subject.

[00227] The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds or contains a formulation and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is the polypeptide. The label or package insert indicates that the composition's use in a subject with specific guidance regarding dosing amounts and intervals of polypeptide and any other drug being provided. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes. In some embodiments, the container is a syringe. In some embodiments, the syringe is further contained within an injection device. In some embodiments, the injection device is an autoinjector.

[00228] A "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications, other therapeutic products to be combined with the packaged product, and/or warnings concerning the use of such therapeutic products.

[00229] Exemplary articles of manufacture containing formulations of the anti-IL13 antibodies described herein are provided in International Patent Pub. No. WO 2013/066866.

[00230] Further details of the invention are illustrated by the following non-limiting Examples. The disclosures of all references in the specification are expressly incorporated herein by reference.

EXAMPLES

[00231] As used in the Examples below and elsewhere herein, "PLB2" and "PLBL2" and "PLBD2" are used interchangeably and refer to the enzyme "phospholipase B-like 2" or its synonym, "phospholipase B-domain-like 2".

EXAMPLE 1 – General Methods

[00232] Materials and methods for all Examples were performed as indicated below unless otherwise noted in the Example.

MAb feedstocks

[00233] MAb feedstocks for all examples were selected from industrial, pilot or small scale cell culture batches at Genentech (South San Francisco, CA, U.S.A.). After a period of cell culture

fermentation, the cells were separated and, in certain instances, the clarified fluid (harvested cell culture fluid, HCCF) was purified by Protein A chromatography and one or more additional chromatography steps and filtration steps as indicated in the Examples below.

MAb quantification

[00234] The concentration of antibody was determined via absorbance at 280 and 320 nm using a UV-visible spectrophotometer (8453 model G1103A; Agilent Technologies; Santa Clara, CA, U.S.A.) or NanoDrop 1000 model ND-1000 (Thermo Fisher Scientific; Waltham, MA, U.S.A.). Species other than antibody (*i.e.* impurities) were too low in concentration to have an appreciable effect on UV absorbance. As needed, samples were diluted with an appropriate non-interfering diluent in the range of 0.1–1.0 absorbance unit. Sample preparation and UV measurements were performed in duplicate and the average value was recorded. The mAb absorption coefficients ranged from 1.42 to 1.645/mg·ml·cm.

Total CHO host cell protein (CHOP) quantification

[00235] An ELISA was used to quantify the levels of the total host cell proteins called CHOP. The ELISAs used to detect CHO proteins in products were based upon a sandwich ELISA format. Affinity-purified polyclonal antibody to CHOP was coated onto a 96-well microtiter plate. Standards, controls, and samples were then loaded in duplicate into separate wells. CHOP, if present in the sample, will bind to the coat antibody (polyclonal anti-CHOP). After an incubation step, anti-CHOP polyclonal antibody-conjugated to horseradish peroxidase (HRP) was added to the plate. After a final wash step, CHOP was quantified by adding a solution of tetramethyl benzidine (TMB), also available as SUREBLUE RESERVE™ from KPL, Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD, cat no. 53-00-03), which when acted on by the HRP enzyme produces a colorimetric signal. The optical density (OD) at 450 nm was measured in each well. A five-parameter curve-fitting program (SOFTMAX® Pro, Molecular Devices, Sunnyvale, CA) was used to generate a standard curve, and sample CHOP concentrations were computed from the standard curve. The assay range for the total CHOP ELISA was from 5 to 320 ng/ml. CHOP concentration, in ng/mL, refers to the amount of CHOP in a sample using the CHOP standard as a calibrator. CHOP ratio (in ng/mg or ppm) refers to the calculated ratio of CHOP concentration to product concentration and, in certain instances, was the reported value for the test methods. The Total CHOP ELISA may be used to quantify total CHOP levels in a sample but does not quantify the concentration of individual proteins.

Murine Monoclonal Anti-Hamster PLBL2 ELISA Assay

[00236] The generation of mouse anti-hamster PLBL2 monoclonal antibodies and development of an ELISA assay for the detection and quantification of PLBL2 in recombinant polypeptide

preparations using such antibodies is described in US Provisional Patent Application Nos. 61/877,503 and 61/991,228. Briefly, the assay is carried out as follows.

[00237] Murine monoclonal antibody 19C10 was coated onto a half area 96-well microtiter plate at a concentration of 0.5 μg/mL in carbonate buffer (0.05M sodium carbonate, pH 9.6), overnight at 2-8°C. After coating, the plate was blocked with Blocking Buffer (0.15M NaCl, 0.1M sodium phosphate, 0.1% fish gelatin, 0.05% polysorbate 20, 0.05% Proclin® 300 [Sigma-Aldrich]; also referred to as Assay Diluent) to prevent non-specific sticking of proteins. Standards, controls, and samples were diluted in Assay Diluent (0.15M NaCl, 0.1M sodium phosphate, 0.1% fish gelatin, 0.05% polysorbate 20, 0.05% Proclin® 300 [Sigma-Aldrich]) then loaded in duplicate into separate wells and incubated for 2 hrs at room temperature (22-27°C). PLBL2, if present in the sample, would bind to the coat (also referred to herein as capture) antibody. After the incubation step described above, unbound materials were washed away using Wash Buffer (0.05% polysorbate 20/PBS [Corning cellgro Cat. No. 99-717-CM]) and the 15G11 anti-PLBL2 murine monoclonal antibody conjugated to biotin was diluted in Assay Diluent to a concentration of 0.03125 μg/mL and added to the wells of the microtiter plate.

[00238] Biotin conjugation was carried out as follows. A biotinylation kit was purchased from Pierce Thermo Scientific, (P/N 20217, E-Z Link NHS-Biotin), and streptavidin-HRP (SA-HRP) from Jackson Immuno Cat. No. 016-030-084. Instructions in the Pierce Kit were followed. Briefly, IgG was dialyzed into PBS, pH 7.4, and biotin was added to the protein and mixed at room temperature for 1 hr. The labeled antibody was then dialized against PBS, pH 7.4 to remove excess biotin, filtered, and protein concentration determined by A280.

After a 2 hr. incubation step with biotinylated 15G11 at room temperature, Streptavidin HRP (1:200,000 dilution in Assay Diluent) was added to the microtiter plate wells. After a final wash step with Wash Buffer (described above), color was developed (for PLBL2 quantification) by adding a solution of TMB (50 μl/well) (SUREBLUE RESERVE™ from KPL, Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD, cat no. 53-00-03) followed by incubation at room temperature for 10-20 minutes. Detection was carried out by assessing optical density (OD) at 450 nm in each well using a Molecular Devices SpectraMax M5e. A four-parameter curve-fitting program (SoftMax Pro v5.2 rev C) was used to generate a standard curve, and sample PLBL2 concentrations were computed from the linear range of the standard curve. Values in the linear range of the standard curve were used to calculate nominal PLBL2 (ng/mg or ppm). The linear range was approximately EC_{10} – EC_{85} or 1.5 – 40 ng/mL as the range varied slightly from plate to plate. Values obtained for PLBL2 using this ELISA were comparable to estimates made

by other methods (e.g., LC-MS/MS, polyclonal PLBL2 ELISA or total CHOP ELISA when diluted to the LOQ of the assay

Rabbit Polyclonal Anti-Hamster PLBL2 ELISA Assay

[00239] The generation of rabbit anti-hamster PLBL2 polyclonal antibodies and development of an ELISA assay for the detection and quantification of PLBL2 in recombinant polypeptide preparations using such antibodies is described in US Provisional Patent Application Nos. 61/877,503 and 61/991,228. Briefly, the assay is carried out as follows.

[00240] Affinity purified rabbit polyclonal antibody was coated onto a half area 96-well microtiter plate at a concentration of 0.5 ug/mL in carbonate buffer (0.05M sodium carbonate, pH 9.6), overnight at 2-8°C. After coating, the plate was blocked with Blocking Buffer (0.15M NaCl, 0.1M sodium phosphate, 0.1% fish gelatin, 0.05% Polysorbate 20, 0.05% Proclin® 300 [Sigma-Aldrich]) to prevent non-specific sticking of proteins. Standards, controls, and samples were diluted in Assay Diluent (0.15M NaCl, 0.1M sodium phosphate, 0.1% fish gelatin, 0.05% Polysorbate 20, 0.05% Proclin® 300 [Sigma-Aldrich]) then loaded in duplicate into separate wells and incubated for 2 hr at room temperature (22-27°C). PLBL2, if present in the sample, would bind to the coat (also referred to herein as capture) antibody. After the incubation step described above, unbound materials were washed away using Wash Buffer (0.05% Polysorbate 20/PBS [Corning Cellgro Cat. No. 99-717-CM]) and the affinity purified rabbit polyclonal antibody conjugated to horseradish peroxidase (HRP) was diluted in Assay Diluent to a concentration of 40 ng/mL and added to the wells of the microtiter plate.

[00241] HRP conjugation was carried out as follows. A HRP conjugation kit was purchased from Pierce Thermo Scientific, (P/N 31489, E-Z Link Plus Activated Peroxidase and Kit). Instructions in the Pierce Kit were followed. Briefly, IgG was dialyzed into Carbonate-Bicarbonate buffer, pH 9.4, and EZ-Link Plus Activated Peroxidase was added to the protein and mixed at room temperature for 1 hr. Sodium cyanoborohydride and Quenching buffer were added subsequently to stabilize the conjugation and quench the reaction. The labeled antibody was then dialyzed against PBS, pH 7.4, filtered, and protein concentration determined by A280.

[00242] After a 2 hr. incubation step with HRP conjugated rabbit polyclonal antibody at room temperature, a final wash step with Wash Buffer (described above) was performed. Afterwards, color was developed (for PLBL2 quantification) by adding a solution of TMB (50 ul/well) (SUREBLUE RESERVE™ from KPL, Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD, cat no. 53-00-03) followed by incubation at room temperature for 10-20 minutes. Detection was carried out by assessing optical density (OD) at 450 nm in each well using a Molecular Devices SpectraMax M5e. A five-parameter curve-fitting program (SoftMax Pro

v5.2 rev C) was used to generate a standard curve, and sample PLBL2 concentrations were computed from the linear range of the standard curve. Values in the linear range of the standard curve were used to calculate nominal PLBL2 (ng/mg or ppm). The quantitative range of the assay was 0.5 -50 ng/mL. Values obtained for PLBL2 using this ELISA were comparable to estimates made by other methods (e.g., murine monoclonal PLBL2 ELISA, LC-MS/MS or total CHOP ELISA when diluted to the LOQ of the assay).

LC-MS/MS Assay

[00243] For quantification of PLBL2 by LC-MS/MS, a Waters Acquity H-Class Bio UPLC and AB Sciex TripleTOF 5600+ mass spectrometer were used. Samples and calibration standards (recombinant PLBL2 spiked into a recombinant humanized monoclonal antibody preparation obtained from a mouse NS0 cell line [the NS0 cell line does not contain hamster PLBL2]) were reduced and digested by trypsin. A total of 40 μg digested sample was injected onto the UPLC, using a Waters BEH300 C18 column, particle size 1.7 μm. A linear gradient of acetonitrile was used to elute the peptides, at a flow rate of 300 μl/min and a column temperature of 60°C.

[00244] Peptides eluting from the UPLC were introduced to the mass spectrometer by electrospray ionization in positive ionization mode. Ion source temperature was set at 400°C, with an IonSpray voltage of 5500 v. and declustering potential of 76 v. A collision energy setting of 32 was used for the fragmentation of selected peptide ions. The mass spectrometer was operated in multiple reaction monitoring high resolution (MRM^{HR}) mode, using four specific PLBL2 peptides and their fragment ion transitions. The parent ions were selected by the quadrupole mass spectrometer with a mass to charge (m/z) selection window of 1.2 amu. Fragment ions of each parent ion were separated by the time-of-flight mass spectrometer and selected for quantification post data acquisition with a selection window of 0.025 amu. The concentration of PLBL2 in samples was determined by measuring the specific signal responses of the four transitions, calibrated by those from the standards in the range of 2-500 ppm using a linear fit. Table 2 below shows the list of PLBL2 peptides monitored by LC-MS/MS.

Table 2. List of PLBL2 Peptides Monitored by LC-MS/MS.

TripleTOF 5600+ Scan Cycle					
Scan #	Scan Type	Peptide	Fragment Ion of Interest	Parent m/z	Fragment m/z
1	TOF MS	N/A	N/A	N/A	N/A
2	Product Ion	SVLLDAASGQLR (SEQ ID NO: 31)	+2y8	615.3461	817.4163
3	Product Ion	GLEDSYEGR (SEQ ID NO: 32)	+2y7	513.2304	855.3479
4	Product Ion	AFIPNGPSPGSR (SEQ ID NO: 33)	+2y9	600.3120	868.4272
5	Product Ion	VTSFSLAK (SEQ ID NO: 34)	+2y6	426.7449	652.3665

EXAMPLE 2 – Improved Purification Process to Reduce Hamster PLBL2

[00245] A purification process for CHO-produced anti-IL13 MAb (lebrikizumab) was established to support early stage clinical trials and is referred to herein as the "Initial Process." The Initial Process employed the following chromatographic steps in order: Protein A affinity chromatography (MABSELECT SURE™) followed by cation exchange (POROS® HS) followed by anion exchange (Q SEPHAROSE™ Fast Flow). Additional virus inactivation and filtration steps were included and a final ultrafiltration-diafiltration (UFDF) step. The final product (drug substance) was formulated at a concentration of 125 mg/mL in 20 mM histidine acetate, 6% sucrose, 0.03% polysorbate 20, pH 5.7.

[00246] Using the Total CHOP ELISA Assay (described in Example 1 above), we observed that in-process intermediates and drug substance purified according to the Initial Process demonstrated atypical dilution-dependent behavior resulting in a > 20% coefficient of variation across a normalized series of sample dilutions. This dilution-dependent behavior is exemplified by the data presented in Table 3 in which each successive two-fold dilution of anti-IL13 MAb product resulted in higher levels of CHOP (expressed in ppm) as determined using the Total CHOP ELISA. Using sensitive analytical methods, such as LC-MS/MS, we determined that a single CHOP species, or HCP, was the cause of this atypical dilution-dependent behavior. In particular, we established that the dilution-dependent behavior on the Total CHOP ELISA was due to antigen excess. Further investigation enabled us to identify the single HCP as an enzyme, hamster phospholipase B-like 2 (PLBL2). By diluting the product samples to the limit of assay quantitation (LOQ), we were able to estimate the level of PLBL2 in clinical lots of lebrikizumab purified using the Initial Process and determined that levels as high as 300 ppm (300 ng/mg) and above were present.

Table 3. Product dilution and CHOP levels.

Fold Dilution	Total CHOP (ppm)
2	0.58
4	1
8	2
16	4
32	7
64	14
128	26
256	49
512	97
1024	147
2048	228
4096	314
8192	346

[00247] This level of impurity (> 300 ppm) of a single CHOP species such as we observed, is considered undesirable in MAb products intended for human clinical and/or therapeutic use, particularly late stage clinical trials and beyond. For example, such levels may be immunogenic when administered to human subjects as described in Example 3.

[00248] Accordingly, we investigated various modifications to the Initial Process as briefly outlined below. Based on the results of these investigations, we developed an improved purification process, described in detail below, and referred to herein as "Improved Process." Use of the Improved Process resulted in purified anti-IL13 MAb (lebrikizumab) product containing substantially reduced levels of PLBL2.

[00249] Efforts for modifying the purification process to reduce PLBL2 included methods orthogonal to the Initial Process including: precipitation, testing various additives to HCCF, additional column washes, hydrophobic interaction and mixed mode chromatography. These efforts were informed by use of one or more of the assays described in Example 1 to monitor the effectiveness of each of the modifications investigated for reduction in total CHOP and/or PLBL2 levels. The various modifications explored are described below.

Precipitation of CHOP in HCCF and Protein A Pool with Caprylic Acid

[00250] Caprylic acid precipitation has been described previously, including use in the monoclonal antibody industry (Wang et al., BioPharm International; Downstream Processing 2010, p4-10, Oct2009; Brodsky et al., Biotechnology and Bioengineering, 109(10):2589, 2012) to selectively precipitate impurities from target proteins of interest. Caprylic acid, also known as octanoic acid, is a saturated fatty acid with eight carbons (formula $CH_3(CH_2)_6COOH$). Studies were done with anti-IL13 MAb to determine whether precipitation of the harvested cell

culture fluid (HCCF) or Protein A pool with caprylic acid would lead to reduced CHOP and/or reduction of dilution-dependent behavior in the Total CHOP ELISA.

[00251] The anti-IL13 MAb starting material for these studies was HCCF and Protein A pools from a 1kL harvest. 1% (v/v) caprylic acid was added to the HCCF and varying concentrations of caprylic acid (0% - 3% v/v) were added to Protein A pools at pH 4.5 or pH 5.0. Samples were mixed for ≥ 5 hours at ambient temperature, 0.2 μm filtered, and diluted with Total CHOP ELISA diluent for detection and quantification using the Total CHOP ELISA. Titer of anti-IL13 MAb in HCCF before and after caprylic acid treatment was determined using an HPLC titer assay performed according to standard methods known in the art.

[00252] Treatment of HCCF with 1% v/v caprylic acid reduced CHOP by approximately 5-fold and resulted in a yield of anti-IL13 MAb of 91%. When Protein A pools were treated with various concentrations of caprylic acid, ranging from 0 – 3% v/v, we observed a loss in yield of > 20% at pH 5.0 and no loss in yield at pH 4.5. When we assessed total CHOP in these caprylic acid-treated Protein A pools, we found a 2-fold to 3-fold reduction of CHOP (Figs. 1A and B). However, as also shown in Figs. 1A and B, dilution-dependence was still present under each of the conditions tested indicating that caprylic acid precipitation was not effective for addressing the dilution-dependent behavior observed in the Total CHOP ELISA and would thus not be effective for reducing PLBL2 levels in this product.

Additives to HCCF

[00253] Previous work by Sisodiya et al., Biotech J. 7:1233 (2012) has demonstrated that additives such as guanidine or sodium chloride to HCCF can reduce the CHOP in the subsequently purified Protein A pools. As arginine has also been shown to reduce CHOP when utilized as a wash on Protein A columns (Millipore Technical Bulletin, Lit. No. TB1024EN00, Rev. A, December, 2005; Millipore Technical Bulletin, Lit. No. 1026EN00, July, 2006, *available at* www(dot)Millipore(dot)com), we included it as an additive to HCCF. Various salts, chaotropes, and caprylic acid were added to the anti-IL13 MAb HCCF to assess the effectiveness of each for reducing the product and CHOP interaction during capture of product on MABSELECT SURE™ (MSS) protein A chromatography. The additives to HCCF tested were: 0.6M guanidine, 0.6M arginine, 0.6M NaCl, phosphate-buffered saline, and 1% caprylic acid.

[00254] Samples that had been treated with each of the HCCF additives were subjected to Protein A chromatography on MSS. Protein A pools were adjusted to pH 4.9 and further purified on the POROS® HS cation exchange chromatography step using the Initial Process conditions. Protein A pools and POROS® HS pools were diluted and submitted to the Total

Chop ELISA. Adjusted Protein A pools were also tested on SEC-HPLC according to methods known in the art for the assessment of %aggregate, %variant species and the like.

[00255] Yields on MABSELECT SURE™ were slightly lower for the runs where guanidine or arginine was added to HCCF. Of all the additives to HCCF tested, guanidine and arginine were the most effective for reducing CHOP levels substantially (see Table 4) and appeared to reduce dilution-dependence on the Protein A pools (data not shown). Further downstream processing of the Protein A pools on POROS® HS, however, showed CHOP ELISA dilution-dependence remaining in the corresponding POROS® pools as shown in Fig. 2. Accordingly, the data demonstrate that addition of guanidine or arginine to HCCF would not be effective for reducing PLBL2 levels in this product.

Table 4. HCCF Additives and effect on CHOP.

Additive	Load pH	Yield (%)	Total CHOP (ppm)
Control (no additive)	7.4	101	3417
0.6M guanidine	7.6	90	892
0.6M arginine	7.1	88	1237
0.6M NaCl	7.7	99	2619
PBS	7.4	98	2773
1% caprylic acid	6	93	3173

Washing of Protein A Column (MABSELECT SURE™)

[00256] It was observed that the more dilution-dependent CHOP eluted in early product-containing fractions on MABSELECT SURE™ (MSS) Protein A chromatography. This suggested that an additional wash step on MSS before elution might further reduce CHOP/PLBL2. Several washes on MSS were tested for their ability to reduce CHOP/PLBL2 in the Protein A pools. For this study, purified anti-IL13 MAb UFDF pool was used as the load material. The UFDF pool was diluted to 1.7 mg/mL (approximate HCCF titer) and loaded onto MSS at 29 g/Lresin. Various washes were tested, for example; 0.5M arginine pH 8.5, 0.5M arginine pH 9.5 with and without 1% polysorbate 20, 0.5M TMAC pH 7.1, 25 mM MOPS pH 7.1, and compared with a high salt wash pH 7.0. Product was eluted under acidic conditions (pH 2.8) and pooled beginning at 0.5 OD (A280) and continuing for a total volume of 2.4 column volumes. Each adjusted pool was diluted and assayed using the Total CHOP ELISA. The summary of these results is that none of the washes adequately reduced CHOP/PLBL2 or dilution-dependence in the Total CHOP ELISA. It thus appeared unlikely we would find protein A wash conditions that would be effective for reducing PLBL2 levels in this anti-IL13 MAb product and we did not investigate these further.

Washing of Cation Exchange Column (POROS® HS)

[00257] Based on theoretical calculations using the amino acid sequences of anti-IL13 MAb and the PLBL2 impurity, we estimated that the pI of PLBL2 is approximately 6.0 and similar to anti-IL13 MAb (pI 6.1). We also estimated that there would be a significant difference in net charge between anti-IL13 MAb and PLBL2 at ≤ pH 4 and ≥ pH 10. As such, we tested various low pH washes on the Initial Process POROS® HS cation exchange step to assess whether these would be effective for selectively reducing total CHOP and/or PLBL2 and dilution-dependence behavior. The following washes were tested at pH 4: (i) acetate gradient, 300 mM – 1,000 mM over 20 column volumes (CV); (ii) citrate gradient, 100 mM – 500 mM over 20 CV; (iii) citrate wash step at 260 mM; and (iv) arginine gradient to 15 mS/cm (conductivity measurement) over 20 CV.

[00258] The results showed that anti-IL13 MAb and CHOP did not elute with the pH 4 acetate gradient up to the tested salt concentration of 1M. Increasing amounts of citrate or acetate resulted in product insolubility and precipitation. All of the pH 4 washes resulted in low yield on the POROS® HS step and none of the washes significantly reduced CHOP dilution-dependence. Accordingly, inclusion of a low pH wash of the cation exchange column was not effective for reducing PLBL2 levels in this product.

Hydroxyapatite Resin and CAPTO™ Adhere Resin

[00259] Ceramic hydroxyapatite (CHT) macroporous resin Type I, 40 um (BioRad) is comprised of calcium phosphate $(Ca_5(PO_4)_3OH)_2$ in repeating hexagonal structures. There are two distinct binding sites; C-sites with sets of 5 calcium ion doublets and P-sites containing pairs of –OH containing phosphate triplets. This resin has mixed mode properties and has been shown to separate challenging impurities such as aggregates (P. Gagnon, New Biotechnology 25(5):287 (2009)).

[00260] To identify initial conditions for running a CHT column, we performed high throughput robot screening of CHT resin Type I, 40 um testing a pH range of 6.5 – 8.0 and varying concentrations of sodium chloride and sodium phosphate for elution. Such high throughput robot screenings have been previously described, for example, in Wensel et al., Biotechnol. Bioeng. 100:839 (2008). Samples from these screenings were tested in the Total CHOP ELISA.

[00261] CAPTO™ Adhere (GE Healthcare) is a mixed mode resin that exhibits both ionic and hydrophobic properties. The base matrix is a rigid agarose, and the ligand is N-benzyl-N-methylethanolamine. The ability of this resin to reduce total CHOP and/or PLBL2 was assessed first with a high-throughput screening study and then with subsequent column conditions.

[00262] Initial studies to identify conditions for running a CAPTO™ Adhere column were done using a high-throughput robot screening method similar to that described above to test binding of anti-IL13 MAb to CAPTO™ Adhere at two load densities (5 g/Lresin and 40 g/Lresin). Salt and pH ranges were also tested; from 25 mM – 200 mM sodium acetate and pH 4.0 – 6.5. The load material was the Initial Process UFDF pool that contained approximately 200 ppm of total CHOP at LOQ by the Total CHOP ELISA. Samples of the unbound (flow-through) on CAPTO™ Adhere were diluted and assayed using the Total CHOP ELISA.

[00263] The results were as follows. For CHT chromatography, none of the tested conditions substantially reduced total CHOP or PLBL2 or affected assay dilution-dependence behavior. In addition, yields were poor and no clearance of high molecular weight species was achieved. For CAPTO™ Adhere chromatography, yields were poor and the assayed material showed substantial dilution-dependence behavior in the Total CHOP ELISA. Accordingly, the use of CHT and CAPTO™ Adhere resins were not explored further as it was clear that we would be unlikely to find conditions using these resins that would be effective for reducing PLBL2 levels in this anti-IL13 MAb product.

Hydrophobic Interaction Chromatography Resins and Membranes

[00264] We initially tested HIC membrane adsorber referred to as Sartobind and manufactured by Sartorius. Sartobind is made with a base matrix of regenerated cellulose and covalently linked hydrophobic phenyl ligand groups.

[00265] The membrane tested was Sartobind HIC 3 mL device (8mm bed height). We adjusted the pool from the Initial Process POROS® HS pool to 0.55M potassium phosphate pH 7.0 and used a flow rate of 10 mL/min. Product was eluted in 0.55M potassium phosphate pH 7.0 (collected in the unbound fractions in 3 mL fractions).

[00266] We observed that the anti-IL13 MAb became hazy and turbid upon conditioning to 0.55M potassium phosphate and required an additional 0.2 um filtration step. The results showed a reduction in total CHOP, however, the remaining CHOP still demonstrated dilution dependent behavior in the Total CHOP ELISA. Use of this membrane was not evaluated further as it seemed unlikely that effective conditions would be identified for reducing PLBL2 levels in this product.

[00267] Next, we employed a high throughput screen to evaluate several different HIC resins. OCTYL-SEPHAROSE® Fast Flow (FF), BUTYL-SEPHAROSE® 4 Fast Flow, PHENYL SEPHAROSE™ 6 Fast Flow (high sub) and PHENYL SEPHAROSE™ 6 Fast Flow (low sub) were obtained from GE Healthcare. These four resins were chosen because they represent a wide range of varying hydrophobicity (OCTYL-SEPHAROSE® Fast Flow is the least

hydrophobic, followed by PHENYL SEPHAROSE™ 6 Fast Flow (low sub) and BUTYL-SEPHAROSE® 4 Fast Flow, with PHENYL SEPHAROSE™ 6 Fast Flow (high sub) the most hydrophobic. We tested several combinations of pH and salt concentrations for their effectiveness at reducing PLBL2 in anti-IL13 MAb preparations. The anti-IL13 MAb preparation employed for the HIC resin experiments was a UFDF pool from a run using the Initial Process. The anti-IL13 MAb concentration was 180 mg/mL and the load density was 40 mg antibody/mL resin. We tested pH 5.5 (25 mM sodium acetate), pH 6.0 (25 mM MES), pH 7.0 (25 mM MOPS), and pH 8.0 (25 mM Tris) and sodium sulfate concentrations between 0 mM and 400 mM. For each condition tested, flow-through samples were collected, diluted and tested using the Total CHOP ELISA assay.

[00268] The results are shown in Figs. 3A-D. With increasing salt, we observed less total CHOP in the flow-through for each resin. The OCTYL-SEPHAROSE® Fast Flow resin (Fig. 3A) showed the highest level of total CHOP while the PHENYL SEPHAROSE™ 6 Fast Flow (high sub) resin reduced total CHOP to very low levels, even with lower amounts of salt (Fig. 3D) and the PHENYL SEPHAROSE™ 6 Fast Flow (low sub) and BUTYL-SEPHAROSE® Fast Flow resins showed intermediate levels of total CHOP. Interestingly, there was also minimal effect of pH on CHOP removal using each of the resins except for PHENYL SEPHAROSE™ 6 Fast Flow (high sub) in low salt conditions (Fig. 3D). For this resin, at low salt conditions, higher pH resulted in higher CHOP in the flow-through fraction (Fig. 3D). Based on these results, PHENYL SEPHAROSE™ 6 Fast Flow (high sub) appeared promising and was chosen for further studies which included running the column in either bind-elute or flow-through mode.

[00269] Operation of HIC using the PHENYL SEPHAROSE™ 6 Fast Flow (high sub) resin in the bind-elute mode required conditioning of the anti-IL13 MAb load with salt to enable binding of the antibody to the resin. Increasing salt increased the dynamic binding capacity (mg of anti-IL13/mL resin) for loading product to the resin. But with increasing salt concentration in the product, we observed increased turbidity and formation of high molecular weight species (HMWs), in particular in combination with lower pH.

[00270] As mentioned above, PHENYL SEPHAROSE™ 6 Fast Flow (high sub) may also be operated in flow-through mode and such operation would require less salt conditioning of the load. From a product quality and product stability viewpoint, for example, product with less turbidity and less HMWs, less salt conditioning would be desirable. Accordingly, we proceeded with process optimization using PHENYL SEPHAROSE™ 6 Fast Flow (high sub) resin in flow-through mode.

[00271] To optimize the process, we investigated numerous parameters for running the HIC column including load concentration, load pH, load salt molarity, load density on the resin, bed height, flowrate, temperature, equilibration buffer pH and molarity. For these experiments, we monitored total CHOP using the Total CHOP ELISA and also PLBL2 by LC-MS/MS. Certain exemplary data is shown in Table 5. The data in Table 5 shows that the HIC column run under the indicated conditions in flow-through mode was effective for substantially reducing PLBL2 levels from the high levels detected in the Protein A pool. The PLBL2 levels after HIC were reduced by several hundred fold compared to the levels in the Protein A pool.

Table 5. Total CHOP and PLBL2 Levels under Varying HIC Column Conditions.

Sample (bed height, flow rate)	% Yield	Total CHOP (ppm by ELISA at LOQ)	PLBL2 (ppm by LC-MS/MS)
Protein A pool (Load for HIC Column)		3324	957
15 cm, 150 cm/hr	88	43	4
25 cm, 150 cm/hr	92	44	2
15 cm, 100 cm/hr	90	67	5
25 cm, 100 cm/hr	92	63	3
15 cm, 200 cm/hr	93	62	6
25 cm, 200 cm/hr	90	72	4
15 cm, 150 cm/hr	54	76	2

[00272] Using the PLBL2 LC-MS/MS assay and other typical product quality assays (e.g., SE-HPLC, CE-SDS, iCIEF) to guide process parameter selections, we identified the following conditions as desirable for running of the HIC column as assessed by product quality attributes and reduction of PLBL2: equilibration and wash buffer: 50 mM sodium acetate, pH 5.0; target load density: 100 g/L, flow rate: 150 cm/hr, 22°C ± 3°C. Certain small variations of these conditions may also be desirable, for example, 25°C ± 3°C or 27°C ± 3°C. Optical density (OD) was monitored by absorbance at 280 nm (A280) and the pool (i.e. the flow-through) was collected between 0.5 OD to 1.5 OD or after 8 column volumes of wash.

[00273] As mentioned above, the Initial Process was: Protein A affinity chromatography (MABSELECT SURE™) followed by cation exchange (POROS® HS) followed by anion exchange (Q SEPHAROSE™ Fast Flow). After developing processes to reduce PLBL2 levels as described above, we next sought to implement process changes in a convenient manner. Accordingly, we explored adding the HIC column to the Initial Process thereby creating a four-column process as well as substituting the HIC column for either the CEX column or the AEX column and finally we explored the order of the columns. We found that a three column process, Protein A affinity chromatography (MABSELECT SURE™), followed by anion

exchange (Q SEPHAROSE™ Fast Flow), followed by HIC operated in flow-through mode (PHENYL SEPHAROSE™ 6 Fast Flow (high sub)) provided the most convenient process and was the most effective for reducing PLBL2 in the final drug substance. This three-column process is described in detail below.

[00274] The first affinity chromatography step was a bind-and-elute process using MABSELECT SURE™ resin. After column equilibration (25 mM sodium chloride, 25 mM Tris pH 7.7), the HCCF was loaded on the column and washed with the equilibration buffer and a high salt pH 7.0 wash buffer. Anti-IL13 MAb was eluted from the column under acidic conditions (pH 2.8).

[00275] The second anion-exchange chromatography step was operated in a bind-and-elute mode using Q SEPHAROSE™ Fast Flow (QSFF) resin. After column equilibration (50 mM Tris, pH 8.0), the anti-IL13 pool from the MABSELECT SURE™ column was adjusted to pH 8.05 and loaded onto the column. The column was washed (50 mM Tris, pH 8.0) and anti-IL13 MAb eluted from the column with 85 mM sodium chloride, 50 mM Tris pH 8.0.

[00276] The third and final hydrophobic interaction chromatography step was operated in a flow-through mode using PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin. After column equilibration (50 mM sodium acetate pH 5.0), the anti-IL13 pool from the QSFF column was adjusted to pH 5.0 and loaded on the column. The anti-IL13 MAb flowed through and the column was also washed with equilibration buffer (50 mM sodium acetate pH 5.0). The anti-IL13 MAb pool was initiated and terminated based on A280 with pooling occurring between 0.5 to 1.5 OD or a maximum of 8 column volumes.

[00277] As with the Initial Process, additional virus inactivation and filtration steps were included and a final ultrafiltration-diafiltration (UFDF) step. The final product (drug substance) was formulated at a concentration of 125 mg/mL in 20 mM histidine acetate, 6% sucrose, 0.03% polysorbate 20, pH 5.7.

[00278] A comparison of the Initial Process to the Improved Process with respect to total CHOP and PLBL2, as measured by the Total CHOP ELISA and the monoclonal PLBL2 ELISA, respectively, is provided in Tables 6 (Initial Process) and 7 (Improved Process). The data in Table 6 clearly shows that the Initial Process resulted in purified product (UFDF pool) containing high levels of total CHOP (179, 310, and 189 ng/mg in three different runs) and high levels of PLBL2 (242, 328, and 273 ng/mg in three different runs) while the data in Table 7 clearly shows that the Improved Process was quite effective for producing purified product with substantially reduced levels of total CHOP (1.1, < 0.9, 2.8, and 3.4 ng/mg in four different runs) and substantially reduced levels of PLBL2 (0.21, 0.42, 0.35, and 0.24 ng/mg in four different

runs). Consistent with the data presented above, the data in Table 7 shows that the HIC column run under the conditions described above was particularly effective for reducing total CHOP and PLBL2 levels in anti-IL13 MAb preparations.

Table 6. Total CHOP and PLBL2 Levels at Various Stages of Purification of Anti-IL13 MAb Using the Initial Process.

In-process sample	Total CHOP (ng/mg at LOQ by ELISA)			PLBL2 (ng/mg by ELISA)		
Run No.	1	2	3	1	2	3
HCCF	620920	541072	608789	1895	3669	2535
ProA Pool	2892	2855	3505	587	769	478
CEX Pool	136	310	138	345	439	287
AEX Pool	104	163	93	291	304	261
UFDF Pool	179	310	189	242	328	273

Table 7. Total CHOP and PLBL2 Levels at Various Stages of Purification of Anti-IL13 MAb Using the Improved Process.

In-process sample	Total CHOP (ng/mg at LOQ by ELISA)				PLBL2 (ng/mg by ELISA)			
Run No.	1	2	3	4	1	2	3	4
HCCF	332132	399157	540134	644549	4084	3770	3077	2986
ProA Pool	2318	2768	3552	3797	1354	1995	1027	975
AEX Pool	495	653	414	377	723	933	677	616
HIC Pool	< 2.1	< 1.9	5.0	7.7	< 0.6	< 0.6	< 0.6	< 0.6
UFDF Pool	1.1	< 0.9	2.8	3.4	0.21	0.42	0.35	0.24

[00279] In summary, faced with the problem of assay non-linear dilution behavior attributable to high levels of a single CHOP species in purified anti-IL13 MAb preparations, we first identified the CHOP species as hamster PLBL2, an impurity which has not been previously described in recombinant protein preparations produced from CHO cells. We next identified purification conditions to effectively reduce the levels of PLBL2 in the anti-IL13 MAb preparations. Finally, we integrated these purification conditions into the overall purification process resulting in an improvement to the prior anti-IL13 MAb purification process. This Improved Process employs a HIC column run in flow-through mode to reduce PLBL2 levels, which is run in combination with an affinity chromatography step and an anion exchange chromatography step. We showed that the Improved Process is robust and effective for substantially reducing hamster PLBL2 levels in anti-IL13 MAb preparations. We showed that the Improved Process reproducibly reduced PLBL2 levels by approximately 1000 fold compared to the Initial Process. Such reduction in PLBL2 levels was important for producing a purified

anti-IL13 MAb product suitable for therapeutic use in patients in late stage clinical trials and beyond.

Purification Process to Reduce Hamster PLBL2 in Anti-Abeta Antibody Preparations

[00280] We next sought to assess whether the purification methods described above, particularly use of a HIC column for a final chromatography step, would similarly be effective for reducing PLBL2 levels in other antibody preparations. For this experiment, we chose an anti-Abeta antibody, which was produced in CHO cells. Exemplary anti-Abeta antibodies and methods of producing such antibodies have been described previously, for example, in WO2008011348, WO2007068429, WO2001062801, and WO2004071408. These particular experiments used the anti-Abeta antibody known as crenezumab. As described for the anti-IL13 MAb, we explored various resins and buffers for the second column after the Protein A affinity column and we explored various buffers and run conditions for the HIC column to identify those that were optimal for anti-Abeta for product quality and stability attributes as well as for removal of hamster PLBL2.

[00281] We found that a three column process, Protein A affinity chromatography (MABSELECT SURE™), followed by use of a mixed mode resin (CAPTO™ Adhere), followed by HIC operated flow-through mode (PHENYL SEPHAROSE™ 6 Fast Flow (high sub)) was convenient and effective for reducing PLBL2 in the final drug substance. This three-column process is described in detail below.

[00282] The first affinity chromatography step was a bind-and-elute process using MABSELECT SURE™ resin similar to that described above for the anti-IL13 MAb.

[00283] The second mixed mode chromatography step was operated in a flow-through mode using CAPTO™ Adhere resin. After column equilibration (20 mM MES, 150 mM sodium acetate, pH 6.25), the anti-Abeta pool from the MABSELECT SURE™ column was adjusted to pH 6.25 and loaded onto the column. Pooling began at 0.5 OD during the load phase. After completing the load, the column was washed with 5 column volumes (CVs) of equilibration buffer (20 mM MES, 150 mM sodium acetate, pH 6.25) and the entire 5 CVs were also collected.

[00284] The third and final hydrophobic interaction chromatography step was operated in a flow-through mode using PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin. After column equilibration (150 mM sodium acetate pH 5.0), the anti-Abeta pool from the CAPTO™ Adhere column was adjusted to pH 5.0 and loaded on the column. The anti-Abeta MAb flowed through and the column was also washed with equilibration buffer (150 mM sodium acetate pH 5.0). The anti-Abeta MAb pool was initiated during the load phase based on A280 with pooling

beginning at 0.5 OD. The column was washed with 10 CVs of equilibration buffer (150 mM sodium acetate pH 5.0) and the entire 10 CVs were also collected. As with the anti-IL13 MAb, additional virus inactivation and filtration steps were included and a final ultrafiltration-diafiltration (UFDF) step.

[00285] The results of using the above process during four different purification runs are shown in Table 8 below.

Table 8. PLBL2 Levels at Various Stages of Purification of Anti-Abeta MAb Using HIC.

In-process sample	PLBL2 (ng/mg by ELISA)			
Run No.	1	2	3	4
HCCF	622	564	1264	553
CpA Pool	7	8	9	2.5
HIC Pool (300 g/L Load density)	0.7	0.6	0.3	0.3
HIC Pool (100 g/L Load density)	< 0.2	< 0.2	< 0.2	Not tested

[00286] The results shown in Table 8 demonstrate that use of a HIC resin as a final chromatography step effectively reduced residual PLBL2 levels in the anti-Abeta MAb preparation to an amount similar to that seen for the anti-IL13 MAb. While a load density of 300 g/L produced desirable results from the viewpoint of both product recovery and reduction in PLBL2, further reduction in residual PLBL2 was seen by reducing the load density for the HIC column from 300 g/L to 100 g/L.

[00287] We also investigated two other conditions for the HIC chromatography step, load pH and load sulfate molarity. For these experiments, we started with a CAPTO^{TM} Adhere pool containing 51 ng/mg PLBL2 (as measured by ELISA), 15 mM sodium acetate pH 5.5. We adjusted the load pH and the load sulfate molarity to the values shown in Table 9 below using 0 mM sodium sulfate or 800 mM sodium sulfate stock solutions at varying pH. We tested each load pH indicated in Table 9 under low sulfate molarity conditions (0 mM) and high sulfate molarity conditions (240 mM). Each condition was tested at a load density of 60 g/L. As shown by the results presented in Table 9, decreasing the load pH to pH 4 or pH 5 or increasing the load sulfate molarity (to 240 mM sulfate) were each effective for reducing the levels of PLBL2 in the final HIC pool. The combination of pH 4.0 and 240 mM sulfate in the load was particularly effective for minimizing the amount of residual PLBL2 in the HIC pool.

Table 9. PLBL2 levels in the HIC pool observed over a range of load pH and sulfate molarity.

Load pH	PLBL2 (ng/mg by ELISA)	
	Low Sulfate Molarity (0 mM)	High Sulfate Molarity (240 mM)
4	4	1
5	10	3
6	27	5
7	64	6

[00288] Accordingly, use of a HIC resin as a final chromatography step in the purification of CHO-produced polypeptides, such as the anti-IL13 MAb and the anti-Abeta MAb described herein, effectively reduced the residual amount of hamster PLBL2 to very low levels, e.g., 1 ng/mg or less in the HIC pool.

Purification Process to Reduce Hamster PLBL2 in IgG1 Antibody Preparations

[00289] Next, we assessed whether the purification methods described for the anti-IL13 and anti-Abeta IgG4 antibody preparations, particularly use of a HIC column for a final chromatography step, would similarly be effective for reducing PLBL2 levels in IgG1 antibody preparations. For these experiments, we first chose an anti-IL17 A/F antibody, which is an IgG1 antibody and which was produced in CHO cells. Exemplary anti-IL17 A/F antibodies and methods of producing such antibodies have been described previously, for example, in WO 2009136286 and U.S. Patent No. 8,715,669. As described for the anti-IL13 and anti-Abeta MAbs, we explored various resin (in particular, PHENYL SEPHAROSE™ FF [low sub] and PHENYL SEPHAROSE™ FF [high sub] and buffer conditions (in particular, 50 mM sodium acetate, pH 5.5 and 50 mM Tris, 85 mM sodium acetate, pH 8.0) for the HIC column to identify those that were optimal for anti-IL17 A/F for product quality and stability attributes as well as for removal of hamster PLBL2.

[00290] We found that a three column process, Protein A affinity chromatography (MABSELECT SURE™), followed by cation exchange chromatography (POROS® 50HS) operated in bind-and-elute mode, and HIC (PHENYL SEPHAROSE™ 6 Fast Flow (high sub)) operated in flow-through mode was convenient and effective for reducing PLBL2 in the final drug substance. This three-column process is described in detail below.

[00291] The first affinity chromatography step was a bind-and-elute process using MABSELECT SURE™ resin similar to that described above for the anti-IL13 and anti-Abeta MAbs. The second cation exchange chromatography step used POROS® 50HS resin and was operated in bind-and-elute mode. After column equilibration (40 mM sodium acetate, pH 5.5), the pH-adjusted anti-IL17 A/F MABSELECT SURE™ pool (pH 5.0) was loaded onto the

column. The column was washed (40 mM sodium acetate, pH 5.5), and then the anti-IL17 A/F antibody was eluted from the column with a conductivity gradient created with 40 and 400 mM sodium acetate, pH 5.5. Pooling was based on A280 and was initiated at ≥ 0.5 OD and ended at ≤ 2.0 OD during the gradient elution phase.

[00292] The third and final hydrophobic interaction chromatography step used PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin and was operated in a flow-through mode. After column equilibration (50 mM sodium acetate pH 5.5), the anti-IL17 A/F pool from the POROS® 50HS column was loaded directly on the column without pH adjustment. The anti-IL17 A/F MAb flowed through. Anti-IL17 A/F MAb pooling was based on A280 and was initiated during the load phase at ≥ 0.5 OD. The column was washed with 10 CVs of equilibration buffer (50 mM sodium acetate, pH 5.5) and pooling ended during this wash phase at ≤ 1.0 OD.

[00293] The results of using the above process during one purification run are shown in Table 10 below.

Table 10. PLBL2 Levels at Various Stages of Purification of Anti-IL17 A/F MAb Using HIC.

In-Process Sample	PLBL2 (ng/mg by ELISA)
Run No.	1
HCCF	713
MABSELECT SURE™ Pool	151
POROS® 50HS Pool	47
HIC Pool (100 g/L load density)	< 0.7

[00294] The results shown in Table 10 demonstrate that use of a HIC resin as a final chromatography step effectively reduced residual PLBL2 levels in the anti-IL17 A/F MAb (IgG1) preparation to an amount similar to that seen for the anti-IL13 and anti-ABeta MAbs (IgG4).

Anti-CMV Antibody

[00295] In addition to testing anti-IL17 A/F, we tested another IgG1 MAb, anti-CMV-MSL antibody, which is also produced in CHO cells. Exemplary anti-CMV antibodies, including anti-CMV-MSL, and methods of producing such antibodies have been described previously, for example, in WO 2012047732.

[00296] Again, we found that a three column process, Protein A affinity chromatography (MABSELECT SURE™), followed by cation exchange chromatography (POROS® 50HS) operated in bind-and-elute mode, and HIC (PHENYL SEPHAROSE™ 6 Fast Flow (high sub)) operated in flow-through mode was convenient and effective for reducing PLBL2 in the final drug substance. This three-column process is described in detail below.

[00297] The first affinity chromatography step was a bind-and-elute process using MABSELECT SURE™ resin similar to that described above for the anti-IL13, anti-Abeta and anti-IL17 A/F MAbs. The second cation exchange chromatography step that used POROS® 50HS resin and was operated in bind-and-elute mode. After column equilibration (40 mM sodium acetate, pH 5.5), the pH-adjusted aCMV-MSL MABSELECT SURE™ pool (pH 5.0) was loaded onto the column. The column was washed (40 mM sodium acetate, pH 5.5), and then the aCMV-MSL antibody was eluted from the column with a conductivity gradient created with 40 and 400 mM sodium acetate, pH 5.5. Pooling was based on A280 and was initiated at ≥ 0.5 OD and ended at ≤ 1.0 OD during the gradient elution phase.

[00298] In this particular run, a viral filtration step was performed in between the cation exchange and hydrophobic interaction chromatography steps using Viresolve Pro as the virus filter and Fluorodyne UEDF filter as the pre-filter.

[00299] The third and final hydrophobic interaction chromatography step used PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin and was operated in a flow-through mode. After column equilibration (50 mM sodium acetate pH 5.5), the anti-CMV-MSL pool from the POROS® 50HS column was loaded directly on the column without pH adjustment. The anti-CMV-MSL MAb flowed through. Anti-CMV-MSL MAb pooling was based on A280 and was initiated during the load phase at ≥ 0.5 OD. The column was washed with 10 CVs of equilibration buffer (50 mM sodium acetate, pH 5.5) and pooling ended during this wash phase at ≤ 0.5 OD.

[00300] The results of using the above process during one purification run is shown in Table 11 below.

Table 11. PLBL2 Levels at Various Stages of Purification of Anti-CMV-MSL MAb Using HIC.

In-Process Sample	PLBL2 (ng/mg by ELISA)
Run No.	1
HCCF	2608
MABSELECT	319

SURE™ Pool	
POROS® 50HS Pool	33
Viresolve Pro Pool	32
HIC Pool (60 g/L load density)	< 0.6

[00301] The results shown in Table 11 demonstrate that use of a HIC resin as a final chromatography step effectively reduced residual PLBL2 levels in the anti-CMV-MSL MAb preparation to an amount similar to that seen for the anti-IL13, anti-ABeta, and anti-IL17 A/F MAbs. Accordingly, use of a HIC resin as a final chromatography step in the purification of CHO-produced polypeptides, such as the anti-IL13 MAb and other MAbs described herein, effectively reduced the residual amount of hamster PLBL2 to very low levels, e.g., less than 1 ng/mg in the HIC pool. Thus, we showed that use of the HIC chromatography step as described herein for reducing PLBL2 levels was as effective for IgG1 MAbs as for IgG4 MAbs, illustrating the general applicability of this method for reducing hamster PLBL2 levels in recombinant polypeptide preparations.

EXAMPLE 3 – Assessment of Human Anti-Hamster PLBL2 Response in Patients Administered Anti-IL13 MAb Compositions Containing Varying Amounts of Hamster PLBL2

[00302] To assess the potential impact of the CHO PLBL2 impurity, we developed an ELISA assay (a bridging ELISA assay) to detect antibodies to hamster PLBL2 in human subjects who had received the anti-IL13 MAb, lebrikizumab. Serum samples from patients who participated in various clinical studies of lebrikizumab were analyzed for evidence of anti-hamster PLBL2 antibodies pre-dose and post-dose as well as in subjects who received placebo. The details of the clinical studies have been described previously (WO 2012/083132, Corren et al., N Engl J Med 365:1088-98 (2011)) and only the most relevant details of these studies are provided below.

[00303] The antibody bridging ELISA assay that was developed and validated to detect antibodies to hamster PLBL2 in human serum used two conjugated reagents to capture all isotypes of antibodies directed against hamster PLBL2: purified hamster PLBL2 conjugated to biotin (Biotin-PLBL2) and purified hamster PLBL2 conjugated to digoxigenin (DIG-PLBL2). Production and purification of hamster PLBL2 was carried out using standard methods known to one skilled in the art is also described in U.S. Provisional Application Nos. 61/877,503 and 61/991,228 and conjugation to biotin or DIG were carried out using standard methods known to one skilled in the art. In this semi-homogenous antibody bridging ELISA assay, 75 µL/well of conjugated solution in assay diluent (PBS/0.5% BSA/0.05% Polysorbate 20/0.05% ProClin 300,

pH 7.4 ± 0.1) containing 3 μg/mL of each Biotin-PLBL2 and DIG-PLBL2 were co-incubated overnight (16 – 24 hours) at ambient temperature with 75 μL/well of 1:20 diluted serum samples and controls in assay diluent in polypropylene micronic tubes (National Scientific Supply Co.; Claremont, CA). After incubation, 100 μL/well of mixture from the micronic tubes were transferred to a streptavidin-coated 96-well microplate (StreptaWell™ High Bind; Roche Diagnostics; Indianapolis, IN) that was washed 3 times with 400 μL/well of wash buffer (PBS/0.05% Polysorbate 20) in an automatic plate washer (BioTek ELx405) and incubated at ambient temperature for 2 hours ± 10 minutes. The plate was washed 4 times with 400 μL/well of wash buffer in the plate washer, Subsequently, 100 μL/well of 400 ng/mL mouse anti-digoxin antibody conjugated with horseradish peroxidase (HRP) (Jackson ImmunoResearch Cat.200-032-156) was added and incubated at ambient temperature for 2 hours ± 10 minutes for detection. After the plate was washed 4 times with 400 μL/well of wash buffer in the plate washer, 100 μL/well of equal mixture solution of peroxidase substrate (tetramethyl benzidine) (0.4 g/L TMB) and Peroxidase Solution B (0.02% hydrogen peroxide) (KPL Cat. 50-76-03) was added and incubated at ambient temperature for 18 – 28 minutes for color development and the reaction was stopped by adding 100 μL/well of 1 M phosphoric acid. The plates were read at 450 nm for detection absorbance and 630 nm for reference absorbance. The positive control for the assay was a monoclonal antibody construct consisting of a murine anti–hamster PLBL2-specific complementarity determining region (CDR) on a human IgG1 framework. The relative sensitivity of the assay using this antibody was determined to be 25 ng/mL. Assay drug tolerance experiments using this antibody demonstrated that up to 50 μg/mL of lebrikizumab or 1 μg/mL of hamster PLBL2 in serum did not cause interference or cross-reactivity in the assay.

[00304] To carry out the assay, serum samples were first screened in the assay at a minimum dilution of 1/20. Samples that screened positive were then confirmed for hamster PLBL2 specificity using a competition confirmatory assay. If the sample was confirmed as positive, it was serially diluted to obtain a titer value. Positive responses were reported in titer units, which is the log10 of the dilution factor at which the sample signal was equal to the signal of the assay cutpoint (threshold for determining positivity).

[00305] The four clinical studies in which patient samples were analyzed using the anti-hamster PLBL2 ELISA described above are briefly described as follows. Study 1 was a Phase II randomized, double-blind, placebo-controlled, proof-of-concept study to evaluate the effects of lebrikizumab in patients with asthma whose disease was inadequately controlled during chronic therapy with inhaled corticosteriods (ICS). A total of 219 patients were randomized, with 106

receiving at least one 250 mg subcutaneous (SC) dose of lebrikizumab and 92 receiving six monthly doses.

[00306] Study 2 was a Phase II randomized, double-blind, placebo-controlled, dose-ranging study in patients with asthma who were not on ICS therapy. Patients received one of three doses (500, 250, or 125 mg) of lebrikizumab or placebo via SC administration. Study drug was administered four times during the 12-week treatment period. A total of 158 patients were exposed to at least one dose of lebrikizumab, and 145 patients received all four doses.

[00307] Study 3 was a Phase I PK study of lebrikizumab in healthy Japanese and Caucasian volunteers. Three discrete cohorts of 20 healthy Japanese and Caucasian subjects (10 subjects in each racial group) were randomized between lebrikizumab (125, 250, and 375 mg SC) and placebo in a 7:3 ratio. Subjects were dosed once on Day 1and were subsequently monitored for 120 days. A total of 42 subjects each received one dose of lebrikizumab.

[00308] In Studies 1-3, a total of 306 subjects, 264 of which were asthma patients, each received at least one dose of material containing hamster PLBL2. Exposure to hamster PLBL2 was variable, depending on the dose of lebrikizumab received.

[00309] Study 4 was a Phase IIb randomized, double-blind, placebo-controlled studies to assess the efficacy and safety of lebrikizumab in patients with uncontrolled asthma who were using ICS and a second controller medication. Patients received one of three doses (250, 125, or 37.5 mg) of lebrikizumab or placebo via SC administration monthly. In Study 4, a total of 463 patients were randomized, with 347 receiving at least one dose of lebrikizumab. Exposure to hamster PLBL2 was variable, depending on the dose of lebrikizumab received.

[00310] Table 12 below provides a summary of each of the Studies 1-4 showing the range of hamster PLBL2 levels the subjects were exposed to and the dose of lebrikizumab.

Table 12. Hamster PLBL2 Exposure in Lebrikizumab Clinical Trials.

Study	Drug Substance PLBL2 (ng/mg)	Lebrikizumab Dose (mg/month)	PLBL2 (µg/dose)
1	34-137[a]	250	9-34
2	34-137[a]	125	4-17
		250	9-34
		500	17-69
3	34	125	4
		250	9
		375	13
4	242	37.5	9
	328	125	41
	328	250	82

[a]Range from four different lots of clinical material.

[00311] A retrospective analysis of selected time points from Study 1 was performed using the anti-hamster PLBL2 antibody assay described above to detect antibodies to hamster PLBL2. Samples from both placebo and dosed subjects were analyzed to determine the level of pre-existing response as well as the development of antibodies in response to lebrikizumab dosing. There were 113 placebo subjects and 106 dosed subjects who received at least one dose of lebrikizumab. Timepoints selected for analysis were Days 0, 29, 85, 141, 225, and early termination. Samples were taken prior to the next dose; therefore, Day 29 samples were taken prior to the administration of the second dose. The percentage of anti–hamster PLBL2 antibody-positive subjects at each timepoint was calculated by taking the number of positive subjects at each timepoint and dividing by the total number of subjects tested at each timepoint. The data is shown in Table 13.

Table 13. Anti-Hamster PLBL2 Antibody Results for Study 1.

	% Positive at Each Timepoint (no. positive subjects/total no. subjects evaluable)					
Study Day:	0	29	85	141	225	Early Termination
Placebo	6 (7/110)	7 (8/107)	9 (9/104)	8 (8/99)	5 (5/97)	25 (2/8)
250 mg dose	5 (5/102)	6 (6/100)	89 (90/101)	98 (92/94)	98 (91/93)	100 (8/8)[a]

[a] Of the 8 lebrikizumab subjects who discontinued study drug early, only 3 reported adverse events as the reason for study drug discontinuation.

[00312] The 6 Study 1 placebo subjects who were positive pre-dose on Day 0 continued to be positive throughout the study. Samples from these subjects were confirmed as positive in a confirmatory competition assay and had titers on Day 0 that ranged from 1.6 to 2.9 titer units. Titers obtained on subsequent visits were similar to those obtained on Day 0. A few additional placebo subjects had low-level positive responses during the Study.

[00313] Among the Study 1 subjects that received lebrikizumab, 98% (104/106) had a positive antibody response after dosing and remained positive through the end of the study, with most subjects becoming positive after receiving at least two doses of lebrikizumab. Titers after dosing ranged from 1.35 to 4.76 titer units, with titers generally increasing over time. The clinical significance of the development of anti–hamster PLBL2 antibodies is not known. No clinically important safety signals were identified in this study and, given the high incidence of antibodies to hamster PLBL2, no correlation with safety events could be made.

[00314] An interim analysis was also performed on samples collected in Study 4. Samples from both placebo and dosed subjects were analyzed to determine the level of pre-existing response as well as the development of anti-hamster PLBL2 antibodies in response to lebrikizumab dosing. There were 116 placebo subjects and 347 dosed subjects who received at least one dose of lebrikizumab. Samples from 92 placebo subjects and 268 dosed subjects are represented in this data set. The results are shown in Table 14.

Table 14. Anti-Hamster PLBL2 Antibody Results for Study 4 for Subjects not Previously Exposed to Lebrikizumab.

	% Positive at Each Timepoint (no. positive subjects/total no. subjects evaluable)					
Study Day:	0	29	85	169	253	Early Termination
Placebo	4 (4/89)	4 (3/78)	4 (2/48)	0 (0/13)	NA	0 (0/5)
37.5 mg dose	9 (8/88)	9 (7/82)	55 (35/64)	79 (27/34)	66 (2/3)	43 (3/7)[a]
125 mg dose	4 (3/81)	11 (8/73)	87 (48/55)	100 (9/9)	NA	0 (0/2)[a]
250 mg dose	5 (4/88)	10 (7/72)	96 (49/51)	100 (13/13)	NA	67 (2/3)[a]

[a] Of the 12 lebrikizumab subjects who discontinued study drug early, only 4 reported adverse events as the reason for study drug discontinuation.

[00315] The four Study 4 placebo subjects that were positive pre-dose on Day 0 had low-level positive responses that were just above the detection limit of the assay. The low-level responses were detectable at some, but not all, subsequent timepoints.

[00316] The 15 Study 4 subjects receiving lebrikizumab that were positive pre-dose on Day 0 continued to be positive at subsequent timepoints, with increasing titers after multiple doses. In addition, there were 10 subjects in Study 4 who previously received lebrikizumab in Study 1. Nine of these subjects were subsequently re-dosed with lebrikizumab in Study 4 while 1 subject received placebo. All 10 subjects were pre-dose positive on Day 0 for Study 4 and continued to be positive at subsequent timepoints. The data from these 10 subjects were excluded from Table 14 due to their previous lebrikizumab exposure.

[00317] Among the Study 4 subjects receiving lebrikizumab, there appear to be differences in positivity rates between dose groups. However, as these data are incomplete, conclusions regarding the significance of these differences cannot be made at this time. Similar to the data from Study 1, the majority of subjects become positive after receiving at least two doses of lebrikizumab. Titers after dosing ranged from 1.68 to 4.55 titer units, with titers generally

increasing over time. Since this is an incomplete data set, positive percentages and titer ranges may change as additional data are accumulated.

[00318] An interim safety assessment of Study 4 showed a safety profile similar those of the earlier completed studies with no clinically significant safety signals, including no reports of anaphylaxis or serious hypersensitivity reactions. Of note, 6 of the 9 patients who received lebrikizumab in Study 1 and were subsequently re-dosed with lebrikizumab in Study 4 had not reported any adverse events at the time of the interim analysis and only 1 patient reported any local injection-site reactions. No clinical sequelae of this anti-hamster PLBL2 antibody response have been identified in the clinical trials to date.

[00319] We also performed an assessment on the 125-mg dose group from Study 2 and those results are shown in Table 15.

Table 15. Anti-Hamster PLBL2 Antibody Results for Study 2.

	% Positive at Each Timepoint (no. positive subjects/total no. subjects evaluable)					
Study Day:	0	29	57	85	141	Early Termination
125 mg dose	4 (2/51)	21 (11/53)	70 (35/50)	88 (45/51)	86 (43/50)	100 (2/2)[a]

[a] Of the 2 subjects who discontinued study drug early, neither reported adverse events as the reason for study drug discontinuation.

[00320] The two Study 2 subjects that were positive pre-dose on Day 0 continued to be positive at all subsequent timepoints, with increasing titers after multiple doses. Among the Study 2 subjects that received 125 mg of lebrikizumab, 87% (46/53) had a positive antibody response after dosing and remained positive through the end of the study, with most subjects becoming positive after receiving at least two doses of lebrikizumab. Titers after dosing ranged from 1.51 to 4.09 titer units, with titers generally increasing over time.

Conclusions

[00321] To assess the potential impact of the CHO PLBL2 impurity, an assay was developed to detect antibodies to hamster PLBL2 in subjects who had received lebrikizumab preparations that contained significant levels of hamster PLBL2. On the basis of the completed data sets from Study 1 and the 125 mg dose group of Study 2 and on the partial data set from Study 4, the presence of hamster PLBL2 in lebrikizumab preparations produced immune responses in most subjects exposed to hamster PLBL2.

[00322] A number of subjects in both the placebo and lebrikizumab dose groups had pre-existing immunoreactivity in the anti–hamster PLBL2 antibody assay. The cause of this pre-

existing response is unknown; antibody reactivity to CHO host cell proteins has previously been characterized and confirmed in normal human serum samples with no known prior exposure to CHO-derived biological products (Xue et al., The AAPS Journal 12(1):98–106 (2010)). However, there are no published data specific to the single species of CHOP, PLBL2.

[00323] For subjects with pre-existing immunoreactivity in the anti–hamster PLBL2 antibody assay at the start of the study, there was a sustained rise in antibody titers after repeat administration with lebrikizumab. For subjects that were antibody negative at the start of the study, the majority of subjects across all four studies became positive after at least two administrations of lebrikizumab and remained positive through all subsequent timepoints.

[00324] The clinical significance of the development of anti–hamster PLBL2 antibodies is not known . Although there was a high incidence of antibodies to hamster PLBL2 in the study subjects, no correlation between safety events could be made. Importantly, there were no safety signals identified in these completed or interim studies and in particular, no reported events of anaphylaxis, anaphylactoid, or serious hypersensitivity reactions. Nevertheless, there remains a concern that long term exposure with repeat dosing could increase the potential for undesirable effects such as anaphylaxis, hypersensitivity, and immune complex deposition, particularly in asthma patient populations and other allergic or hypersensitive patient populations. Accordingly, it is important to dose patients in late stage clinical studies and beyond, where there may be such repeat dosing over a long period of time, with anti-IL13 MAb (e.g., lebrikizumab) preparations containing substantially reduced levels of hamster PLBL2 so as to minimize immunogenicity as much as possible.

[00325] Additional antibody sequences are provided in Table 16 below.

Table 16. Anti-IL17 A/F antibody amino acid sequences (SEQ ID NOS.: 15-22) and anti-Abeta antibody amino acid sequences (SEQ ID NOS.: 23-30).

CDR-H1 (SEQ ID NO.:15)	Asp Tyr Ala Met His
CDR-H2 (SEQ ID NO.:16)	Gly Ile Asn Trp Ser Ser Gly Gly Ile Gly Tyr Ala Asp Ser Val Lys Gly
CDR-H3 (SEQ ID NO.:17)	Asp Ile Gly Gly Phe Gly Glu Phe Tyr Trp Asn Phe Gly Leu
CDR-L1 (SEQ ID NO.:18)	Arg Ala Ser Gln Ser Val Arg Ser Tyr Leu Ala
CDR-L2 (SEQ ID NO.:19)	Asp Ala Ser Asn Arg Ala Thr
CDR-L3 (SEQ ID NO.:20)	Gln Gln Arg Ser Asn Trp Pro Pro Ala Thr
VH (SEQ ID NO.:21)	Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Gly Ile Asn Trp Ser Ser Gly Gly Ile Gly Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg Asp Ile Gly Gly Phe Gly Glu Phe Tyr Trp Asn Phe Gly Leu Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser
VL (SEQ ID NO.:22)	Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Arg Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Pro Ala Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
CDR-H1 (SEQ ID NO.:23)	GFTFSSYGMS
CDR-H2 SEQ ID NO.:24)	SINSNGGSTY YPDSVK
CDR-H3 SEQ ID NO.: 25)	GDY
CDR-L1 (SEQ ID NO.:26)	RSSQSLVYSN GDTYLH

CDR-L2 (SEQ ID NO.: 27)	KVSNRFS
CDR-L3 (SEQ ID NO.: 28)	SQSTHVPWT
VH (SEQ ID NO.: 29)	EVQLVESGGG LVQPGGSLRL SCAASGFTFS SYGMSWVRQA PGKGLELVAS INSNGGSTYY PDSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCASGD YWGQGTTVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT KTYTCNVDHK PSNTKVDKRV ESKYGPPCPP CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQ KSLSLSLG
VL (SEQ ID NO.: 30)	DIVMTQSPLS LPVTPGEPAS ISCRSSQSLV YSNGDTYLHW YLQKPGQSPQ LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCSQSTHVP WTFGQGTKVE IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK VQWKVDNALQ SGNSQESVTE QDSKDSTYSL SSTLTLSKAD YEKHKVYACE VTHQGLSSPV TKSFNRGEC

WHAT IS CLAIMED IS:

1. A composition comprising an anti-IL13 monoclonal antibody purified from Chinese hamster ovary host cells, wherein the composition comprises the anti-IL13 antibody and a residual amount of hamster PLBL2, wherein the amount of hamster PLBL2 is less than 20 ng/mg, or less than 15 ng/mg, or less than 10 ng/mg, or less than 8 ng/mg, or less than 5 ng/mg, or less than 3 ng/mg, or less than 2 ng/mg, or less than 1 ng/mg, or less than 0.5 ng/mg.

2. The composition of claim 1, wherein the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6.

3. The composition of claim 2, wherein the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7.

4. The composition of claim 2, wherein the anti-IL13 antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 9.

5. The composition of claim 3, wherein the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10.

6. The composition of claim 4, wherein the anti-IL13 antibody comprises a light chain having the amino acid sequence of SEQ ID NO.: 14.

7. The composition of claim 2, wherein the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7 and a light chain variable region having the amino acid sequence of SEQ ID NO.: 9.

8. The composition of claim 7, wherein the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10 and a light chain having the amino acid sequence of SEQ ID NO.: 14.

9. The composition of claim 1, wherein the amount of hamster PLBL2 was quantified using an immunoassay or a mass spectrometry assay.

10. The composition of claim 9, wherein the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA.

11. The composition of claim 9, wherein the mass spectrometry assay is LC-MS/MS.

12. A purified anti-IL13 monoclonal antibody preparation isolated from Chinese hamster ovary host cells, wherein the preparation is purified by a process comprising a hydrophobic interaction chromatography (HIC) step thereby producing a purified preparation, wherein the

purified preparation comprises the anti-IL13 antibody and a residual amount of hamster PLBL2, wherein the amount of hamster PLBL2 is less than 20 ng/mg, or less than 15 ng/mg, or less than 10 ng/mg, or less than 8 ng/mg, or less than 5 ng/mg, or less than 3 ng/mg, or less than 2 ng/mg, or less than 1 ng/mg, or less than 0.5 ng/mg.

13. The anti-IL13 antibody preparation of claim 12, wherein the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin.

14. The anti-IL13 antibody preparation of claim 13, wherein the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode.

15. The anti-IL13 antibody preparation of claim 14, wherein the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 50 mM sodium acetate pH 5.0.

16. The anti-IL13 antibody preparation of claim 15, wherein the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected between 0.5 OD to 1.5 OD.

17. The anti-IL13 antibody preparation of claim 15, wherein the flow-through is collected for a maximum of 8 column volumes.

18. The anti-IL13 antibody preparation of claim 12, wherein the process further comprises an affinity chromatography step.

19. The anti-IL13 antibody preparation of claim 18, wherein the affinity chromatography is protein A chromatography.

20. The anti-IL13 antibody preparation of claim 12, wherein the process further comprises an ion exchange chromatography step.

21. The anti-IL13 antibody preparation of claim 20, wherein the ion exchange chromatography is anion exchange chromatography.

22. A purified anti-IL13 monoclonal antibody preparation isolated from Chinese hamster ovary cells, wherein the antibody preparation is purified by a process comprising a first Protein A affinity chromatography step, a second anion exchange chromatography step, and a third hydrophobic interaction chromatography (HIC) step thereby producing a purified preparation, wherein the purified preparation comprises the anti-IL13 antibody and a residual amount of hamster PLBL2, wherein the amount of hamster PLBL2 is less than 20 ng/mg, or less than 15 ng/mg, or less than 10 ng/mg, or less than 8 ng/mg, or less than 5 ng/mg, or less than 3 ng/mg, or less than 2 ng/mg, or less than 1 ng/mg, or less than 0.5 ng/mg.

23. The anti-IL13 antibody preparation of claim 22, wherein the affinity chromatography step comprises MABSELECT SURE™ resin, the anion exchange chromatography step

comprises Q SEPHAROSE™ Fast Flow resin, and the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (high sub) resin.

24. The anti-IL13 antibody preparation of claim 23, wherein:

the affinity chromatography step comprises operating a MABSELECT SURE™ resin-containing column in bind-elute mode;

the anion exchange chromatography step comprises operating a Q SEPHAROSE™ Fast Flow resin-containing column in bind-elute mode; and

the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode.

25. The anti-IL13 antibody preparation of claim 12 or claim 22, wherein the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6.

26. The anti-IL13 antibody preparation of claim 25, wherein the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7.

27. The anti-IL13 antibody preparation of claim 25, wherein the anti-IL13 antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 9.

28. The anti-IL13 antibody preparation of claim 26, wherein the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10.

29. The anti-IL13 antibody preparation of claim 27, wherein the anti-IL13 antibody comprises a light chain having the amino acid sequence of SEQ ID NO.: 14.

30. The anti-IL13 antibody preparation of claim 25, wherein the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7 and a light chain variable region having the amino acid sequence of SEQ ID NO.: 9.

31. The anti-IL13 antibody preparation of claim 30, wherein the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10 and a light chain having the amino acid sequence of SEQ ID NO.: 14.

32. The anti-IL13 antibody preparation of claim 12 or claim 22, wherein the amount of hamster PLBL2 was quantified using an immunoassay or a mass spectrometry assay.

33. The anti-IL13 antibody preparation of claim 32, wherein the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA.

34. The anti-IL13 antibody preparation of claim 32, wherein the mass spectrometry assay is LC-MS/MS.

35. A method of purifying a recombinant polypeptide produced in Chinese hamster ovary host cells, wherein the method provides a purified preparation comprising the recombinant polypeptide and a residual amount of hamster PLBL2.

36. The method of claim 35, wherein the amount of hamster PLBL2 is less than 20 ng/mg, or less than 15 ng/mg, or less than 10 ng/mg, or less than 8 ng/mg, or less than 5 ng/mg, or less than 3 ng/mg, or less than 2 ng/mg, or less than 1 ng/mg, or less than 0.5 ng/mg.

37. The method of claim 36 comprising a hydrophobic interaction chromatography (HIC) step.

38. The method of claim 37, wherein the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin.

39. The method of claim 38, wherein the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode.

40. The method of claim 37, wherein the recombinant polypeptide is selected from a growth factor, a cytokine, an antibody, an antibody fragment, and an immunoadhesin.

41. The method of claim 40, wherein the recombinant polypeptide is an antibody.

42. The method of claim 41, wherein the antibody is a humanized monoclonal antibody.

43. The method of claim 42, wherein the antibody is IgG1, or IgG2, or IgG3, or IgG4.

44. The method of claim 43, wherein the antibody is IgG4.

45. The method of claim 41, wherein the antibody is anti-IL13.

46. The method of claim 44, wherein the antibody is lebrikizumab.

47. The method of claim 45, wherein the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6.

48. The method of any one of claims 45, 46, or 47, wherein the HIC step comprises operating a resin-containing column in flow-through mode and an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 50 mM sodium acetate pH 5.0.

49. The method of claim 48, wherein the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected between 0.5 OD to 1.5 OD.

50. The method of claim 48, wherein the flow-through is collected for a maximum of 8 column volumes.

51. The method of claim 48, further comprising an affinity chromatography step.

52. The method of claim 51, wherein the affinity chromatography is protein A chromatography.

53. The method of claim 48, further comprising an ion exchange chromatography step.

54. The method of claim 53, wherein the ion exchange chromatography is anion exchange chromatography.

55. The method of claim 48 comprising a first Protein A affinity chromatography step and a second anion exchange chromatography step prior to the hydrophobic interaction chromatography (HIC) step.

56. The method of claim 55, wherein the affinity chromatography step comprises MABSELECT SURE™ resin, the anion exchange chromatography step comprises Q SEPHAROSE™ Fast Flow, and the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (high sub).

57. The method of claim 56, wherein:

the affinity chromatography step comprises operating a MABSELECT SURE™ resin-containing column in bind-elute mode;

the anion exchange chromatography step comprises operating a Q SEPHAROSE™ Fast Flow resin-containing column in bind-elute mode, and

the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode.

58. The method of claim 41, wherein the antibody is anti-Abeta.

59. The method of claim 44, wherein the antibody is crenezumab.

60. The method of claim 58, wherein the anti-Abeta antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.:23, CDR-H2 having the amino acid sequence of SEQ ID NO.:24, and CDR-H3 having the amino acid sequence of SEQ ID NO.:25, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.:26, CDR-L2 having the amino acid sequence of SEQ ID NO.:27, and CDR-L3 having the amino acid sequence of SEQ ID NO.:28.

61. The method of any one of claims 58, 59, or 60, wherein the HIC step comprises operating a resin-containing column in flow-through mode and an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 150 mM sodium acetate pH 5.0.

62. The method of claim 61, wherein the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected beginning at 0.5 OD and for 10 column volumes.

63. The method of claim 61, further comprising an affinity chromatography step.

64. The method of claim 63, wherein the affinity chromatography is protein A chromatography.

65. The method of claim 61, further comprising a mixed mode chromatography step.

66. The method of claim 61 comprising a first Protein A affinity chromatography step and a second mixed mode chromatography step prior to the hydrophobic interaction chromatography (HIC) step.

67. The method of claim 66, wherein the affinity chromatography step comprises MABSELECT SURE™ resin, the mixed mode chromatography step comprises CAPTO™ Adhere, and the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (high sub).

68. The method of claim 67, wherein:

the affinity chromatography step comprises operating a MABSELECT SURE™ resin-containing column in bind-elute mode;

the mixed mode chromatography step comprises operating a CAPTO™ Adhere resin-containing column in flow-through mode, and

the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode.

69. The method of claim 36, wherein the amount of hamster PLBL2 is quantified using an immunoassay or a mass spectrometry assay.

70. The method of claim 69, wherein the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA.

71. The method of claim 69, wherein the mass spectrometry assay is LC-MS/MS.

72. A composition comprising an anti-Abeta monoclonal antibody purified from Chinese hamster ovary host cells, wherein the composition comprises the anti-Abeta antibody and a residual amount of hamster PLBL2, wherein the amount of hamster PLBL2 is less than 20 ng/mg, or less than 15 ng/mg, or less than 10 ng/mg, or less than 8 ng/mg, or less than 5 ng/mg, or less than 3 ng/mg, or less than 2 ng/mg, or less than 1 ng/mg, or less than 0.5 ng/mg.

73. The composition of claim 72, wherein the anti-Abeta antibody is crenezumab.

74. The composition of claim 72, wherein the anti-Abeta antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.:23, CDR-H2 having the amino acid sequence of SEQ ID NO.:24, and CDR-H3 having the amino acid sequence of SEQ ID NO.:25, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID

NO.:26, CDR-L2 having the amino acid sequence of SEQ ID NO.:27, and CDR-L3 having the amino acid sequence of SEQ ID NO.:28.

75. The composition of claim 74, wherein the anti-Abeta antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:29.

76. The composition of claim 74, wherein the anti-Abeta antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.:30.

77. The composition of claim 74, wherein the anti-Abeta antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:29 and a light chain variable region having the amino acid sequence of SEQ ID NO.:30.

78. The method of claim 43, wherein the antibody is IgG1.

79. The method of claim 41, wherein the antibody is anti-IL17 A/F.

80. The method of claim 79, wherein the antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.:15, CDR-H2 having the amino acid sequence of SEQ ID NO.:16, and CDR-H3 having the amino acid sequence of SEQ ID NO.:17, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.:18, CDR-L2 having the amino acid sequence of SEQ ID NO.:19 and CDR-L3 having the amino acid sequence of SEQ ID NO.:20.

81. The method of claim 79 or claim 80, wherein the HIC step comprises operating a resin-containing column in flow-through mode and an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 50 mM sodium acetate pH 5.5.

82. The method of claim 81, wherein the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected beginning at 0.5 OD and for 10 column volumes.

83. The method of claim 81, further comprising an affinity chromatography step.

84. The method of claim 83, wherein the affinity chromatography is protein A chromatography.

85. The method of claim 81, further comprising a cation exchange chromatography step.

86. The method of claim 81 comprising a first Protein A affinity chromatography step and a second cation exchange chromatography step prior to the hydrophobic interaction chromatography (HIC) step.

87. The method of claim 86, wherein the affinity chromatography step comprises MABSELECT SURE™ resin, the cation exchange chromatography step comprises POROS® 50HS resin, and the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (high sub) resin.

88. The method of claim 87, wherein:

the affinity chromatography step comprises operating a MABSELECT SURE™ resin-containing column in bind-elute mode;

the cation exchange chromatography step comprises operating a POROS® 50HS resin-containing column in bind-elute mode, and

the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode.

89. A composition comprising an anti-IL17 A/F monoclonal antibody purified from Chinese hamster ovary host cells, wherein the composition comprises the anti-IL17 A/F antibody and a residual amount of hamster PLBL2, wherein the amount of hamster PLBL2 is less than 20 ng/mg, or less than 15 ng/mg, or less than 10 ng/mg, or less than 8 ng/mg, or less than 5 ng/mg, or less than 3 ng/mg, or less than 2 ng/mg, or less than 1 ng/mg, or less than 0.5 ng/mg.

90. The composition of claim 89, wherein the anti-IL17 A/F antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.:15, CDR-H2 having the amino acid sequence of SEQ ID NO.:16, and CDR-H3 having the amino acid sequence of SEQ ID NO.:17, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.:18, CDR-L2 having the amino acid sequence of SEQ ID NO.:19, and CDR-L3 having the amino acid sequence of SEQ ID NO.:20.

91. The composition of claim 90, wherein the anti-IL17 A/F antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:21.

92. The composition of claim 90, wherein the anti-IL17 A/F antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.:22.

93. The composition of claim 90, wherein the anti-IL17 A/F antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:21 and a light chain variable region having the amino acid sequence of SEQ ID NO.:22.

94. A method of treating an IL-13-mediated disorder in a patient comprising administering a treatment composition to the patient, wherein the treatment composition comprises the composition of any one of claims 1-8.

95. The method of claim 94, wherein administration of the treatment composition is less immunogenic for hamster PLBL2 compared to administration of a reference composition, wherein the reference composition comprises an anti-IL13 monoclonal antibody purified from Chinese hamster ovary host cells and a residual amount of hamster PLBL2 of greater than 30 ng/mg, or greater than 50 ng/mg, or greater than 100 ng/mg, or greater than 200 ng/mg, or greater than 300 ng/mg.

96. The method claim 94, wherein the treatment composition is administered subcutaneously once every four weeks, or once every eight weeks, or once every 12 weeks.

97. The method of claim 96, wherein the patient is treated once every four weeks for at least one month, or at least three months, or at least six months, or at least nine months, or at least twelve months, or at least 18 months, or at least two years, or more than two years.

98. The method of claim 94, wherein the IL-13-mediated disorder is selected from asthma, idiopathic pulmonary fibrosis and atopic dermatitis.

99. The method of claim 94, wherein the IL-13-mediated disorder is selected from allergic asthma, non-allergic asthma, allergic rhinitis, allergic conjunctivitis, eczema, urticaria, food allergies, chronic obstructive pulmonary disease, ulcerative colitis, RSV infection, uveitis, scleroderma, and osteoporosis.

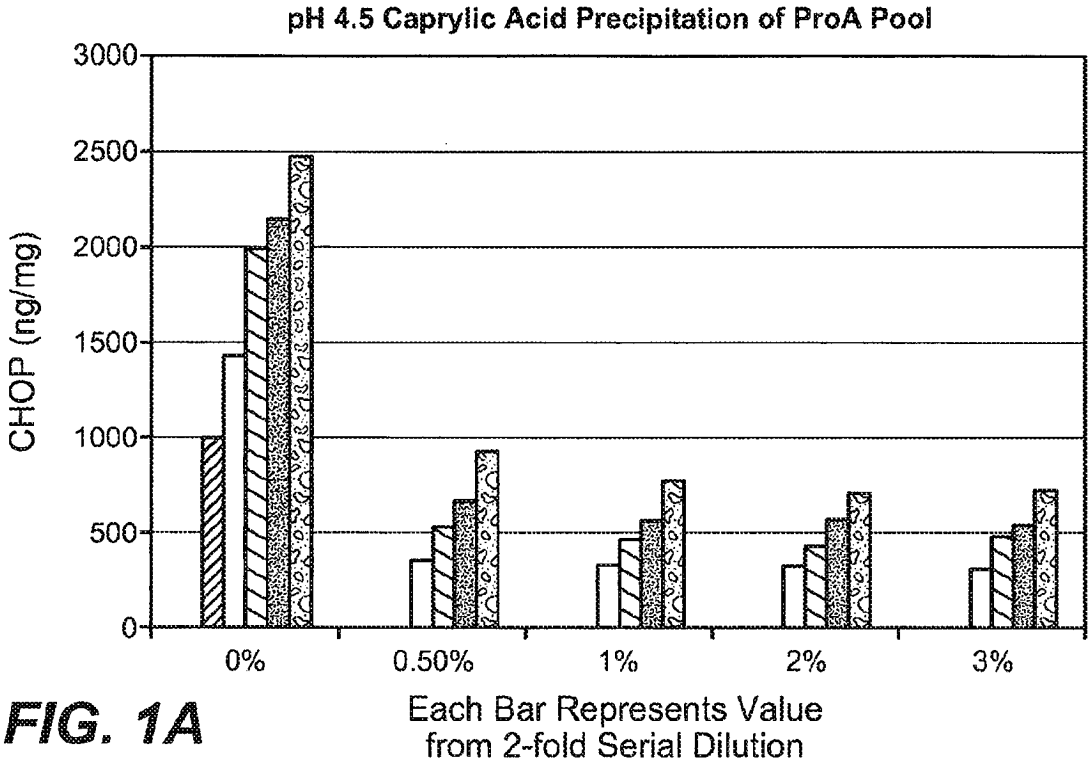
pH 4.5 Caprylic Acid Precipitation of ProA Pool
CHOP (ng/mg)
3000
2500
2000
1500
1000
500
0
0%
0.50%
1%
2%
3%
Each Bar Represents Value
from 2-fold Serial Dilution

FIG. 1A

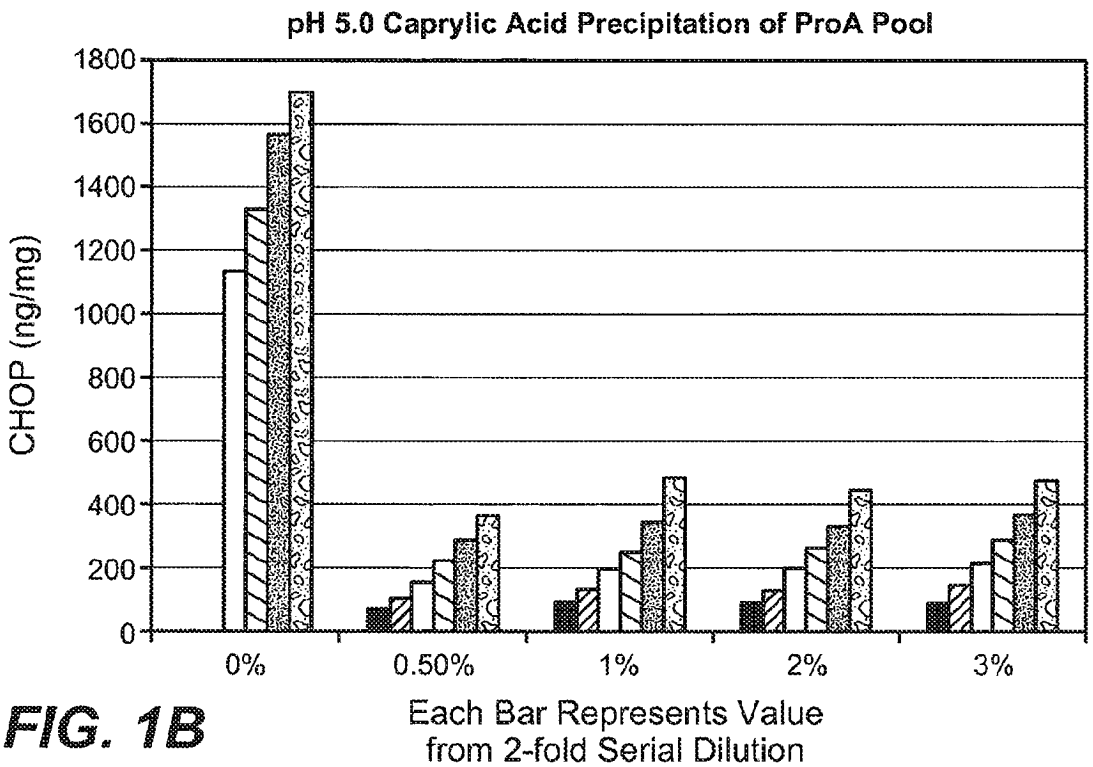
pH 5.0 Caprylic Acid Precipitation of ProA Pool
CHOP (ng/mg)
1800
1600
1400
1200
1000
800
600
400
200
0
0%
0.50%
1%
2%
3%
Each Bar Represents Value
from 2-fold Serial Dilution

FIG. 1B

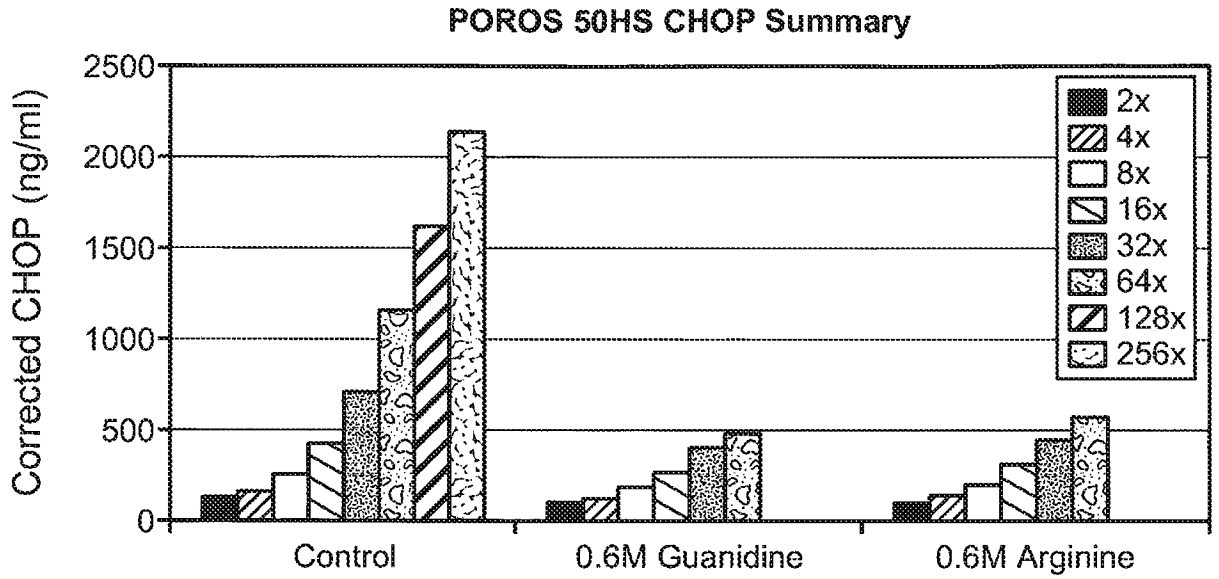
POROS 50HS CHOP Summary
Corrected CHOP (ng/ml)
2500
2000
1500
1000
500
0
2x
4x
8x
16x
32x
64x
128x
256x
Control
0.6M Guanidine
0.6M Arginine

FIG. 2

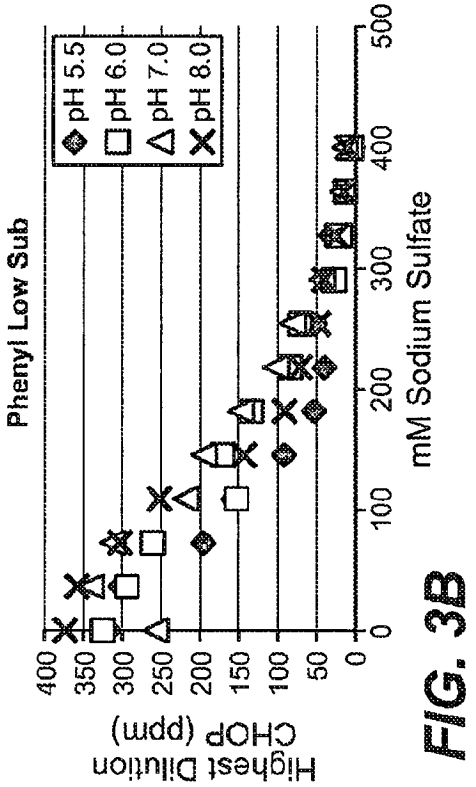

FIG. 3A

FIG. 3B

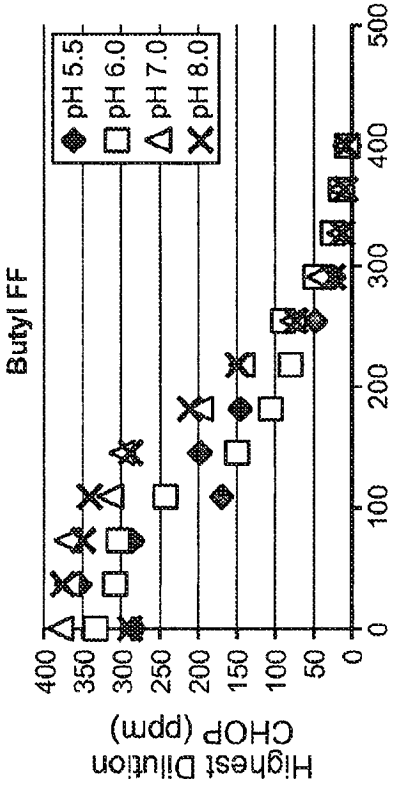

FIG. 3C

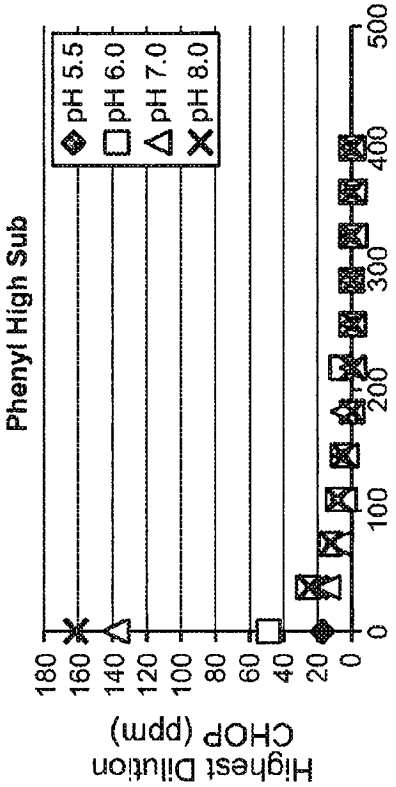

FIG. 3D

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/055387

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/395 (2014.01)
CPC - A61K 2039/505 (2014.12)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 39/395, 49/00; A61P 17/00, 25/28 (2014.01)
USPC - 424/130.1, 141.1; 530/389.2;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - A61K 2039/505; C07K 16/18, 16/244; (2014.12) (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar, Google, PubMed

Search terms used: IL-13; IL-17A/F; Abeta; PLBL2, PLBD2, p76, LAMA-like; CHO; monoclonal antibody

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2005/062967 A2 (TANOX, INC) 14 July 2005 (14.07.2005), entire document	1-99
A	WO 2012/075037 A1 (GENENTECH, INC) 07 JUNE 2012 (07.06.2012), entire document	1-99
A	US 2010/0055103 A1 (CHEN et al) 04 March 2010 (04.03.2010), entire document	1-99
A	BECKER et al. "Unraveling the Chinese hamster ovary cell line transcriptome by next-generation sequencing," Journal of Biotechnology, 17 September 2011 (17.09.2011), Vol. 156, Pgs. 227- 235. entire document	1-99

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 December 2014	20 JAN 2015
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs1-6 and 8-10 were searched.

(19)中华人民共和国国家知识产权局

(12)发明专利申请

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(43)申请公布日 2016.06.29

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2016.05.12
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(87)PCT国际申请的公布数据
WO2015/038888 EN 2015.03.19
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代理人 陈迎春 黄革生
(51)Int.Cl.
A61K 39/395(2006.01)

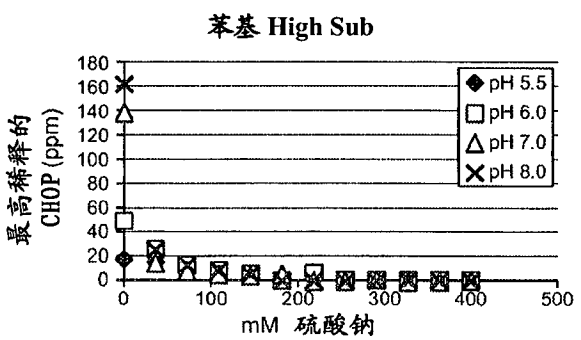
权利要求书6页 说明书59页
序列表23页 附图3页

(54)发明名称

包含纯化的重组多肽的方法和组合物

(57)摘要

本发明提供了从中国仓鼠卵巢宿主细胞分离的纯化的重组多肽，包括抗体（如治疗抗体），以及该多肽的制备方法和使用方法。



CN 105722532 A

1.一种包含从中国仓鼠卵巢宿主细胞纯化的抗IL-13单克隆抗体的组合物，其中所述组合物包含抗IL-13抗体和残留量的仓鼠PLBL2，其中仓鼠PLBL2的量小于20ng/mg，或小于15ng/mg，或小于10ng/mg，或小于8ng/mg，或小于5ng/mg，或小于3ng/mg，或小于2ng/mg，或小于1ng/mg，或小于0.5ng/mg。

2.根据权利要求1所述的组合物，其中抗IL-13抗体包含三个重链CDR和三个轻链CDR，所述三个重链CDR是具有氨基酸序列SEQ ID NO:1的CDR-H1、具有氨基酸序列SEQ ID NO:2的CDR-H2和具有氨基酸序列SEQ ID NO:3的CDR-H3，所述三个轻链CDR是具有氨基酸序列SEQ ID NO:4的CDR-L1、具有氨基酸序列SEQ ID NO:5的CDR-L2和具有氨基酸序列SEQ ID NO:6的CDR-L3。

3.根据权利要求2所述的组合物，其中抗IL-13抗体包含具有氨基酸序列SEQ ID NO:7的重链可变区。

4.根据权利要求2所述的组合物，其中抗IL-13抗体包含具有氨基酸序列SEQ ID NO:9的轻链可变区。

5.根据权利要求3所述的组合物，其中抗IL-13抗体包含具有氨基酸序列SEQ ID NO:10的重链。

6.根据权利要求4所述的组合物，其中抗IL-13抗体包含具有氨基酸序列SEQ ID NO:14的轻链。

7.根据权利要求2所述的组合物，其中抗IL-13抗体包含具有氨基酸序列SEQ ID NO:7的重链可变区和具有氨基酸序列SEQ ID NO:9的轻链可变区。

8.根据权利要求7所述的组合物，其中抗IL-13抗体包含具有氨基酸序列SEQ ID NO:10的重链和具有氨基酸序列SEQ ID NO:14的轻链。

9.根据权利要求1所述的组合物，其中使用免疫测定法或质谱测定法定量仓鼠PLBL2的量。

10.根据权利要求9所述的组合物，其中免疫测定法是总中国仓鼠卵巢蛋白ELISA或仓鼠PLBL2ELISA。

11.根据权利要求9所述的组合物，其中质谱测定法是LC-MS/MS。

12.一种从中国仓鼠卵巢宿主细胞分离的经纯化的抗IL-13单克隆抗体制剂，其中所述制剂通过包括疏水相互作用色谱法(HIC)步骤的方法纯化，从而生成纯化的制剂，其中纯化的制剂包含抗IL-13抗体和残留量的仓鼠PLBL2，其中仓鼠PLBL2的量小于20ng/mg，或小于15ng/mg，或小于10ng/mg，或小于8ng/mg，或小于5ng/mg，或小于3ng/mg，或小于2ng/mg，或小于1ng/mg，或小于0.5ng/mg。

13.根据权利要求12所述的抗IL-13抗体制剂，其中HIC步骤包括PHENYL SEPHAROSE™ 6Fast Flow(High Sub)树脂。

14.根据权利要求13所述的抗IL-13抗体制剂，其中HIC步骤包括在流出模式操作含PHENYL SEPHAROSE™ 6Fast Flow(High Sub)树脂的柱。

15.根据权利要求14所述的抗IL-13抗体制剂，其中HIC步骤包括平衡缓冲液和洗涤缓冲液，其中平衡缓冲液和洗涤缓冲液各包含50mM乙酸钠pH 5.0。

16.根据权利要求15所述的抗IL-13抗体制剂，其中在280纳米的吸光度监测流出液和收集0.5OD至1.5OD之间的流出液。

17.根据权利要求15所述的抗IL-13抗体制剂，其中收集最多8个柱体积的流出液。

18.根据权利要求12所述的抗IL-13抗体制剂，其中方法进一步包括亲和色谱法步骤。

19.根据权利要求18所述的抗IL-13抗体制剂，其中亲和色谱法是蛋白A色谱法。

20.根据权利要求12所述的抗IL-13抗体制剂，其中方法进一步包括离子交换色谱法步骤。

21.根据权利要求20所述的抗IL-13抗体制剂，其中离子交换色谱法为阴离子交换色谱法。

22.一种从中国仓鼠卵巢细胞分离的经纯化的抗IL-13单克隆抗体制剂，其中所述抗体制剂通过包括第一蛋白A亲和色谱法步骤、第二阴离子交换色谱法步骤、和第三疏水相互作用色谱法(HIC)步骤的方法纯化，从而产生纯化的制剂，其中纯化的制剂包含抗IL-13抗体和残留量的仓鼠PLBL2，其中仓鼠PLBL2的量小于20ng/mg，或小于15ng/mg，或小于10ng/mg，或小于8ng/mg，或小于5ng/mg，或小于3ng/mg，或小于2ng/mg，或小于1ng/mg，或小于0.5ng/mg。

23.根据权利要求22所述的抗IL-13抗体制剂，其中亲和色谱法步骤包括MABSELECT SURE™树脂，阴离子交换色谱法步骤包括Q SEPHAROSE™ Fast Flow树脂，和HIC步骤包括PHENYL SEPHAROSE™ 6Fast Flow(High Sub)树脂。

24.根据权利要求23所述的抗IL-13抗体制剂，其中

亲和色谱法步骤包括在结合-洗脱模式操作含MABSELECT SURE™树脂的柱；

阴离子交换色谱法步骤包括在结合-洗脱模式操作含Q SEPHAROSE™ Fast Flow树脂的柱；

HIC步骤包括在流出模式操作含PHENYL SEPHAROSE™ 6Fast Flow(High Sub)树脂的柱。

25.根据权利要求12或权利要求22所述的抗IL-13抗体制剂，其中抗IL-13抗体包含三个重链CDR和三个轻链CDR，其中所述三个重链CDR的具有氨基酸序列SEQ ID NO:1的CDR-H1、具有氨基酸序列SEQ ID NO:2的CDR-H2和具有氨基酸序列SEQ ID NO:3的CDR-H3，所述三个轻链CDR是具有氨基酸序列SEQ ID NO:4的CDR-L1、具有氨基酸序列SEQ ID NO:5的CDR-L2和具有氨基酸序列SEQ ID NO:6的CDR-L3。

26.根据权利要求25所述的抗IL-13抗体制剂，其中抗IL-13抗体包含具有氨基酸序列SEQ ID NO:7的重链可变区。

27.根据权利要求25所述的抗IL-13抗体制剂，其中抗IL-13抗体包含具有氨基酸序列SEQ ID NO:9的轻链可变区。

28.根据权利要求26所述的抗IL-13抗体制剂，其中抗IL-13抗体包含具有氨基酸序列SEQ ID NO:10的重链。

29.根据权利要求27所述的抗IL-13抗体制剂，其中抗IL-13抗体包含具有氨基酸序列SEQ ID NO:14的轻链。

30.根据权利要求25所述的抗IL-13抗体制剂，其中抗IL-13抗体包含具有氨基酸序列SEQ ID NO:7的重链可变区和具有氨基酸序列SEQ ID NO:9的轻链可变区。

31.根据权利要求30所述的抗IL-13抗体制剂，其中抗IL-13抗体包含具有氨基酸序列SEQ ID NO:10的重链和具有氨基酸序列SEQ ID NO:14的轻链。

32.根据权利要求12或权利要求22所述的抗IL-13抗体制剂，其中使用免疫测定法或质谱测定法定量仓鼠PLBL2的量。

33.根据权利要求32所述的抗IL-13抗体制剂，其中免疫测定法是总中国仓鼠卵巢蛋白ELISA或仓鼠PLBL2ELISA。

34.根据权利要求32所述的抗IL-13抗体制剂，其中质谱测定法是LC-MS/MS。

35.一种纯化中国仓鼠卵巢宿主细胞中生产的重组多肽的方法，其中所述方法提供了包含重组多肽和残留量仓鼠PLBL2的纯化制剂。

36.根据权利要求35所述的方法，其中仓鼠PLBL2的量小于20ng/mg，或小于15ng/mg，或小于10ng/mg，或小于8ng/mg，或小于5ng/mg，或小于3ng/mg，或小于2ng/mg，或小于1ng/mg，或小于0.5ng/mg。

37.根据权利要求36所述的方法，其包括疏水相互作用色谱法(HIC)步骤。

38.根据权利要求37所述的方法，其中HIC步骤包括PHENYLSEPHAROSE™ 6 Fast Flow (High Sub)树脂。

39.根据权利要求38所述的方法，其中HIC步骤包括在流出模式操作含PHENYL SEPHAROSE™ 6Fast Flow(High Sub)树脂的柱。

40.根据权利要求37所述的方法，其中重组多肽选自生长因子、细胞因子、抗体、抗体片段和免疫粘附素。

41.根据权利要求40所述的方法，其中重组多肽是抗体。

42.根据权利要求41所述的方法，其中抗体是人源化单克隆抗体。

43.根据权利要求42所述的方法，其中抗体是IgG1、或IgG2或IgG3或IgG4。

44.根据权利要求43所述的方法，其中抗体是IgG4。

45.根据权利要求41所述的方法，其中抗体是抗IL-13。

46.根据权利要求44所述的方法，其中抗体是lebrikizumab。

47.根据权利要求45所述的方法，其中抗IL-13抗体包含三个重链CDR和三个轻链CDR，所述三个重链CDR是具有氨基酸序列SEQ ID NO:1的CDR-H1、具有氨基酸序列SEQ ID NO:2的CDR-H2和具有氨基酸序列SEQ ID NO:3的CDR-H3，所述三个轻链CDR是具有氨基酸序列SEQ ID NO:4的CDR-L1、具有氨基酸序列SEQ ID NO:5的CDR-L2和具有氨基酸序列SEQ ID NO:6的CDR-L3。

48.根据权利要求45、46或47任一项所述的方法，其中HIC步骤包括在流出模式操作含树脂柱，包括平衡缓冲液和洗涤缓冲液，其中平衡缓冲液和洗涤缓冲液各包含50mM乙酸钠pH 5.0。

49.根据权利要求48所述的方法，其中在280纳米的吸光度监测流出液和收集0.5OD至1.5OD之间的流出液。

50.根据权利要求48所述的方法，其中收集最多8个柱体积的流出液。

51.根据权利要求48所述的方法，进一步包括亲和色谱法步骤。

52.根据权利要求51所述的方法，其中亲和色谱法是蛋白A色谱法。

53.根据权利要求48所述的方法，进一步包括离子交换色谱法步骤。

54.根据权利要求53所述的方法，其中离子交换色谱法为阴离子交换色谱法。

55.根据权利要求48所述的方法，包括第一蛋白A亲和色谱法步骤和第二阴离子交换色

谱法步骤以及之后的疏水相互作用色谱法(HIC)步骤。

56.根据权利要求55所述的方法,其中亲和色谱法步骤包括MABSELECT SURE™树脂,阴离子交换色谱法步骤包括Q SEPHAROSE™ Fast Flow和包括PHENYL SEPHAROSE™ 6Fast Flow(high sub)的HIC步骤。

57.根据权利要求56所述的方法,其中

亲和色谱法步骤包括在结合-洗脱模式操作含MABSELECT SURE™树脂的柱;

阴离子交换色谱法步骤包括在结合-洗脱模式操作含Q SEPHAROSE™ Fast Flow树脂的柱,和

HIC步骤包括在流出模式操作含PHENYL SEPHAROSE™ 6Fast Flow(High Sub)树脂的柱。

58.根据权利要求41所述的方法,其中抗体是抗Aβ。

59.根据权利要求44所述的方法,其中抗体是crenezumab。

60.根据权利要求58所述的方法,其中抗Aβ抗体包含三个重链CDR和三个轻链CDR,所述三个重链CDR是具有氨基酸序列SEQ ID NO:23的CDR-H1,具有氨基酸序列SEQ ID NO:24的CDR-H2和具有氨基酸序列SEQ ID NO:25的CDR-H3,所述三个轻链CDR是具有氨基酸序列SEQ ID NO:26的CDR-L1,具有氨基酸序列SEQ ID NO:27的CDR-L2和具有氨基酸序列SEQ ID NO:28的CDR-L3。

61.根据权利要求58、59或60任一项所述的方法,其中HIC步骤包括在流出模式操作含树脂柱,和HIC步骤包括平衡缓冲液和洗涤缓冲液,其中平衡缓冲液和洗涤缓冲液各包含150mM乙酸钠pH 5.0。

62.根据权利要求61所述的方法,其中在280纳米吸光度下监测流出液并在0.5OD开始收集流出液,收集持续10个柱体积。

63.根据权利要求61所述的方法,其进一步包括亲和色谱法步骤。

64.根据权利要求63所述的方法,其中亲和色谱法是蛋白A色谱法。

65.根据权利要求61所述的方法,其包括混合模式色谱法步骤。

66.根据权利要求61所述的方法,其包括第一蛋白A亲和色谱法步骤和第二混合模式色谱法步骤和之后的疏水相互作用色谱法(HIC)步骤。

67.根据权利要求66所述的方法,其中亲和色谱法步骤包括MABSELECT SURE™树脂,混合模式色谱法步骤包括CAPTO™Adhere,HIC步骤包括PHENYL SEPHAROSE™6Fast Flow(high sub)。

68.根据权利要求67所述的方法,其中

亲和色谱法步骤包括在结合-洗脱模式操作含MABSELECT SURE™树脂的柱;

混合模式色谱法步骤包括在流出模式操作含CAPTO™Adhere树脂的柱,和

HIC步骤包括在流出模式操作含PHENYL SEPHAROSE™6Fast Flow(high sub)树脂的柱。

69.根据权利要求36所述的方法,其中使用免疫测定法或质谱测定法定量仓鼠PLBL2的量。

70.根据权利要求69所述的方法,其中免疫测定法是总中国仓鼠卵巢蛋白ELISA或仓鼠PLBL2ELISA。

71.根据权利要求69所述的方法,其中质谱测定法是LC-MS/MS。

72.一种包含从中国仓鼠卵巢宿主细胞纯化的抗Aβ单克隆抗体的组合物，其中组合物包含抗Aβ抗体和残留量的仓鼠PLBL2，其中仓鼠PLBL2的量小于20ng/mg，或小于15ng/mg，或小于10ng/mg，或小于8ng/mg，或小于5ng/mg，或小于3ng/mg，或小于2ng/mg，或小于1ng/mg，或小于0.5ng/mg。

73.根据权利要求72所述的组合物，其中抗Aβ抗体是crenezumab。

74.根据权利要求72所述的组合物，其中抗Aβ抗体包含三个重链CDR和三个轻链CDR，所述三个重链CDR是具有氨基酸序列SEQ ID NO:23的CDR-H1，具有氨基酸序列SEQ ID NO:24的CDR-H2和具有氨基酸序列SEQ ID NO:25的CDR-H3，所述三个轻链CDR是具有氨基酸序列SEQ ID NO:26的CDR-L1，具有氨基酸序列SEQ ID NO:27的CDR-L2和具有氨基酸序列SEQ ID NO:28的CDR-L3。

75.根据权利要求74所述的组合物，其中抗Aβ抗体包含具有氨基酸序列SEQ ID NO:29的重链可变区。

76.根据权利要求74所述的组合物，其中抗Aβ抗体包含具有氨基酸序列SEQ ID NO:30的轻链可变区。

77.根据权利要求74所述的组合物，其中抗Aβ抗体包含具有氨基酸序列SEQ ID NO:29的重链可变区和具有氨基酸序列SEQ ID NO:30的轻链可变区。

78.根据权利要求43所述的方法，其中抗体是IgG1。

79.根据权利要求41所述的方法，其中抗体是抗IL17A/F。

80.根据权利要求79所述的方法，其中抗体包含三个重链CDR和三个轻链CDR，所述三个重链CDR是具有氨基酸序列SEQ ID NO:15的CDR-H1，具有氨基酸序列SEQ ID NO:16的CDR-H2和具有氨基酸序列SEQ ID NO:17的CDR-H3，所述三个轻链CDR是具有氨基酸序列SEQ ID NO:18的CDR-L1，具有氨基酸序列SEQ ID NO:19的CDR-L2和具有氨基酸序列SEQ ID NO:20的CDR-L3。

81.根据权利要求79或权利要求80所述的方法，其中HIC步骤包括在流出模式操作含树脂柱，和HIC步骤包括平衡缓冲液和洗涤缓冲液，其中平衡缓冲液和洗涤缓冲液各包含50mM乙酸钠pH 5.5。

82.根据权利要求81所述的方法，其中在280纳米的吸光度监测流出液和在0.5OD开始收集流出液，收集持续10个柱体积。

83.根据权利要求81所述的方法，进一步包括亲和色谱法步骤。

84.根据权利要求83所述的方法，其中亲和色谱法是蛋白A色谱法。

85.根据权利要求81所述的方法，进一步包括阳离子交换色谱法步骤。

86.根据权利要求81所述的方法，其包括第一蛋白A亲和色谱法步骤和第二阳离子交换色谱法步骤和之后的疏水相互作用色谱法(HIC)步骤。

87.根据权利要求86所述的方法，其中亲和色谱法步骤包括MABSELECT SURE™树脂，阳离子交换色谱法步骤包括**POROS®**50HS树脂，和HIC步骤包括PHENYL SEPHAROSE™ 6Fast Flow(high sub)树脂。

88.根据权利要求87所述的方法，其中

亲和色谱法步骤包括在结合-洗脱模式操作含MABSELECT SURE™树脂的柱；

阳离子交换色谱法步骤包括在结合-洗脱模式操作含**POROS®**50HS树脂的柱，和

HIC步骤包括在流出模式操作含PHENYL SEPHAROSE™ 6Fast Flow(High Sub)树脂的柱。

89.一种包含由中国仓鼠卵巢宿主细胞纯化的抗IL17A/F单克隆抗体的组合物，其中所述组合物包含抗IL17A/F抗体和残留量的仓鼠PLBL2，其中仓鼠PLBL2的量小于20ng/mg，或小于15ng/mg，或小于10ng/mg，或小于8ng/mg，或小于5ng/mg，或小于3ng/mg，或小于2ng/mg，或小于1ng/mg，或小于0.5ng/mg。

90.根据权利要求89所述的组合物，其中抗IL17A/F抗体包含三个重链CDR和三个轻链CDR，所述三个重链CDR是具有氨基酸序列SEQ ID NO:15的CDR-H1，具有氨基酸序列SEQ ID NO:16的CDR-H2和具有氨基酸序列SEQ ID NO:17的CDR-H3，所述三个轻链CDR是具有氨基酸序列SEQ ID NO:18的CDR-L1，具有氨基酸序列SEQ ID NO:19的CDR-L2和具有氨基酸序列SEQ ID NO:20的CDR-L3。

91.根据权利要求90所述的组合物，其中抗IL17A/F抗体包含具有氨基酸序列SEQ ID NO:21的重链可变区。

92.根据权利要求90所述的组合物，其中抗IL17A/F抗体包含具有氨基酸序列SEQ ID NO:22的轻链可变区。

93.根据权利要求90所述的组合物，其中抗IL17A/F抗体包含具有氨基酸序列SEQ ID NO:21的重链可变区和具有氨基酸序列SEQ ID NO:22的轻链可变区。

94.一种治疗患者中IL-13介导的疾患的方法，所述方法包括向患者施用治疗组合物，其中治疗组合物包含权利要求1-8任一项的治疗组合物。

95.根据权利要求94所述的方法，其中施用的治疗组合物相比于施用参考组合物，对仓鼠PLBL2的免疫原性更低，其中所述参考组合物包含从中国仓鼠卵巢宿主细胞纯化的抗IL-13单克隆抗体和残留量大于30ng/mg、或大于50ng/mg、或大于100ng/mg、或大于200ng/mg、或大于300ng/mg的仓鼠PLBL2。

96.根据权利要求94所述的方法，其中治疗组合物每四周皮下施用一次、或每八周皮下施用一次、每12周皮下施用一次。

97.根据权利要求96所述的方法，其中患者每四周治疗一次，持续至少一个月，或持续至少三个月，或持续至少六个月，或持续至少九个月，或持续至少12个月，或持续至少18个月，或持续至少两年，或持续超过两年。

98.根据权利要求94所述的方法，其中IL-13介导的疾患选自哮喘、特发性肺纤维化和特应性皮炎。

99.根据权利要求94所述的方法，其中IL-13介导的疾患选自过敏性哮喘、非过敏性哮喘、过敏性鼻炎、过敏性结膜炎、湿疹、荨麻疹、食物过敏、慢性阻塞性肺疾病、溃疡性结肠炎、RSV感染、葡萄膜炎、硬皮病和骨质疏松症。

包含纯化的重组多肽的方法和组合物

[0001] 相关申请的交叉参考

[0002] 本申请要求2013年9月13日提交的美国临时申请号61/877,517的优先权的权益，在此通过引用以其整体并入。

[0003] 序列表

[0004] 本申请包含已通过EFS-Web提交的序列表，并且在此通过引用以其整体并入。所述ASCII副本创建于2014年8月28日，被命名为2014.AUG.28P5704R1-WO Sequence Listing.txt，大小34,811字节。

技术领域

[0005] 提供了从中国仓鼠卵巢宿主细胞分离的纯化的重组多肽(包括抗体，如治疗性抗体)以及制备和使用此类多肽的方法。

背景技术

[0006] 许多药物用于治疗哮喘和其他呼吸疾患已经上市或正在开发中。用于哮喘治疗的一个靶标是IL-13。IL-13是由激活的T细胞、NKT细胞、嗜碱性粒细胞、嗜酸性粒细胞和肥大细胞产生的多效性TH2细胞因子，在临床前模型中已强烈暗示其参与哮喘的发病机理。以前已描述了IL-13拮抗剂，包括抗IL-13抗体。某些此类抗体也已经被开发用于人治疗。最近，一些研究表明针对IL-13的单克隆抗体在治疗哮喘中的临床活性(例如，见Corren等人，2011,N.Engl.J.Med.365,1088-1098;Gauvreau等人,2011,Am.J.Respir.Crit.Care Med.183,1007-1014;Ingram and Kraft,2012,J.Allergy Clin.Immunol.130,829-42;Webb,2011,Nat Biotechnol 29,860-863)。在这些单克隆抗体中，lebrikizumab，一种中和IL-13活性的人源化IgG4抗体，改善了对大多数尽管用吸入糖皮质激素和长效β2-肾上腺素受体激动剂治疗了但仍然有症状的哮喘患者的肺功能(Corren等人，2011，N.Engl.J.Med.365,1088-1098)。

[0007] 此外，已暗示IL-13参与许多其他过敏性和纤维化疾患。例如，由IL13介导的这类疾病和/或病症(condition)包括但不限于，过敏性哮喘、非过敏性(内源性)哮喘、过敏性鼻炎、过敏性皮炎、过敏性结膜炎、湿疹、荨麻疹、食物过敏、慢性阻塞性肺病、溃疡性结肠炎、RSV感染、葡萄膜炎、硬皮病和骨质疏松。

[0008] 为了使重组生物制药蛋白被接受用于人患者施用，重要的是从最终生物产物中除去制造和纯化方法中产生的残余杂质。这些方法组分包括培养基蛋白、免疫球蛋白亲和配体、病毒、内毒素、DNA和宿主细胞蛋白。这些宿主细胞杂质包括方法特定的宿主细胞蛋白(HCP)，其是来自重组DNA技术的生物制剂中的方法相关杂质/污染物。尽管HCP通常少量(以每百万预期重组蛋白中的若干份或每毫克预期重组蛋白中的纳克)存在于最终药品(drug substance)中，公认HCP是不所期望的的、其数量应减到最小。例如，美国食品和药物管理局(FDA)要求用于人体内的生物制药应该尽可能地不含外来杂质，并且要求检测和定量潜在污染物/杂质(如HCP)的测试。

[0009] 从细胞碎片纯化蛋白的步骤首先取决于蛋白质表达的位点。一些蛋白质直接从细胞分泌到周围的生长培养基;另一些蛋白质在细胞内生成。对于后者,纯化方法的第一步包括细胞裂解,这可以通过多种方法进行,包括机械剪切、渗透压休克、或酶处理。这种破坏使细胞的全部内容物释放到匀浆中,并且另外产生由于其尺寸小而难以除去的亚细胞碎片。这些通常通过离心或过滤除去。直接分泌的蛋白质也出现同样的问题,原因是细胞的自然死亡和蛋白质生产方法运行中细胞内宿主细胞蛋白质的释放。

[0010] 一旦获得含有目的蛋白质的溶液,通常使用不同色谱法技术的组合试图从细胞产生的其他蛋白质中分离该目的蛋白质。通常情况下,这些技术基于蛋白质的电荷、疏水性程度或大小分离蛋白质混合物。对于这些技术中的每种可采用几种不同的色谱法树脂,该色谱法树脂允许为所包含的特定蛋白质精确定制纯化方案。这些分离方法中每个的本质是,或者可以使蛋白质以不同的速率沿着柱向下移动、当其进一步向下通过柱时,实现物理分离的增加;或者使蛋白质选择性地粘附到分离介质,然后通过不同溶剂差异洗脱。在一些情况下,当杂质特异地粘附至柱、而感性趣的蛋白质并不特异地粘附至柱时,所期望的蛋白质从杂质分离,即,目的蛋白质存在于"流出液(flow-through)"。

[0011] 离子交换色谱法,针对可交换的抗衡离子(counter ion)命名,是适用于可电离分子的纯化方法。电离分子基于其带电基团与附着至固相支持基质的相反电荷的分子的非特异性静电相互作用被分离,由此阻滞那些与固相更强地相互作用的电离分子。每类电离分子的净电荷和其对基质的亲和力,根据带电基团的数量、各基团的电荷、与带电固相基质竞争相互作用的分子的性质而变化。这些差异导致离子交换色谱法对不同类型分子的分离。在使用离子交换色谱法典型的蛋白质纯化中,如在哺乳动物细胞培养物中的来自宿主细胞的许多蛋白质的混合物,被施加到离子交换柱。非结合分子被洗掉后,例如通过以逐步或梯度模式改变pH值、抗衡离子浓度等调节条件,从固相释放目的非特异性保留或阻滞的电离蛋白质,并将其从具有不同带电特性的蛋白质中分离出来。阴离子交换色谱法涉及目的阴离子分子在特定分离方法的pH和条件下与负性抗衡离子竞争与附着至固相基质上的带正电荷的分子的相互作用。与此相反,阳离子交换色谱法涉及目的阳离子分子在特定分离方法的pH和条件下与正性抗衡离子竞争与附着至固相基质上的带负电荷的分子的相互作用。混合模式离子交换色谱法(也称为多模式离子交换色谱法)涉及在相同的步骤中使用阳离子和阴离子交换色谱法介质的组合。特别是,"混合模式"指的是共价附着阳离子交换、阴离子交换、以及疏水相互作用部分的混合物的固相支持基质。

[0012] 蛋白质的羟磷灰石色谱法涉及蛋白质的带电氨基或羧基与羟磷灰石上带相反电荷基团的非特异性相互作用,其中羟磷灰石和蛋白质的净电荷被缓冲液pH值控制。通过用离子如Ca2+或Mg2+置换非特异性蛋白质-羟磷灰石对完成洗脱。带负电的蛋白质基团由带负电的化合物(如磷酸盐)置换,从而洗脱净负电荷的蛋白质。

[0013] 疏水相互作用色谱法(HIC)通常根据其表面疏水性的差异用于纯化和分离分子,如蛋白质。蛋白质的疏水基团与耦合到色谱法基质的疏水基团非特异性地相互作用。蛋白质表面疏水基团的数量和性质的差异导致蛋白质在HIC柱上差异阻滞,从而分离蛋白质混合物中的蛋白质。

[0014] 亲和色谱法,利用了待纯化蛋白质和固定的捕获剂之间特异的结构上依赖(即,空间互补)的相互作用,其是一些蛋白质(例如抗体)的标准纯化选择。例如蛋白A是蛋白质(如

抗体，其包含Fc区）亲和色谱法的有用的吸附剂。蛋白A是来自金黄色葡萄球菌(Staphylococcus aureas)的41kD细胞壁蛋白，其以高亲和力(对人IgG约10^{-8}M)结合抗体的Fc区。

[0015] 重组多肽的纯化通常是使用结合-洗脱色谱法(B/E)或流出液(flow-through)(F/T)色谱法进行。以下简要描述这些方法。

[0016] 结合-洗脱色谱法(B/E)：在B/E色谱法中，通常以最大化动态结合容量(DBC)上样产物至色谱法材料，然后确定洗涤和洗脱条件，从而在洗出液中获得最大产物纯度。

[0017] 已描述了各种使用蛋白A亲和色谱法的B/E方法，包括各种中间洗涤液。例如，美国专利号6,127,526和6,333,398描述了蛋白A色谱法期间使用疏水性电解质(例如，四甲基氯化铵(TMAC)和四乙基氯化铵(TEAC))的中间洗涤步骤，以除去污染物，而不是结合至蛋白A柱的固定的蛋白A或目的蛋白质。美国专利号6,870,034描述了使用蛋白A亲和色谱法的另外的方法和洗涤液。

[0018] 流出液色谱法(F/T)：使用F/T色谱法，确认其中杂质强力结合到色谱法材料，同时产物流出的上样条件。F/T色谱法允许标准单克隆抗体制剂(MAb)的高上样密度。

[0019] 我们在总CHOP ELISA测定法中、在CHO细胞中产生的重组抗IL13MAb制剂和某些其他重组多肽中、确定了一种酶，磷脂酶B样2，作为单个CHOP种类过量存在于可用抗体。如本文中所使用的，“PLB2”和“PLBL2”和“PLBD2”可互换使用并且是指“磷脂酶B样2”或其同义词，“磷脂酶B结构域样2”。关于PLBL2的某些科学出版物包括Lakomek,K.等人，BMC Structural Biology 9:56(2009);Deuschi,等人，FEBS Lett 580:5747-5752(2006)。PLBL2作为亲本MW约66,000的前酶原被合成。其具有被去除的初始前导序列和潜在的6甘露糖-6-磷酸(M-6-P)基团在翻译后修饰过程中加入。M-6-P是靶向修饰的，将此酶通过M-6-P受体导向溶酶体。PLBL2含有6个半胱氨酸，其中两个具有游离的巯基、四个形成二硫键。在酸性环境中，PLBL2被进一步剪切成分别具有32,000和45,000MW的N-和C-末端片段。通过与其他溶酶体酶类推，这种切割是活化步骤，允许底物进入活性位点。

[0020] 酶的仓鼠和人形式之间具有约80％PLBL2氨基酸序列同源性。认为酶的活性是从构成细胞膜的磷脂切割任一脂肪酸链。存在其他的具有不同底物切割特异性的磷脂酶。在微生物中存在相似的酶活性，其中，它们经常是毒力因子。尽管微生物具有类似的酶活性，但是产生该活性的蛋白质是不同的，在微生物和哺乳动物PLBL2酶之间具有低的序列同源性。磷脂酶产生游离脂肪酸(FFA)，作为底物水解的一个产物。游离脂肪酸本身是潜在的免疫信号传导因子。脱氢作用转化FFA为花生四烯酸，其可能参与涉及花生酸类的炎症级联。

[0021] 确定了PLBL2作为CHO细胞中产生的重组抗IL13 MAb制剂和某些其他重组多肽的单一HCP(CHOP)，我们开发了特异、灵敏和定量测定抗IL-13Mab制剂(和其他重组多肽产物)中和纯化的各阶段中PLBL2水平的试剂、方法和试剂盒。这些将在下面的实施例中以及美国临时专利申请号61/877,503和61/991,228中简单说明。此外，开发大规模、稳健和有效地纯化抗IL-13 Mab(和其他重组多肽产物)的方法、以获得足以用于人治疗用途(包括后期临床和商业用途)的纯度的MAb(包括除去PLBL2)，存在艰巨的挑战。本文描述的发明满足某些上述需要，并提供了其他益处。

[0022] 本文引用的所有参考文献，包括专利申请和出版物，通过引用将其全文并入。

发明内容

[0023] 本发明是基于，至少部分地基于用于纯化中国仓鼠卵巢(CHO)细胞中产生的重组多肽的方法的改进，所述方法提供具有大幅降低的仓鼠PLBL2水平的纯化产物。根据本发明的方法纯化的重组多肽，包括治疗性抗体，例如抗IL-13抗体，当施用于人受试者可能具有降低的免疫原性。

[0024] 因此，在一个方面，提供了包含从含有抗IL-13抗体的CHO细胞纯化的抗IL-13单克隆抗体的组合物，所述组合物包含残留量的仓鼠PLBL2。在某些实施方案中，仓鼠PLBL2的量小于20ng/mg。在某些实施方案中，仓鼠PLBL2的量小于15ng/mg。在某些实施方案中，仓鼠PLBL2的量小于10ng/mg。在某些实施方案中，仓鼠PLBL2的量小于8ng/mg。在某些实施方案中，仓鼠PLBL2的量小于5ng/mg。在某些实施方案中，仓鼠PLBL2的量小于3ng/mg。在某些实施方案中，仓鼠PLBL2的量小于2ng/mg。在某些实施方案中，仓鼠PLBL2的量小于1ng/mg。在某些实施方案中，仓鼠PLBL2的量小于0.5ng/mg。在某些实施方案中，仓鼠PLBL2的量在0.5ng/mg和20ng/mg之间，或0.5ng/mg和15ng/mg之间，或0.5ng/mg和10ng/mg之间，或0.5ng/mg和8ng/mg之间，或0.5ng/mg和5ng/mg之间，或0.5ng/mg和3ng/mg之间，或0.5ng/mg和2ng/mg之间，或0.5ng/mg和1ng/mg之间，或定量测定法的极限(LOQ)和1ng/mg之间。在某些实施方案中，抗IL-13抗体包含三个重链CDR，具有氨基酸序列SEQ ID NO:1的CDR-H1，具有氨基酸序列SEQ ID NO:2的CDR-H2和具有氨基酸序列SEQ ID NO:3的CDR-H3，和三个轻链CDR，具有氨基酸序列SEQ ID NO:4的CDR-L1，具有氨基酸序列SEQ ID NO:5的CDR-L2和具有氨基酸序列SEQ ID NO:6的CDR-L3。在某些实施方案，抗IL-13抗体包含具有氨基酸序列SEQ ID NO:7的重链可变区。在某些实施方案中，抗IL-13抗体包含具有氨基酸序列SEQ ID NO:9的轻链可变区。在某些实施方案中，抗IL-13抗体包含具有氨基酸序列SEQ ID NO:10的重链。在某些实施方案中，抗IL-13抗体包含具有氨基酸序列SEQ ID NO:14的轻链。在某些实施方案中，抗IL-13抗体包含具有氨基酸序列SEQ ID NO:7的重链可变区和具有氨基酸序列SEQ ID NO:9的轻链可变区。在某些实施方案中，抗IL-13抗体包含具有氨基酸序列SEQ ID NO:10的重链和具有氨基酸序列SEQ ID NO:14的轻链。在某些实施方案中，使用免疫测定法或质谱测定法定量组合物中仓鼠PLBL2的量。在某些实施方案中，免疫测定法是总中国仓鼠卵巢蛋白质ELISA或仓鼠PLBL2 ELISA。在某些实施方案中，质谱测定法是LC-MS/MS。

[0025] 在另一方面，提供了使用包括疏水相互作用色谱法(HIC)步骤的方法从CHO细胞分离和纯化的抗IL-13单克隆抗体制剂。在某些实施方案中，纯化的制剂包含抗IL-13抗体和残留量的仓鼠PLBL2。在某些实施方案中，仓鼠PLBL2的量小于20ng/mg。在某些实施方案中，仓鼠PLBL2的量小于15ng/mg。在某些实施方案中，仓鼠PLBL2的量小于10ng/mg。在某些实施方案中，仓鼠PLBL2的量小于8ng/mg。在某些实施方案中，仓鼠PLBL2的量小于5ng/mg。在某些实施方案中，仓鼠PLBL2的量小于3ng/mg。在某些实施方案中，仓鼠PLBL2的量小于2ng/mg。在某些实施方案中，仓鼠PLBL2的量小于1ng/mg。在某些实施方案中，仓鼠PLBL2的量小于0.5ng/mg。在某些实施方案中，仓鼠PLBL2的量在0.5ng/mg和20ng/mg之间，或0.5ng/mg和15ng/mg之间，或0.5ng/mg和10ng/mg之间，或0.5ng/mg和8ng/mg之间，或0.5ng/mg和5ng/mg之间，或0.5ng/mg和3ng/mg之间，或0.5ng/mg和2ng/mg之间，或0.5ng/mg和1ng/mg之间，或定量测定法的极限(LOQ)和1ng/mg之间。在某些实施方案中，HIC步骤包括PHENYL

SEPHAROSE™6Fast Flow(High Sub)树脂。在某些实施方案中,HIC步骤包括在流出模式操作含树脂柱。在某些实施方案中,HIC步骤包括平衡缓冲液和洗涤缓冲液,其中平衡缓冲液和洗涤缓冲液的每个包含50mM乙酸钠pH5.0。在某些实施方案中,在280纳米的吸光度监测流出液和收集0.5OD至1.5OD之间的流出液。在某些实施方案中,收集最多8个柱体积的流出液。在某些实施方案中,方法进一步包括亲和色谱法步骤。在某些实施方案中,亲和色谱法是蛋白A色谱法。在某些实施方案中,方法进一步包括离子交换色谱法步骤。在某些实施方案中,离子交换色谱法为阴离子交换色谱法。在某些实施方案中,抗IL-13抗体包含三个重链CDR,具有氨基酸序列SEQ ID NO:1的CDR-H1,具有氨基酸序列SEQ ID NO:2的CDR-H2和具有氨基酸序列SEQ ID NO:3的CDR-H3,和三个轻链CDR,具有氨基酸序列SEQ ID NO:4的CDR-L1,具有氨基酸序列SEQ ID NO:5的CDR-L2和具有氨基酸序列SEQ ID NO:6的CDR-L3。在某些实施方案,抗IL-13抗体包含具有氨基酸序列SEQ ID NO:7的重链可变区。在某些实施方案中,抗IL-13抗体包含具有氨基酸序列SEQ ID NO:9的轻链可变区。在某些实施方案中,抗IL-13抗体包含具有氨基酸序列SEQ ID NO:10的重链。在某些实施方案中,抗IL-13抗体包含具有氨基酸序列SEQ ID NO:14的轻链。在某些实施方案中,抗IL-13抗体包含具有氨基酸序列SEQ ID NO:7的重链可变区和具有氨基酸序列SEQ ID NO:9的轻链可变区。在某些实施方案中,抗IL-13抗体包含具有氨基酸序列SEQ ID NO:10的重链和具有氨基酸序列SEQ ID NO:14的轻链。在某些实施方案中,使用免疫测定法或质谱测定法定量仓鼠PLBL2的量。在某些实施方案中,免疫测定法是总中国仓鼠卵巢蛋白质ELISA或仓鼠PLBL2ELISA。在某些实施方案中,质谱测定法是LC-MS/MS。

[0026] 在又一方面,提供了从CHO细胞中分离的纯化抗IL-13单克隆抗体制剂。在某些实施方案中,该抗体制剂通过包括第一蛋白A亲和色谱法步骤,第二阴离子交换色谱法步骤,和第三疏水相互作用色谱法(HIC)步骤的方法纯化,从而产生纯化的制剂。在某些实施方案中,纯化的制剂包含抗IL-13抗体和残留量的仓鼠PLBL2。在某些实施方案中,仓鼠PLBL2的量小于20ng/mg。在某些实施方案中,仓鼠PLBL2的量小于15ng/mg。在某些实施方案中,仓鼠PLBL2的量小于10ng/mg。在某些实施方案中,仓鼠PLBL2的量小于8ng/mg。在某些实施方案中,仓鼠PLBL2的量小于5ng/mg。在某些实施方案中,仓鼠PLBL2的量小于3ng/mg。在某些实施方案中,仓鼠PLBL2的量小于2ng/mg。在某些实施方案中,仓鼠PLBL2的量小于1ng/mg。在某些实施方案中,仓鼠PLBL2的量小于0.5ng/mg。在某些实施方案中,仓鼠PLBL2的量在0.5ng/mg和20ng/mg之间,或0.5ng/mg和15ng/mg之间,或0.5ng/mg和10ng/mg之间,或0.5ng/mg和8ng/mg之间,或0.5ng/mg和5ng/mg之间,或0.5ng/mg和3ng/mg之间,或0.5ng/mg和2ng/mg之间,或0.5ng/mg和1ng/mg之间,或定量测定法的极限(LOQ)和1ng/mg之间。在某些实施方案中,亲和色谱法步骤包括MABSELECT SURE™树脂,阴离子交换色谱法步骤包括Q SEPHAROSE™Fast Flow,和HIC步骤包括PHENYL SEPHAROSE™6Fast Flow(High Sub)。在某些实施方案中,亲和色谱法步骤包括在结合-洗脱模式操作含MABSELECT SURE™树脂的柱,阴离子交换色谱法步骤包括在结合-洗脱模式操作含Q SEPHAROSE™Fast Flow树脂的柱,以及HIC步骤包括在流出模式操作含PHENYL SEPHAROSE™6Fast Flow(High Sub)树脂的柱。在某些实施方案中,抗IL-13抗体包含三个重链CDR,具有氨基酸序列SEQ ID NO:1的CDR-H1,具有氨基酸序列SEQ ID NO:2的CDR-H2和具有氨基酸序列SEQ ID NO:3的CDR-H3,和三个轻链CDR,具有氨基酸序列SEQ ID NO:4的CDR-L1,具有氨基酸序列SEQ ID NO:5的CDR-L2和具

有氨基酸序列SEQ ID NO:6的CDR-L3。在某些实施方案，抗IL-13抗体包含具有氨基酸序列SEQ ID NO:7的重链可变区。在某些实施方案中，抗IL-13抗体包含具有氨基酸序列SEQ ID NO:9的轻链可变区。在某些实施方案中，抗IL-13抗体包含具有氨基酸序列SEQ ID NO:10的重链。在某些实施方案中，抗IL-13抗体包含具有氨基酸序列SEQ ID NO:14的轻链。在某些实施方案中，抗IL-13抗体包含具有氨基酸序列SEQ ID NO:7的重链可变区和具有氨基酸序列SEQ ID NO:9的轻链可变区。在某些实施方案中，抗IL-13抗体包含具有氨基酸序列SEQ ID NO:10的重链和具有氨基酸序列SEQ ID NO:14的轻链。在某些实施方案中，使用免疫测定法或质谱测定法定量仓鼠PLBL2的量。在某些实施方案中，免疫测定法是总中国仓鼠卵巢蛋白质ELISA或仓鼠PLBL2ELISA。在某些实施方案中，质谱测定法是LC-MS/MS。

[0027] 在又一方面，提供了纯化CHO细胞中生产的重组多肽的方法，其中所述方法提供了包含重组多肽和残留量仓鼠PLBL2的纯化制剂。在某些实施方案中，仓鼠PLBL2的量小于20ng/mg。在某些实施方案中，仓鼠PLBL2的量小于15ng/mg。在某些实施方案中，仓鼠PLBL2的量小于10ng/mg。在某些实施方案中，仓鼠PLBL2的量小于8ng/mg。在某些实施方案中，仓鼠PLBL2的量小于5ng/mg。在某些实施方案中，仓鼠PLBL2的量小于3ng/mg。在某些实施方案中，仓鼠PLBL2的量小于2ng/mg。在某些实施方案中，仓鼠PLBL2的量小于1ng/mg。在某些实施方案中，仓鼠PLBL2的量小于0.5ng/mg。在某些实施方案中，仓鼠PLBL2的量在0.5ng/mg和20ng/mg之间，或0.5ng/mg和15ng/mg之间，或0.5ng/mg和10ng/mg之间，或0.5ng/mg和8ng/mg之间，或0.5ng/mg和5ng/mg之间，或0.5ng/mg和3ng/mg之间，或0.5ng/mg和2ng/mg之间，或0.5ng/mg和1ng/mg之间，或定量测定法的极限(LOQ)和1ng/mg之间。在某些实施方案中，重组多肽选自生长因子、细胞因子、抗体、抗体片段和免疫粘附素。在某些实施方案中，重组多肽是抗体。在某些实施方案中，抗体是人源化单克隆抗体。在某些实施方案中，抗体是IgG1、或IgG2或IgG3或IgG4。在某些实施方案中，抗体是IgG1。在某些实施方案中，抗体是IgG2。在某些实施方案中，抗体是IgG3。在某些实施方案中，抗体是IgG4。在某些实施方案中，方法包括疏水相互作用色谱法(HIC)步骤。在某些实施方案中，HIC步骤包括PHENYL SEPHAROSE™6Fast Flow(High Sub)树脂。

[0028] 在上述纯化方法的某些实施方案中，纯化的抗体是抗IL-13。在某些实施方案中，抗体是lebrikizumab。在某些实施方案中，HIC步骤包括在流出模式操作含树脂柱。在某些实施方案中，HIC步骤包括平衡缓冲液和洗涤缓冲液，其中每个平衡缓冲液和洗涤缓冲液包含50mM乙酸钠pH5.0。在某些实施方案中，在280纳米的吸光度监测流出液和收集0.5OD至1.5OD之间的流出液。在某些实施方案中，收集最多8个柱体积的流出液。在某些实施方案中，方法进一步包括亲和色谱法步骤。在某些实施方案中，亲和色谱法是蛋白A色谱法。在某些实施方案中，方法进一步包括离子交换色谱法步骤。在某些实施方案中，离子交换色谱法为阴离子交换色谱法。在某些实施方案中，方法包括第一蛋白A亲和色谱法步骤，第二阴离子交换色谱法步骤和第三疏水相互作用色谱法(HIC)步骤。在某些实施方案中，亲和色谱法步骤包括MABSELECT SURE™树脂，阴离子交换色谱法步骤包括Q SEPHAROSE™Fast Flow和HIC步骤包括PHENYL SEPHAROSE™6Fast Flow(high sub)。在某些实施方案中，亲和色谱法步骤包括在结合-洗脱模式操作含MABSELECT SURE™树脂的柱，阴离子交换色谱法步骤包括在结合-洗脱模式操作含Q SEPHAROSE™Fast Flow树脂的柱，以及HIC步骤包括在流出模式操作含PHENYL SEPHAROSE™6Fast Flow(High Sub)树脂的柱。在某些实施方案中，使用免疫

测定法或质谱测定法定量仓鼠PLBL2的量。在某些实施方案中，免疫测定法是总中国仓鼠卵巢蛋白ELISA或仓鼠PLBL2 ELISA。在某些实施方案中，质谱测定法是LC-MS/MS。

[0029] 在上述纯化方法的某些实施方案中，纯化的抗体是抗Aβ。在某些实施方案中，抗Aβ抗体是crenezumab。在某些实施方案中，抗Aβ抗体包含三个重链CDR，具有氨基酸序列SEQ ID NO:23的CDR-H1，具有氨基酸序列SEQ ID NO:24的CDR-H2和具有氨基酸序列SEQ ID NO:25的CDR-H3，和三个轻链CDR，具有氨基酸序列SEQ ID NO:26的CDR-L1，具有氨基酸序列SEQ ID NO:27的CDR-L2和具有氨基酸序列SEQ ID NO:28的CDR-L3。在某些实施方案，抗Aβ抗体包含具有氨基酸序列SEQ ID NO:29的重链可变区。在某些实施方案中，抗Aβ抗体包含具有氨基酸序列SEQ ID NO:30的轻链可变区。在某些实施方案中，抗Aβ抗体包含具有氨基酸序列SEQ ID NO:29的重链可变区和具有氨基酸序列SEQ ID NO:30的轻链可变区。在某些实施方案中，HIC步骤包括在流出模式操作含树脂柱。在某些实施方案中，HIC步骤包括平衡缓冲液和洗涤缓冲液，其中每个平衡缓冲液和洗涤缓冲液包含150mM乙酸钠pH5.0。在某些实施方案中，HIC步骤包括平衡缓冲液和洗涤缓冲液，其中每个平衡缓冲液和洗涤缓冲液包含150mM乙酸钠pH4.0。在某些实施方案中，HIC步骤包括平衡缓冲液和洗涤缓冲液，其中每个平衡缓冲液和洗涤缓冲液包含150mM乙酸钠、240mM硫酸钠pH4.0。在某些实施方案中，HIC步骤包括平衡缓冲液和洗涤缓冲液，其中每个平衡缓冲液和洗涤缓冲液包括150mM乙酸钠、240mM硫酸钠pH5.0。在某些实施方案中，上样密度为300g/L。在某些实施方案中，上样密度为100g/L。在某些实施方案中，在280纳米吸光度下监测流出液并在0.5OD开始收集流出液，收集持续10个柱体积。在某些实施方案中，方法进一步包括亲和色谱法步骤。在某些实施方案中，亲和色谱法是蛋白A色谱法。在某些实施方案中，方法还包括混合模式色谱法步骤。在某些实施方案中，方法包括第一蛋白A亲和色谱法步骤，第二混合模式色谱法步骤和第三疏水相互作用色谱法(HIC)步骤。在某些实施方案中，亲和色谱法步骤包括MABSELECT SURE™树脂，混合模式色谱法步骤包括CAPTO™Adhere树脂，HIC步骤包括PHENYL SEPHAROSE™6Fast Flow(high sub)树脂。在某些实施方案中，亲和色谱法步骤包括在结合-洗脱模式操作含MABSELECT SURE™树脂的柱，混合模式色谱法步骤包括在流出模式操作含CAPTO™Adhere树脂的柱，HIC步骤包括在流出模式操作含PHENYL SEPHAROSE™6Fast Flow(high sub)树脂的柱。在某些实施方案中，使用免疫测定法或质谱测定法定量仓鼠PLBL2的量。在某些实施方案中，免疫测定法是总中国仓鼠卵巢蛋白ELISA或仓鼠PLBL2 ELISA。在某些实施方案中，质谱测定法是LC-MS/MS。

[0030] 在上述纯化方法的又一方面，纯化的抗体是IgG1。在一些实施方案中，抗体是抗IL17A/F。在一些实施方案中，抗IL17A/F抗体包含三个重链CDR，具有氨基酸序列SEQ ID NO:15的CDR-H1，具有氨基酸序列SEQ ID NO:16的CDR-H2和具有氨基酸序列SEQ ID NO:17的CDR-H3，和三个轻链CDR，具有氨基酸序列SEQ ID NO:18的CDR-L1，具有氨基酸序列SEQ ID NO:19的CDR-L2和具有氨基酸序列SEQ ID NO:20的CDR-L3。在某些实施方案，抗IL17A/F抗体包含具有氨基酸序列SEQ ID NO:21的重链可变区。在某些实施方案中，抗IL17A/F抗体包含具有氨基酸序列SEQ ID NO:22的轻链可变区。在某些实施方案中，抗IL17A/F抗体包含具有氨基酸序列SEQ ID NO:21的重链可变区和具有氨基酸序列SEQ ID NO:22的轻链可变区。在某些实施方案中，HIC色谱法步骤包括平衡缓冲液和洗涤缓冲液，其中每个平衡缓冲液和洗涤缓冲液包含50mM乙酸钠pH5.5。在某些实施方案中，在280纳米吸光度下监测流出

液并在0.5OD开始收集流出液，收集持续10个柱体积。在某些实施方案中，方法进一步包括亲和色谱法步骤。在某些实施方案中，亲和色谱法是蛋白A色谱法。在某些实施方案中，方法还包括阳离子交换色谱法步骤。在某些实施方案中，方法包括第一蛋白A亲和色谱法步骤，第二阳离子交换色谱法步骤和之后的疏水相互作用色谱法(HIC)步骤。在某些实施方案中，亲和色谱法步骤包括MABSELECT SURE™树脂，阳离子交换色谱法步骤包括POROS 50HS树脂，HIC步骤包括PHENYL SEPHAROSE™6Fast Flow(high sub)树脂。在某些实施方案中，亲和色谱法步骤包括在结合-洗脱模式操作含MABSELECT SURE™树脂的柱，阳离子交换色谱法步骤包括在结合-洗脱模式操作含POROS 50HS树脂的柱，HIC步骤包括在流出模式操作含PHENYL SEPHAROSE™6Fast Flow(High Sub)树脂的柱。

[0031] 在又一方面，提供了通过包括疏水相互作用色谱法(HIC)步骤的方法从CHO细胞纯化的抗Aβ单克隆抗体制剂。在某些实施方案中，纯化的制剂包含抗Aβ抗体和残留量的仓鼠PLBL2。在某些实施方案中，仓鼠PLBL2的量小于20ng/mg。在某些实施方案中，仓鼠PLBL2的量小于15ng/mg。在某些实施方案中，仓鼠PLBL2的量小于10ng/mg。在某些实施方案中，仓鼠PLBL2的量小于8ng/mg。在某些实施方案中，仓鼠PLBL2的量小于5ng/mg。在某些实施方案中，仓鼠PLBL2的量小于3ng/mg。在某些实施方案中，仓鼠PLBL2的量小于2ng/mg。在某些实施方案中，仓鼠PLBL2的量小于1ng/mg。在某些实施方案中，仓鼠PLBL2的量小于0.5ng/mg。在某些实施方案中，仓鼠PLBL2的量为0.5ng/mg和20ng/mg之间，或0.5ng/mg和15ng/mg之间，或0.5ng/mg和10ng/mg之间，或0.5ng/mg和8ng/mg之间，或0.5ng/mg和5ng/mg之间，或0.5ng/mg和3ng/mg之间，或0.5ng/mg和2ng/mg之间，或0.5ng/mg和1ng/mg之间，或定量测定法的极限(LOQ)和1ng/mg之间。在某些实施方案中，HIC步骤包括PHENYL SEPHAROSE™6Fast Flow(High Sub)树脂。在某些实施方案中，HIC步骤包括在流出模式操作含树脂的柱。在某些实施方案中，HIC步骤包括平衡缓冲液和洗涤缓冲液，其中每个平衡缓冲液和洗涤缓冲液包含150mM乙酸钠pH5.0。在某些实施方案中，HIC步骤包括平衡缓冲液和洗涤缓冲液，其中每个平衡缓冲液和洗涤缓冲液包含150mM乙酸钠pH4.0。在某些实施方案中，HIC步骤包括平衡缓冲液和洗涤缓冲液，其中每个平衡缓冲液和洗涤缓冲液包含150mM乙酸钠、240mM硫酸钠pH4.0。在某些实施方案中，HIC步骤包括平衡缓冲液和洗涤缓冲液，其中每个平衡缓冲液和洗涤缓冲液包含150mM乙酸钠、240mM硫酸钠pH5.0。在某些实施方案中，上样密度为300g/L。在某些实施方案中，上样密度为100g/L。在某些实施方案中，在280纳米吸光度下监测流出液并在0.5OD开始收集流出液，收集持续10个柱体积。在某些实施方案中，方法进一步包括亲和色谱法步骤。在某些实施方案中，亲和色谱法是蛋白A色谱法。在某些实施方案中，方法还包括混合模式色谱法步骤。在某些实施方案中，抗Aβ抗体包含三个重链CDR，具有氨基酸序列SEQ ID NO:23的CDR-H1，具有氨基酸序列SEQ ID NO:24的CDR-H2和具有氨基酸序列SEQ ID NO:25的CDR-H3，和三个轻链CDR，具有氨基酸序列SEQ ID NO:26的CDR-L1，具有氨基酸序列SEQ ID NO:27的CDR-L2和具有氨基酸序列SEQ ID NO:28的CDR-L3。在某些实施方案，抗Aβ抗体包含具有氨基酸序列SEQ ID NO:29的重链可变区。在某些实施方案中，抗Aβ抗体包含具有氨基酸序列SEQ ID NO:30的轻链可变区。在某些实施方案中，抗Aβ抗体包含具有氨基酸序列SEQ ID NO:29的重链可变区和具有氨基酸序列SEQ ID NO:30的轻链可变区。在某些实施方案中，使用免疫测定法或质谱测定法定量仓鼠PLBL2的量。在某些实施方案中，免疫测定法是总中国仓鼠卵巢蛋白ELISA或仓鼠PLBL2ELISA。在某些实施方案中，质谱

测定法是LC-MS/MS。

[0032] 在一个方面，提供了通过包括疏水相互作用色谱法(HIC)步骤的方法从CHO细胞分离和纯化的抗IL17A/F单克隆抗体制剂。在某些实施方案中，纯化的制剂包含抗IL17A/F抗体和残留量的仓鼠PLBL2。在某些实施方案中，仓鼠PLBL2的量小于20ng/mg。在某些实施方案中，仓鼠PLBL2的量小于15ng/mg。在某些实施方案中，仓鼠PLBL2的量小于10ng/mg。在某些实施方案中，仓鼠PLBL2的量小于8ng/mg。在某些实施方案中，仓鼠PLBL2的量小于5ng/mg。在某些实施方案中，仓鼠PLBL2的量小于3ng/mg。在某些实施方案中，仓鼠PLBL2的量小于2ng/mg。在某些实施方案中，仓鼠PLBL2的量小于1ng/mg。在某些实施方案中，仓鼠PLBL2的量小于0.5ng/mg。在某些实施方案中，仓鼠PLBL2的量为0.5ng/mg和20ng/mg之间，或0.5ng/mg和15ng/mg之间，或0.5ng/mg和10ng/mg之间，或0.5ng/mg和8ng/mg之间，或0.5ng/mg和5ng/mg之间，或0.5ng/mg和3ng/mg之间，或0.5ng/mg和2ng/mg之间，或0.5ng/mg和1ng/mg之间，或定量测定法的极限(LOQ)和1ng/mg之间。在某些实施方案中，HIC步骤包括PHENYL SEPHAROSE™6Fast Flow(High Sub)树脂。在某些实施方案中，HIC步骤包括在流出模式操作含树脂的柱。在某些实施方案中，HIC步骤包括平衡缓冲液和洗涤缓冲液，其中每个平衡缓冲液和洗涤缓冲液包含50mM乙酸钠pH5.5。在某些实施方案中，在280纳米的吸光度监测流出液和在0.5OD之间收集流出液，收集持续10个柱体积。在某些实施方案中，方法进一步包括亲和色谱法步骤。在某些实施方案中，亲和色谱法是蛋白A色谱法。在某些实施方案中，方法还包括阳离子交换色谱法步骤。在某些实施方案中，抗IL17A/F抗体包含三个重链CDR，具有氨基酸序列SEQ ID NO:15的CDR-H1，具有氨基酸序列SEQ ID NO:16的CDR-H2和具有氨基酸序列SEQ ID NO:17的CDR-H3，和三个轻链CDR，具有氨基酸序列SEQ ID NO:18的CDR-L1，具有氨基酸序列SEQ ID NO:19的CDR-L2和具有氨基酸序列SEQ ID NO:20的CDR-L3。在某些实施方案，抗IL17A/F抗体包含具有氨基酸序列SEQ ID NO:21的重链可变区。在某些实施方案中，抗IL17A/F抗体包含具有氨基酸序列SEQ ID NO:22的轻链可变区。在某些实施方案中，抗IL17A/F抗体包含具有氨基酸序列SEQ ID NO:21的重链可变区和具有氨基酸序列SEQ ID NO:32的轻链可变区。在某些实施方案中，使用免疫测定法或质谱测定法定量仓鼠PLBL2的量。在某些实施方案中，免疫测定法是总中国仓鼠卵巢蛋白ELISA或仓鼠PLBL2 ELISA。在某些实施方案中，质谱测定法是LC-MS/MS。

[0033] 在又一方面，提供了包含从含有抗Aβ抗体的CHO细胞纯化的抗Aβ单克隆抗体的组合物，所述组合物包含残留量的仓鼠PLBL2。在某些实施方案中，仓鼠PLBL2的量小于20ng/mg。在某些实施方案中，仓鼠PLBL2的量小于15ng/mg。在某些实施方案中，仓鼠PLBL2的量小于10ng/mg。在某些实施方案中，仓鼠PLBL2的量小于8ng/mg。在某些实施方案中，仓鼠PLBL2的量小于5ng/mg。在某些实施方案中，仓鼠PLBL2的量小于3ng/mg。在某些实施方案中，仓鼠PLBL2的量小于2ng/mg。在某些实施方案中，仓鼠PLBL2的量小于1ng/mg。在某些实施方案中，仓鼠PLBL2的量小于0.5ng/mg。在某些实施方案中，仓鼠PLBL2的量为0.5ng/mg和20ng/mg之间，或0.5ng/mg和15ng/mg之间，或0.5ng/mg和10ng/mg之间，或0.5ng/mg和8ng/mg之间，或0.5ng/mg和5ng/mg之间，或0.5ng/mg和3ng/mg之间，或0.5ng/mg和2ng/mg之间，或0.5ng/mg和1ng/mg之间，或定量测定法的极限(LOQ)和1ng/mg之间。在某些实施方案中，抗Aβ抗体是crenezumab。在某些实施方案中，抗Aβ抗体包含三个重链CDR，具有氨基酸序列SEQ ID NO:23的CDR-H1，具有氨基酸序列SEQ ID NO:24的CDR-H2和具有氨基酸序列SEQ ID NO:

25的CDR-H3，和三个轻链CDR，具有氨基酸序列SEQ ID NO:26的CDR-L1，具有氨基酸序列SEQ ID NO:27的CDR-L2和具有氨基酸序列SEQ ID NO:28的CDR-L3。在某些实施方案，抗Aβ抗体包含具有氨基酸序列SEQ ID NO:29的重链可变区。在某些实施方案中，抗Aβ抗体包含具有氨基酸序列SEQ ID NO:30的轻链可变区。在某些实施方案中，抗Aβ抗体包含具有氨基酸序列SEQ ID NO:29的重链可变区和具有氨基酸序列SEQ ID NO:30的轻链可变区。

[0034] 在又一方面，提供了包含由含有抗IL17A/F抗体的CHO细胞纯化的抗IL17A/F单克隆抗体的组合物，所述组合物包含残留量的仓鼠PLBL2。在某些实施方案中，组合物包含抗IL17A/F抗体和残留量的仓鼠PLBL2，其中仓鼠PLBL2的量小于20ng/mg，或小于15ng/mg，或小于10ng/mg，或小于8ng/mg，或小于5ng/mg，或小于3ng/mg，或小于2ng/mg，或小于1ng/mg，或小于0.5ng/mg。在某些实施方案中，仓鼠PLBL2的量小于20ng/mg。在某些实施方案中，仓鼠PLBL2的量小于15ng/mg。在某些实施方案中，仓鼠PLBL2的量小于10ng/mg。在某些实施方案中，仓鼠PLBL2的量小于8ng/mg。在某些实施方案中，仓鼠PLBL2的量小于5ng/mg。在某些实施方案中，仓鼠PLBL2的量小于3ng/mg。在某些实施方案中，仓鼠PLBL2的量小于2ng/mg。在某些实施方案中，仓鼠PLBL2的量小于1ng/mg。在某些实施方案中，仓鼠PLBL2的量小于0.5ng/mg。在某些实施方案中，仓鼠PLBL2的量为0.5ng/mg和20ng/mg之间，或0.5ng/mg和15ng/mg之间，或0.5ng/mg和10ng/mg之间，或0.5ng/mg和8ng/mg之间，或0.5ng/mg和5ng/mg之间，或0.5ng/mg和3ng/mg之间，或0.5ng/mg和2ng/mg之间，或0.5ng/mg和1ng/mg之间，或定量测定法的极限(LOQ)和1ng/mg之间。在某些实施方案中，抗IL17A/F抗体包含三个重链CDR，具有氨基酸序列SEQ ID NO:15的CDR-H1，具有氨基酸序列SEQ ID NO:16的CDR-H2和具有氨基酸序列SEQ ID NO:17的CDR-H3，和三个轻链CDR，具有氨基酸序列SEQ ID NO:18的CDR-L1，具有氨基酸序列SEQ ID NO:19的CDR-L2和具有氨基酸序列SEQ ID NO:20的CDR-L3。在某些实施方案，抗IL17A/F抗体包含具有氨基酸序列SEQ ID NO:21的重链可变区。在某些实施方案中，抗IL17A/F抗体包含具有氨基酸序列SEQ ID NO:22的轻链可变区。在某些实施方案中，抗IL17A/F抗体包含具有氨基酸序列SEQ ID NO:21的重链可变区和具有氨基酸序列SEQ ID NO:22的轻链可变区。

[0035] 在一个方面，提供了治疗IL-13介导的疾患的方法，所述方法包括施用包含从CHO细胞纯化的抗IL-13单克隆抗体和残留量的仓鼠PLBL2的治疗组合物。在某些实施方案中，仓鼠PLBL2的量小于20ng/mg。在某些实施方案中，仓鼠PLBL2的量小于15ng/mg。在某些实施方案中，仓鼠PLBL2的量小于10ng/mg。在某些实施方案中，仓鼠PLBL2的量小于8ng/mg。在某些实施方案中，仓鼠PLBL2的量小于5ng/mg。在某些实施方案中，仓鼠PLBL2的量小于3ng/mg。在某些实施方案中，仓鼠PLBL2的量小于2ng/mg。在某些实施方案中，仓鼠PLBL2的量小于1ng/mg。在某些实施方案中，仓鼠PLBL2的量小于0.5ng/mg。在某些实施方案中，仓鼠PLBL2的量为0.5ng/mg和20ng/mg之间，或0.5ng/mg和15ng/mg之间，或0.5ng/mg和10ng/mg之间，或0.5ng/mg和8ng/mg之间，或0.5ng/mg和5ng/mg之间，或0.5ng/mg和3ng/mg之间，或0.5ng/mg和2ng/mg之间，或0.5ng/mg和1ng/mg之间，或定量测定法的极限(LOQ)和1ng/mg之间。在某些实施方案中，抗IL-13抗体包含三个重链CDR，具有氨基酸序列SEQ ID NO:1的CDR-H1，具有氨基酸序列SEQ ID NO:2的CDR-H2和具有氨基酸序列SEQ ID NO:3的CDR-H3，和三个轻链CDR，具有氨基酸序列SEQ ID NO:4的CDR-L1，具有氨基酸序列SEQ ID NO:5的CDR-L2和具有氨基酸序列SEQ ID NO:6的CDR-L3。在某些实施方案，抗IL-13抗体包含具有

氨基酸序列SEQ ID NO:7的重链可变区。在某些实施方案中，抗IL-13抗体包含具有氨基酸序列SEQ ID NO:9的轻链可变区。在某些实施方案中，抗IL-13抗体包含具有氨基酸序列SEQ ID NO:10的重链。在某些实施方案中，抗IL-13抗体包含具有氨基酸序列SEQ ID NO:14的轻链。在某些实施方案中，抗IL-13抗体包含具有氨基酸序列SEQ ID NO:7的重链可变区和具有氨基酸序列SEQ ID NO:9的轻链可变区。在某些实施方案中，抗IL-13抗体包含具有氨基酸序列SEQ ID NO:10的重链和具有氨基酸序列SEQ ID NO:14的轻链。在某些实施方案中，治疗组合物每四周皮下施用一次。在某些实施方案中，治疗组合物每八周皮下施用一次。在某些实施方案中，治疗组合物每12周皮下施用一次。在某些实施方案中，患者每四周治疗一次，持续至少一个月。在某些实施方案中，患者每四周治疗一次，持续至少三个月。在某些实施方案中，患者每四周治疗一次，持续至少六个月。在某些实施方案中，患者每四周治疗一次，持续至少九个月。在某些实施方案中，患者每四周治疗一次，持续至少12个月。在某些实施方案中，患者每四周治疗一次，持续至少18个月。在某些实施方案中，患者每四周治疗一次，持续至少两年。在某些实施方案中，患者每四周治疗一次，持续超过两年。在某些实施方案中，IL-13介导的疾患是哮喘。在某些实施方案中，IL-13介导的疾患是特发性肺纤维化。在某些实施方案中，IL-13介导的疾患是特应性皮炎。在某些实施方案中，IL-13介导的疾患选自过敏性哮喘、非过敏性哮喘、过敏性鼻炎、过敏性结膜炎、湿疹、荨麻疹、食物过敏、慢性阻塞性肺疾病、溃疡性结肠炎、RSV感染、葡萄膜炎、硬皮病和骨质疏松症。

[0036] 另一个方面，根据任何上述方法向患者施用的治疗组合物相比于施用参考组合物，对仓鼠PLBL2的免疫原性更低，其中所述参考组合物包含从中国仓鼠卵巢宿主细胞纯化的抗IL-13单克隆抗体和残留量大于30ng/mg的仓鼠PLBL2。在某些实施方案中，参考组合物中的仓鼠PLBL2的量大于50ng/mg。在某些实施方案中，参考组合物中的仓鼠PLBL2的量大于100ng/mg。在某些实施方案中，参考组合物中的仓鼠PLBL2的量大于200ng/mg。在某些实施方案中，参考组合物中的仓鼠PLBL2的量大于300ng/mg。在某些实施方案中，参考组合物中的仓鼠PLBL2的量为30ng/mg和300ng/mg之间，或30ng/mg和200ng/mg之间，或30ng/mg和100ng/mg之间，或30ng/mg和50ng/mg之间。

附图说明

[0037] 图1示出如实施例2所述，抗IL-13MAb的辛酸处理的蛋白A池中总CHOP的水平。(A)蛋白A池在pH4.5的辛酸沉淀。(B)蛋白A池在pH5.0的辛酸沉淀。沿纵轴指示以ng/mg计的CHOP水平；沿横轴示出辛酸的百分比，每个棒代表来自2倍连续稀释的值。

[0038] 图2示出如实施例2所示，在蛋白A色谱法以及其后的**POROS®**50HS上的阳离子交换色谱法后，在添加剂处理的HCCF抗IL-13Mab中总CHOP的水平。纵轴显示以ng/ml计的修正的CHOP水平；横轴表示添加剂(对照、0.6M胍、或0.6M精氨酸)，每个棒代表所示的来自2倍连续稀释的值。

[0039] 图3示出如实施例2所示，在不同的盐和pH条件下、进行不同的HIC树脂的抗IL-13MAb的UFDF池中总CHOP的水平。(A)**OCTYL-SEPHAROSE®**Fast Flow树脂；(B)PHENYL SEPHAROSE™6Fast Flow(low sub)树脂；(C)**BUTYL-SEPHAROSE®**4Fast Flow树脂；(D)PHENYL SEPHAROSE™6Fast Flow(high sub)树脂；最高稀释的CHOP(以ppm计)示于纵轴，硫酸钠的浓度示于横轴；pH值(5.5,6.0,7.0或8.0由图例指示。

[0040] 详细说明

[0041] 除非另外定义，本文所用的技术和科学术语具有与本发明所属领域中的普通技术人员通常所理解的相同的含义。Singleton等人，Dictionary of Microbiology and Molecular Biology第二版，J.Wiley&Sons(New York,N.Y.1994)，以及March,Advanced Organic Chemistry Reactions,Mechanisms and Structure第四版，John Wiley&Sons(New York,N.Y.1992)，为本领域技术人员提供本申请使用的许多术语的一般指导。

[0042] 某些定义

[0043] 为了解释本说明书的目的，将应用下列定义，在任何适当的时候，以单数使用的术语也包括复数，反之亦然。如果下面阐述的任何定义与通过引用并入本文的任何文件发生冲突，以下面提出的定义为准。

[0044] 如在本说明书和所附权利要求书中所使用的，单数形式“一”，“一个”和“该”包括复数对象，除非上下文另有明确说明。因此，例如，提及“一种蛋白质”或“一种抗体”分别包括多个蛋白质或抗体；提及“一种细胞”包括细胞的混合物，等等。

[0045] 术语“检测”在本文中用于最广泛的意义，包括靶分子的定性和定量测量。检测包括仅识别靶分子在样品中的存在，以及确定所述靶分子是否在样品中以可检测的水平存在。

[0046] “样品”指的是大量材料的一小部分。通常，根据本文所述的方法对样品进行测试。样品典型地从获得的重组多肽制备物得到，例如，重组多肽制备物从培养的宿主细胞得到。样品可以从，例如但不限于从收获的细胞培养液得到、从纯化方法的特定步骤中、进程(in-process)池中得到，或者从最终纯化产物得到。

[0047] 如本文所描述的术语“产物”是待由各种色谱方法纯化的物质；例如，多肽。

[0048] 术语“多肽”或“蛋白质”在本文中可互换使用，指任何长度的氨基酸聚合物。该聚合物可以是直链或支链，其可以包含修饰的氨基酸，并且其可被非氨基酸中断。该术语还包括天然地或通过介入修饰的氨基酸聚合物；例如，二硫键形成、糖基化、脂质化、乙酰化、磷酸化或任何其他操作或修饰，如与标记组分缀合。还包括在该定义内的是，例如，含有一种或多种氨基酸类似物的多肽(包括例如，非天然氨基酸等)，以及本领域已知的其他修饰。如本文所用的术语“多肽”和“蛋白质”特别涵盖抗体。

[0049] “纯化的”多肽(例如，抗体或免疫粘附素)是指该多肽已经增加了纯度，使得其以比其在其天然环境和/或初始合成时和/或在实验室条件下扩增时更纯的形式存在。纯度是一个相对的术语，并不一定意味着绝对纯度。

[0050] 术语“标记表位”在用于本文时是指嵌合多肽，其包含融合到“标签多肽”的多肽。标签多肽具有足够的残基以提供表位，针对这些表位可制备抗体，但其也足够短使得它不干扰与它融合的多肽的活性。标签多肽优选还是相当独特的，使得抗体基本上不与其他表位发生交叉反应。合适的标签多肽通常具有至少六个氨基酸残基，通常在约8和50个氨基酸残基之间(在某些情况下，约10至20个氨基酸残基之间)。

[0051] 本文中“活性的”或“活性”是指保留目的生物和/或免疫活性的多肽形式，其中“生物”活性指由多肽引起的生物学功能(抑制的或刺激的)，而不是诱导抗多肽具有的抗原表位的抗体产生的能力，“免疫学”活性指诱导抗多肽具有的抗原表位的抗体产生的能力。

[0052] 术语“拮抗剂”以最广的意义使用，包括部分或完全阻断、抑制、或中和天然多肽

(例如,细胞因子)的生物活性的任何分子。以类似的方式,术语"激动剂"以最广的意义使用,包括模拟天然多肽的生物活性的任何分子。合适的激动剂或拮抗剂分子具体地包括激动或拮抗性抗体或抗体片段、天然多肽的片段或氨基酸序列变体,等等。用于鉴定多肽的激动剂或拮抗剂的方法可包括使多肽与候选激动剂或拮抗剂分子接触,测量通常与该多肽相关的一种或多种生物活性的可检测变化。

[0053] "结合"目的抗原(例如肿瘤相关的多肽抗原靶)的多肽,是以足够的亲和力结合抗原的多肽,使得该多肽可作为检测试剂、诊断和/或治疗剂用于靶向含有抗原的样品、表达抗原的细胞或组织,并且不显著地与其他多肽交叉反应。

[0054] 关于多肽与靶分子的结合,术语"特异的结合"或"特异性结合"或"特异于"特定多肽或特定多肽靶标上的表位是指结合可检测程度地不同于非特异性相互作用。特异的结合,例如,可通过与对照分子的结合相比较测定分子的结合来确定,对照分子通常为结构类似的不具有结合活性的分子。例如,通过与类似于靶标的对照分子的竞争来确定特异的结合,例如,过量的未标记靶标。在这种情况下,如果标记的靶标与探针的结合被过量的未标记靶标竞争性抑制,则表明特异性结合。

[0055] 术语"抗体"在本文中以最广的意义使用,包括各种抗体结构,包括但不限于单克隆抗体、多克隆抗体、由至少两个完整抗体形成的多特异性抗体(例如双特异性抗体)和抗体片段,只要其表现出所期望的生物活性。术语"免疫球蛋白"(Ig)在本文中与抗体互换使用。

[0056] 抗体是天然存在的免疫球蛋白分子,其具有不同的结构,全部基于免疫球蛋白折叠。例如,IgG抗体有二硫键键合的两个"重"链和两个"轻"链,形成功能性抗体。每条重链和轻链本身包含"恒定区"(C)和"可变区"(V)。V区决定抗体的抗原结合特异性,而C区提供结构支撑和与免疫效应子非抗原特异性相互作用中的功能。抗体或抗体的抗原结合片段的抗原结合特异性是抗体特异性结合特定抗原的能力。

[0057] 抗体的抗原结合特异性由V区的结构特性决定。可变性并非均匀分布在可变结构域的110个氨基酸跨度。相反,V区由称为构架区(FR)的15-30个氨基酸的相对不变的片段和称为"高变区"的、每个为9-12个氨基酸长的极端可变性的较短区域组成,构架区被高变区分开。天然重链和轻链的每个可变结构域包含四个FR,其主要采取β折叠构型,通过三个高变区连接,形成环连接,在某些情况中形成β折叠结构的一部分。每条链中的高变区通过FR非常接近地保持在一起,并与来自另一条链的高变区一起促成抗体的抗原结合位点的形成(参见Kabat等人,Sequences of Proteins of Immunological Interest,第五版,Public Health Service,National Institutes of Health,Bethesda,Md.(1991))。恒定结构域不直接参与抗体与抗原的结合,但表现出多种效应子功能,如抗体参与的抗体依赖性细胞毒性(ADCC)。

[0058] 每个V区通常包含三个互补决定区("CDR",其每个包含一个"高变环")和四个构架区。因此抗体结合位点(以大量亲和力结合具体所期望的抗原所必须的最小结构单元)通常包括三个CDR,和至少三个,优选四个,穿插其间的框架区,以使CDR保持和呈现在适当的构象中。经典的四链抗体具有V_H和V_L结构域协同限定的抗原结合位点。某些抗体,如骆驼和鲨鱼抗体缺乏轻链,依靠仅由重链形成的结合位点。可以制备单结构域工程化的免疫球蛋白,其中结合位点单独由重链或轻链形成,不存在V_H和V_L之间的协同。

[0059] 在用于本文时，术语“高变区”是指负责抗原结合的抗体的某些氨基酸残基。高变区可包含来自如上讨论的“互补决定区”或“CDR”的氨基酸残基(例如，在V_L中约为残基24-34(L1)，50-56(L2)和89-97(L3)和在V_H中约为31-35B(H1)，50-65(H2)和95-102(H3)(Kabat等人，Sequences of Proteins of Immunological Interest，5th Ed.Public Health Service，National Institutes of Health，Bethesda，Md.(1991))和/或来自“高变环”的那些残基(例如，在V_L中残基26-32(L1)，50-52(L2)和91-96(L3)和在V_H中26-32(H1)，52A-55(H2)和96-101(H3)(Chothia and Lesk J.Mol.Biol.196:901-917(1987))。

[0060] “框架区”或“FR”残基是除文中定义的高变区残基之外的那些可变结构域残基。

[0061] “抗体片段”包含完整抗体的一部分，优选包含其抗原结合区。抗体片段的实例包括Fab，Fab'，$F(ab')_2$和Fv片段；双抗体；串联双抗体(taDb)，线性抗体(例如，美国专利号5，641，870，实施例2；Zapata等人，Protein Eng.8(10):1057-1062(1995))；单臂抗体，单可变结构域抗体，微抗体，单链抗体分子；由抗体片段形成的多特异性抗体(例如，包括但不限于Db-Fc，taDb-Fc，taDb-CH3，(scFV)4-Fc，di-scFv，bi-scFv，或串联(di，tri)-scFv)；和双特异性T细胞接合器(BiTE)。

[0062] 抗体的木瓜蛋白酶消化产生两个相同的抗原结合片段(称为“Fab”片段，每个具有单抗原结合位点)和残余的“Fc”片段(其名称反映出其易于结晶的能力)。胃蛋白酶处理产生$F(ab')_2$片段，其具有两个抗原结合位点且仍能够交联抗原。

[0063] “Fv”是包含完整抗原识别和抗原结合位点的最小抗体片段。该区域由一个重链和一个轻链可变结构域紧密、非共价结合的二聚体组成。正是在这种构型中，各可变结构域的三个高变区相互作用以限定V_H-V_L二聚体表面上的抗原结合位点。六个高变区共同赋予抗体抗原结合特异性。然而，即使是单个可变结构域(或仅包含特异于抗原的三个高变区的半个Fv)也具有识别和结合抗原的能力，尽管与完整结合位点相比亲和力更低。

[0064] Fab片段还包含轻链的恒定结构域和重链的第一恒定结构域(CH1)。Fab'片段不同于Fab片段，其在重链CH1结构域的羧基末端增加了几个残基，包括来自抗体铰链区的一个或多个半胱氨酸。Fab'-SH在本文中是对其中恒定结构域的半胱氨酸残基携带至少一个游离巯基的Fab'的命名。$F(ab')_2$抗体片段最初作为其之间具有铰链半胱氨酸的成对Fab'片段生成。抗体片段的其他化学偶联也是已知的。

[0065] 来自任何脊椎动物物种的抗体(免疫球蛋白)的“轻链”可归入基于其恒定结构域的氨基酸序列的两种截然不同类型中的一种，称为kappa(κ)和lambda(λ)。

[0066] 根据其重链恒定结构域的氨基酸序列，抗体可归入不同的种类。完整抗体有五大类：IgA，IgD，IgE，IgG和IgM，其中有些可进一步分为亚类(同种型)，例如IgG1，IgG2，IgG3，IgG4，IgA和IgA2。对应于不同类抗体的重链恒定结构域分别称作α，δ，ε，γ 和μ。不同种类免疫球蛋白的亚基结构和三维构型是众所周知的。

[0067] “单链Fv”或“scFv”抗体片段包含抗体的V_H和V_L结构域，其中这些结构域存在于单一多肽链中。在一些实施方案中，Fv多肽进一步包含在V_H和V_L结构域之间的多肽接头，使得scFv能够形成用于抗原结合的所期望的结构。关于scFv的综述参见Plückthun in The Pharmacology of Monoclonal Antibodies，vol.113，Rosenburg and Moore eds.，Springer-Verlag，New York，pp.269-315(1994)。

[0068] 术语“双抗体”指具有两个抗原结合位点的小抗体片段，该片段包含在同一多肽链

(V_H-V_L)中连接到轻链可变结构域(V_L)的重链可变结构域(V_H)。通过使用太短而不允许相同链上的两个结构域之间配对的接头，迫使结构域与另一条链的互补结构域配对，并产生两个抗原结合位点。双抗体更完整的描述于，例如EP 404,097;WO 93/11161;和Hollinger等人,Proc.Natl.Acad.Sci.USA,90:6444-6448(1993)。

[0069] 术语“多特异性抗体”以最广的意义使用，特别地覆盖具有多表位特异性的抗体。这样的多特异性抗体包括但不限于，包含重链可变结构域(V_H)和轻链可变结构域(V_L)的抗体，其中V_HV_L单元具有多表位特异性；具有两个或更多个V_L和V_H结构域的抗体，其中每个V_HV_L单元结合不同的表位；具有两个或更多个单可变结构域的抗体，其中每个单可变结构域结合不同的表位；全长抗体；抗体片段如Fab,Fv,dsFv,scFv,双抗体，双特异性双抗体，三抗体，三官能抗体，已被共价或非共价连接的抗体片段。“多表位特异性”指的是特异性结合相同或不同靶标上的两个或更多个不同表位的能力。“单特异性”指的是仅结合一个表位的能力。根据一个具体实施方案，多特异性抗体是以亲和力5μM至0.001pM,3μM至0.001pM,1μM至0.001pM,0.5μM至0.001pM,或0.1μM至0.001pM结合每个表位的IgG抗体。

[0070] 表述“单结构域抗体”(sdAbs)或“单可变结构域(SVD)抗体”通常是指其中单可变结构域(VH或VL)可赋予抗原结合的抗体。换句话说，所述单可变结构域不需要为了识别靶抗原与另一个可变结构域相互作用。单结构域抗体的例子包括来自骆驼科(lamas和骆驼)和软骨鱼类(例如，铰口鲨)的和由重组方法来自人和小鼠抗体的那些(Nature(1989)341:544-546;Dev Comp Immunol(2006)30:43-56;Trend Biochem Sci(2001)26:230-235;Trends Biotechnol(2003):21:484-490;WO 2005/035572;WO 03/035694;Febs Lett(1994)339:285-290;WO00/29004;WO 02/051870)。

[0071] 如文中使用的术语“单克隆抗体”指从一群基本上同质的抗体获得的抗体，构成群的各个抗体是相同的和/或结合相同的表位，除了生产单克隆抗体的过程中出现的可能的变体，这种变体通常少量存在。与典型地包含针对不同决定簇(表位)的不同抗体的多克隆抗体制剂相反，各单克隆抗体针对抗原上的单一决定簇。除了其特异性，单克隆抗体的优点在于其未被其他免疫球蛋白污染。修饰语“单克隆”表示由于从基本上同质群的抗体获得的抗体特征，而不应被解释为需要通过任何特定方法生产抗体。例如，根据本文提供的方法中使用的单克隆抗体可通过Kohler等人,Nature 256:495(1975)最初描述的杂交瘤方法制备，或者可通过重组DNA方法制备(参见，例如，美国专利号4,816,567)。该“单克隆抗体”也可以使用例如Clackson等人,Nature 352:624-628(1991)和Marks等人,J.Mol.Biol.222:581-597(1991)描述的技术从噬菌体抗体文库分离。

[0072] 单克隆抗体在本文中具体包括“嵌合”抗体(免疫球蛋白)，其中重链和/或轻链的一部分与源自特定物种或属于特定抗体类别或亚类的抗体中的相应序列相同或同源，而链的剩余部分与源自另一物种或属于另一抗体类别或亚类的抗体中的相应序列相同或同源，以及此类抗体的片段，只要其表现出期望生物活性(美国专利号4,816,567;Morrison等人,Proc.Natl.Acad.Sci.USA 81:6851-6855(1984))。本文中目的嵌合抗体包括包含源自非人灵长类动物的可变结构域抗原结合序列(例如，旧大陆猴，如狒狒、恒河猴或猕猴)和人恒定区序列(美国专利号5,693,780)的“灵长类化”抗体。

[0073] 非人(例如，鼠)抗体的“人源化”形式是包含源自非人免疫球蛋白的最小序列的嵌合抗体。在大多数情况下，人源化抗体是人免疫球蛋白(受体抗体)，其中来自受体高变区的

残基被来自非人物种(供体抗体)的高变区替代,如具有所期望的特异性、亲和力和能力的小鼠、大鼠、兔或非人灵长类。在一些情况下,人免疫球蛋白的框架区(FR)残基被相应的非人残基替代。此外,人源化抗体可包含受体抗体中或供体抗体中不存在的残基。做出这些修饰是为了进一步改进抗体的性能。一般地,除了以上提及的FR取代,人源化抗体基本上包含至少一个、通常两个可变结构域的全部,其中高变环的全部或基本上全部对应于非人免疫球蛋白的那些,以及FR的全部或基本上全部是人免疫球蛋白序列的那些。人源化抗体任选还将包含免疫球蛋白恒定区的至少部分,通常是人免疫球蛋白的恒定区。为了解更多详情,参见Jones等人,Nature 321:522-525(1986);Riechmann等人,Nature 332:323-329(1988);and Presta,Curr.Op.Struct.Biol.2:593-596(1992)。

[0074] 为了本文的目的,"完整抗体"是包含重链和轻链可变结构域和Fc区的抗体。恒定结构域可以是天然序列恒定结构域(例如人天然序列恒定结构域)或其氨基酸序列变体。优选地,完整抗体具有一种或多种效应子功能。

[0075] "天然抗体"通常是约150,000道尔顿的异四聚体糖蛋白,其由两条相同的轻(L)链和两条相同的重(H)链组成。每条轻链通过一个共价二硫键连接到重链,而在不同免疫球蛋白同种型的重链间二硫键联的数量不同。每条重链和轻链还具有规则间隔的链内二硫键桥。每条重链在一端具有可变结构域(V_H),随后是多个恒定结构域。每条轻链在一端具有可变结构域(V_L),在另一端具有恒定结构域;轻链的恒定结构域与重链的第一恒定结构域排列在一起,轻链可变结构域与重链的可变结构域排列在一起。特定氨基酸残基被认为形成轻链和重链可变结构域之间的交界。

[0076] 相对于参考多肽序列的"百分比(%)氨基酸序列同一性"定义为在比对序列后,候选序列中与参考多肽序列中的氨基酸残基相同的氨基酸残基的百分比,如果需要引入缺口,达到最大百分比的序列同一性,并且不将任何保守取代视为序列同一性的一部分。可以本领域技术范围内的各种方式实现测定百分比氨基酸序列同一性目的的比对,例如,使用公众可得到的计算机软件,诸如BLAST,BLAST-2,ALIGN或Megalign(DNASTAR)软件来实现。本领域技术人员可确定用于比对序列的适当的参数,包括在被比较序列的全长上实现最大比对所需要的任何算法。但是,对于本文的目的,使用序列比较计算机程序ALIGN-2生成%氨基酸序列同一性值。ALIGN-2序列比较计算机程序由Genentech,Inc.编写,并且源代码已经与用户文档提交给美国版权局,华盛顿,20559,其以美国版权注册号TXU510087注册。ALIGN-2程序可公开获自Genentech,Inc.,南圣弗朗西斯科,加利福尼亚,或者可以从源代码编译。ALIGN-2程序应当编译成在UNIX操作系统,包括数字UNIX V4.0D上使用。所有序列比较参数由ALIGN-2程序设定且不变。

[0077] 当ALIGN-2用于氨基酸序列比较的情况中,给定氨基酸序列A相较于、与、或针对给定氨基酸序列B(或者可表述为相较于、与、或针对给定氨基酸序列B具有或包含一定%氨基酸序列同一性的给定氨基酸序列A)的%氨基酸序列同一性如下计算:

[0078] 100乘以分数X/Y

[0079] 其中X是在A和B的程序比对中由序列对比程序ALIGN-2打分为相同匹配的氨基酸残基的数目,其中Y是B中氨基酸残基的总数。应当理解,当氨基酸序列A的长度不等于氨基酸序列B的长度时,A相较于B的%氨基酸序列同一性将不等于B相较于A的%氨基酸序列同一性。除非另有具体说明,本文所用的所有%氨基酸序列同一性值如紧接在前面的段落中

所述，使用ALIGN-2计算机程序获得。

[0080] 术语“抗IL-13抗体”和“结合IL-13的抗体”是指能够以足够的亲和力结合IL-13的抗体，使得该抗体用作为靶向IL-13的诊断和/或治疗剂。在一些实施方案中，抗IL-13抗体结合至不相关的、非IL-13蛋白的程度小于所测量(例如，通过放射免疫测定法(RIA))的抗体与IL-13结合的约10％。在某些实施方案中，结合IL-13的抗体具有≤1μM、≤100nM、≤10nM、≤1nM、≤0.1nM、≤0.01nM或≤0.001nM的离解常数(Kd)(例如，10^{-8}M或更小，例如从10^{-8}M至10^{-13}M，例如从10^{-9}M至10^{-13}M)。在特定实施方案中，抗IL-13抗体结合来自不同物种的IL-13中保守的IL-13表位。

[0081] “IL-13介导的疾患”是指与过量IL-13水平或活性相关的疾患，其中由于身体局部和/或全身的IL-13水平或活性可表现非典型症状。IL-13介导的疾患的实例包括：癌症(例如，非霍奇金淋巴瘤，胶质母细胞瘤)、特应性皮炎、过敏性鼻炎、哮喘、纤维变性、炎性肠病、克罗恩病、肺的炎性疾患(包括肺纤维化，如IPF)、COPD、和肝纤维化。

[0082] 术语“呼吸系统疾患”包括但不限于，哮喘(例如，过敏性和非过敏性哮喘(例如，由于如呼吸道合胞病毒(RSV)感染，例如在年幼的儿童))；支气管炎(例如，慢性支气管炎)；慢性阻塞性肺疾病(COPD)(例如，肺气肿(例如，香烟引起的肺气肿)；涉及气道炎症的病症，嗜酸粒细胞增多症，纤维化和过量粘液产生，例如，囊性纤维化，肺纤维化和过敏性鼻炎。可由气道炎症、过度气道分泌、以及气道阻塞表征的疾病的实例包括哮喘、慢性支气管炎、支气管扩张、和囊性纤维化。

[0083] 术语“治疗剂”是指用于治疗疾病的任何试剂。治疗剂可以是，例如，多肽(例如，抗体、免疫粘附素或肽体)，可结合蛋白质或核酸分子(其可以结合编码靶标的核酸分子(即，siRNA))的适体或小分子，等等。

[0084] “裸抗体”是不缀合异源分子(如细胞毒性部分或放射性标记)的抗体(如本文定义)。

[0085] 术语“宿主细胞”、“宿主细胞系”和“宿主细胞培养物”可互换使用，是指其中已引入外源核酸的细胞，包括该细胞的后代。宿主细胞包括“转化体”和“转化细胞”，包括原代转化细胞和不考虑传代数目的来自原代转化细胞的后代。后代可能在核酸含量上与亲代细胞不完全相同，但也可以含有突变。本文包括具有与针对最初转化细胞所筛选或选择的相同的功能或生物活性的突变后代。

[0086] 如本文所用的术语“载体”，是指能够繁殖其连接的另一核酸的核酸分子。该术语包括作为自我复制的核酸结构的载体，以及掺入其所导入的宿主细胞的基因组内的载体。某些载体能指导表达其可操作地连接的核酸。这样的载体在本文中称为“表达载体”。

[0087] “分离的”抗体是从其天然环境成分中分离的抗体。在一些实施方案中，抗体纯化至纯度大于95％或99％，如通过，例如，电泳(例如，SDS-PAGE、等电点聚焦(IEF)、毛细管电泳)或色谱法(例如，离子交换或反相HPLC)所测定的。对于评估抗体纯度方法的综述，见，例如，Flatman等人，J.Chromatogr.B 848:79-87(2007)。

[0088] 如本文中所用的关于色谱法的术语“相继的”指的是具有第一色谱法以及随后的第二色谱法。第一色谱法和第二色谱法之间可以包括额外步骤。

[0089] 如本文中所用的关于色谱法的术语“连续的”指的是具有直接连接或一些其他机制连接的第一色谱法材料和第二色谱法材料，所述其他机制允许在两个色谱法材料之间连

续流动。

[0090] “杂质”和“污染物”是指不同于所期望的多肽产物的物质。杂质和污染物包括但不限于：宿主细胞物质，如CHOP，包括单一CHOP物种；过滤的蛋白A；核酸；所期望的多肽的变体、片段、聚集体或衍生物；其他多肽；内毒素；病毒污染物；细胞培养基成分等。在一些实例中，污染物可能是宿主细胞蛋白(HCP)，所述宿主细胞蛋白来自例如，但不限于，细菌细胞，如大肠杆菌细胞、昆虫细胞、原核细胞、真核细胞、酵母细胞、哺乳动物细胞、禽类细胞、真菌细胞。

[0091] 术语“中国仓鼠卵巢细胞蛋白”和“CHOP”可互换使用，指的是来自中国仓鼠卵巢(“CHO”)细胞培养物的宿主细胞蛋白(“HCP”)的混合物。HCP或CHOP一般作为包含目的蛋白(如CHO细胞中表达的抗体或免疫粘附素)的细胞培养基或裂解物(例如，收获的细胞培养液(“HCCF”))中的杂质存在。包含目的蛋白质的混合物中CHOP存在的量提供目的蛋白质的纯度程度的量度。HCP或CHOP包括但不限于，宿主细胞，如CHO宿主细胞表达的目的蛋白质。通常情况下，蛋白质混合物中CHOP的量以相对于混合物中目的蛋白质的量的每百万份表示。可以理解的是，其中宿主细胞是其他哺乳动物细胞类型、大肠杆菌、酵母、昆虫细胞、或植物细胞时，HCP是指存在于宿主细胞裂解物中的不是靶蛋白的蛋白质。

[0092] 术语“每百万份”或“ppm”在本文中可互换使用，指本发明方法纯化的目的蛋白质的纯度的量度。单位ppm指的是每以毫克/毫升计的目的蛋白质中纳克/毫升的HCP或CHOP的量(即，CHOP ppm＝(CHOP ng/ml)/(目的蛋白质mg/ml)，其中蛋白质在溶液中)。其中蛋白质被干燥时(例如通过冻干)，ppm指(CHOP ng)/(目的蛋白质mg))。杂质也可以表示为“ng/mg”，其与ppm可互换使用。

[0093] 从包含多肽和一种或多种杂质的组合物中“纯化”多肽是指通过从组合物中除去(完全或部分)至少一种杂质增加组合物中多肽的纯度程度。

[0094] “纯化步骤”可以是产生“均质”组合物的整个纯化方法中的一部分，“均质”组合物在本文中用于指在含有目的蛋白质的组合物中包含小于100ppm HCP(100ng/mg)、或小于90ppm(90ng/mg)、或小于80ppm(80ng/mg)、或小于70ppm(70ng/mg)、或小于60ppm(60ng/mg)、或小于50ppm 50ng/mg)、或小于40ppm(40ng/mg)、或小于30ppm(30ng/mg)、或小于20ppm(20ng/mg)、或小于10ppm(10ng/mg)、或小于5ppm(5ng/mg)、或小于3ppm(3ng/mg)或小于1ppm(1ng/mg)的杂质的组合物。在某些实施方案中，HCP是单一HCP种类。在一个实施方案中，单一HCP种类是仓鼠PLBL2。

[0095] 文中待纯化“组合物”包含目的多肽和一种或多种杂质或污染物。该组合物可被“部分纯化”(即，已经经过一个或多个纯化步骤)，或者可直接获得自产生多肽的宿主细胞或生物体(例如，组合物可包含收获的细胞培养液)。

[0096] 术语“蛋白质A”和“ProA”在本文中可互换使用，包括从其天然来源物回收的蛋白A、合成(例如，通过肽合成或通过重组技术)产生的蛋白A、以及保留了结合具有CH2/CH3区(如Fc区)的蛋白质的能力的其变体。蛋白A可商购自各种来源。蛋白A通常固定在固相载体材料上。术语“ProA”还指含有共价附着蛋白A的色谱固体支持物基质的亲和色谱法树脂或柱。

[0097] 术语“色谱法”是指这样的方法，由于在移动相的影响下、或在结合-洗脱过程中，混合物中的各溶质通过静止介质的迁移速率不同，从而通过该方法从混合物的其他溶质中

分离混合物中的目的溶质。

[0098] 术语“亲和色谱法”和“蛋白质亲和色谱法”在本文中可互换使用，是指一种蛋白质分离技术，其中目的蛋白质或目的抗体可逆地和特异性地结合至生物特异性配体。通常，所述生物特异性配体共价附着于色谱法固相材料，当溶液与色谱法固相材料接触时，溶液中的目的蛋白质可接近该生物特异性配体。在色谱法步骤中目的蛋白质(例如，抗体、酶或受体蛋白)保留其对生物特异性配体(例如，抗原、底物、辅因子、或激素)的特异性结合亲和力，而混合物中的其他溶质和/或蛋白质不明显或特异性地结合至配体。目的蛋白质与固定的配体的结合允许污染的蛋白质或蛋白质杂质通过色谱法介质流出而目的蛋白质仍然特异性地结合于固相材料上固定的配体。然后利用低pH、高pH、高盐、竞争性配体等从固定的配体除去活性形式的特异性结合的目的蛋白质，并利用洗脱缓冲液使目的蛋白质流过色谱柱，其不含较早允许流过柱的污染性蛋白质或蛋白质杂质。任何组分可作为配体用于纯化它的各自特异性结合蛋白，例如抗体。

[0099] 术语“非亲和色谱法”和“非亲和纯化”指其中未利用亲和色谱法的纯化方法。非亲和色谱法包括依赖于目的分子(如蛋白质，例如抗体)和固相基质之间的非特异性相互作用的色谱法技术。

[0100] 如本文在色谱法上下文中使用的术语“特异性结合”，如描述目的分子和结合于固相基质的配体之间的相互作用，指的是目的蛋白质通过蛋白质和配体结构在结合位点的空间互补性加上在结合位点的静电力、氢键键合、疏水力和/或范德华力的组合效应，通常可逆地结合配体。空间互补性越大、在结合位点的其他力越强，蛋白质与其相应配体的结合特异性越大。非限制性的特异性结合包括抗体-抗原结合、酶-底物结合、酶-辅因子结合、金属离子螯合、DNA结合蛋白-DNA结合、调控蛋白-蛋白相互作用，等。典型地，在亲和色谱法上发生在自由溶液中亲和力约10^{-4}至10^{-8}M的特异性结合。

[0101] 在本文色谱法的上下文中使用的术语“非特异性结合”，如描述目的分子和结合于固相基质的配体或其他化合物之间的相互作用，是指目的蛋白通过在相互作用位点上的静电力、氢键、疏水力和/或范德华力结合固相基质上的配体或化合物，但缺乏增强非结构性力的效应的结构互补性。非特异性相互作用的实例包括但不限于，静电力、疏水力和范德华力以及氢键键合。

[0102] “盐”是由酸和碱的相互作用形成的化合物。示例性的盐包括但不限于，乙酸盐(例如乙酸钠)、柠檬酸盐(例如柠檬酸钠)、氯盐(例如氯化钠)、硫酸盐(例如，硫酸钠)、或钾盐。

[0103] 如本文所用，“溶剂”是指能够溶解或分散一种或多种其他物质，以提供溶液的液体物质。溶剂包括水和有机溶剂，其中，某些有机溶剂包括非极性溶剂、乙醇、甲醇、异丙醇、乙腈、己二醇、丙二醇、和2,2-硫二甘醇。

[0104] 术语“去污剂”是指离子和非离子表面活性剂，如聚山梨醇酯(例如聚山梨醇酯20或80)；泊洛沙姆(例如泊洛沙姆188)；Triton；十二烷基硫酸钠(SDS)；月桂基磺酸钠；辛钠配糖体；月桂基-、十四烷基-、亚油基-、或硬脂基-磺基甜菜碱；月桂基-、十四烷基-、亚油基-或硬脂基肌氨酸；亚油基-、十四烷基-、或十六烷基甜菜碱；月桂酰胺丙基-、椰油酰胺丙基-、亚油酰胺丙基-、肉豆蔻酰胺丙基-，palmidopropyl-、或异硬脂酰胺丙基甜菜碱(例如月桂酰胺丙基)；肉豆蔻酰胺丙基-、palmidopropyl-、或异硬脂酰胺丙基二甲胺；钠甲基椰油酰基-、或二钠甲基油烯基月桂酸酯；和MONAQUAT(tm)系列(Mona Industries,Inc.,

Paterson,New Jersey),聚山梨醇酯,如聚山梨酯20(TWEEN20(r))或聚山梨醇酯80(TWEEN80(r))。

[0105] 在本文中"聚合物"是由两种或更多种单体共价连接形成的分子,其中所述单体不是氨基酸残基。聚合物的例子包括但不限于,聚乙二醇、聚丙二醇、和共聚物(例如PLURONICS™,PF68等),聚乙二醇(PEG),例如PEG 400和PEG 8000。

[0106] 术语"离子交换"和"离子交换色谱法"指的是色谱方法,其中混合物中目的溶质(如蛋白质)与连结(例如通过共价连接)至固相离子交换材料的带电化合物相互作用,使得目的溶质比混合物中溶质杂质或污染物更多或更少地非特异性地与带电化合物相互作用。混合物中的污染溶质比目的溶质更快或更慢从离子交换材料柱洗脱,或相对于目的溶质结合树脂或从树脂排出。"离子交换色谱法"具体包括阳离子交换、阴离子交换和混合模式色谱法。

[0107] 短语"离子交换材料"是指带负电荷(即,阳离子交换树脂)或带正电荷(即,阴离子交换树脂)的固相。电荷可通过将一个或多个带电配体附着(例如可以共价连接)至固相提供。可替代地,或另外地,电荷可以是固相的固有特性(例如,如二氧化硅的情况下,其具有整体负电荷)。

[0108] "固相"是指非水性基质,其可附着一个或多个带电配体。固相可以是纯化柱、离散颗粒的不连续相、膜、或者过滤器等。用于形成固相的材料的例子包括多糖(如琼脂糖和纤维素);和其他机械稳定的基质如二氧化硅(例如可控孔度玻璃),聚(苯乙烯二乙烯)苯,聚丙烯酰胺,陶瓷颗粒和上述任何的衍生物。

[0109] "阳离子交换树脂"是指带负电荷的固相,因此其具有游离的阳离子与流经或流过固相的水性溶液中的阳离子交换。附着到固相、以形成阳离子交换树脂的带负电荷的配体可以是,例如,羧酸盐或磺酸盐。市售的阳离子交换树脂包括但不限于:固定在琼脂糖上的羧基甲基纤维素,磺丙基(SP)(例如SP-SEPHAROSE FAST FLOW(或SP-SEPHAROSE HIGH PERFORMANCE))和固定在琼脂糖和**POROS®**HS上的磺酰基(例如,S-SEPHAROSE FAST FLOW)。

[0110] "混合模式离子交换树脂"是指共价修饰具有阳离子、阴离子、和疏水部分的固相。混合模式离子交换也被称为"多模式离子交换"。市售的混合模式离子交换树脂是可用的,例如,BAKERBOND ABX,其含有弱阳离子交换基团,低浓度的阴离子交换基团,和附着至二氧化硅固相支持基质的水性配体。另外的示例性混合模式离子交换树脂包括但不限于,CAPTO™Adhere树脂、QMA树脂、CAPTO™MMC树脂、MEP HyperCel树脂、HEA HyperCel树脂、PPA HyperCel树脂、或ChromaSorb膜或Sartobind STIC。在一些实施方案中,混合模式材料是CAPTO™Adhere树脂。

[0111] 术语"阴离子交换树脂"在本文中用于指带正电的固相,例如,其具有与其附着的一个或多个带正电荷的配体,如季氨基团。市售的阴离子交换树脂包括DEAE纤维素、QAE SEPHADEX和FAST Q SEPHAROSE™和Q SEPHAROSE™FAST FLOW。

[0112] "缓冲液"是通过其酸碱缀合组分的作用抗pH变化的溶液。可使用的各种缓冲液,例如,取决于所期望的缓冲液pH,描述于Buffers.A Guide for the Preparation and Use of Buffers in Biological Systems,Gueffroy,D.,ed.Calbiochem Corporation(1975)。在某些情况下,缓冲液具有的pH值范围从约2至约9,或者从约3至约8,或者从约4至约7,或

者从约5至约7。将pH控制在该范围内的缓冲液的非限制性例子包括MES、MOPS、MOPSO、Tris、HEPES、磷酸盐、乙酸盐、柠檬酸盐、琥珀酸盐、以及铵缓冲液、以及它们的组合。

[0113] 术语“疏水相互作用色谱法”或“HIC”在本文中用于指基于分子的疏水性分离分子的色谱方法。可用于HIC的示例性树脂包括但不限于苯基-、丁基-、辛基-SEPHAROSE、**BUTYL-SEPHAROSE®**4Fast Flow、PHENYL SEPHAROSE™High Performance、PHENYL SEPHAROSE™6Fast Flow(low sub)、和PHENYL SEPHAROSE™6Fast Flow(high sub)。通常，将高盐缓冲液中的样品分子上样到HIC柱。缓冲液中的盐与水分子相互作用以降低溶液中分子的溶剂化，由此暴露样品分子的疏水区，其随后由HIC柱吸附。分子疏水性越强，促进结合所需的盐越少。通常情况下，使用递减的盐梯度从柱上洗脱样品。由于离子强度降低，分子亲水区的暴露增加，分子从柱上洗脱以增加疏水性。也可以通过向洗脱缓冲液中加入温和的有机改性剂或去污剂实现样品的洗脱。

[0114] “上样缓冲液”是用来向离子交换树脂上样包含目的多肽分子和一种或多种杂质的组合物的缓冲液。上样缓冲液具有导电性和/或pH，以使目的多肽分子(通常以及一种或多种杂质)结合至离子交换树脂，或使目的蛋白质流过柱而杂质结合至树脂。

[0115] “中间缓冲液”用于在洗脱目的多肽分子之前从离子交换树脂洗脱一种或多种杂质。中间缓冲液的导电性和/或pH使得一种或多种杂质从离子交换树脂洗脱，但目的多肽的量不显著。

[0116] 当用于本文时，术语“洗涤缓冲液”是指在目的多肽分子洗脱前用于洗涤或再平衡离子交换树脂的缓冲液。在某些情况下，为方便起见，洗涤缓冲液和上样缓冲液可以是相同的，但是这不是必需的。

[0117] 在“洗脱缓冲液”用于从固相洗脱目的多肽。洗脱缓冲液的导电性和/或pH使得目的多肽从离子交换树脂洗脱。

[0118] “再生缓冲液”可用于再生离子交换树脂，使得它可以被重新使用。再生缓冲液具有从离子交换树脂基本上除去所有杂质和目的多肽所需的导电性和/或pH。

[0119] 术语“导电性”是指水性溶液在两个电极之间传导电流的能力。在溶液中，电流通过离子传输流动。因此，随着水性溶液中存在的离子含增的增加，溶液将具有较高的导电性。导电性的测量单位为每厘米milliSeimens(mS/cm)，并且可以使用例如，Orion出售的电导仪测量。可以通过改变其中的离子浓度改变溶液的导电性。例如，为了实现期望的导电性，可以改变溶液中的缓冲液浓度和/或盐(例如NaCl或KCl)浓度。

[0120] 多肽的“pI”或“等电点”是指该多肽的正电荷与其负电荷平衡的pH。pI可从氨基酸残基的净电荷计算，或从附着多肽的碳水化合物的唾液酸残基计算，或者可以通过等电点聚焦来确定。

[0121] 分子与离子交换材料“结合”是指在合适的条件(pH/导电性)下使分子暴露于离子交换材料，使得所述分子通过分子与离子交换材料的带电基团(一个或多个)之间的离子相互作用可逆地固定在离子交换材料内或上。

[0122] “洗涤”离子交换材料是指使适当的缓冲液流过或流经离子交换材料。

[0123] 至于从离子交换材料“洗脱”分子(例如多肽或杂质)是指，通过改变环绕离子交换材料的缓冲液的离子强度，使得缓冲液与分子竞争离子交换材料上的带点位点，而从离子交换材料上除去分子。

[0124] “超滤”是膜过滤的形式，其中静压迫使液体透过半透膜。悬浮固体和高分子量溶质保留，而水和低分子量溶质流过膜。在一些实例中，超滤膜具有1至100nm范围的孔径大小。术语“超滤膜”和“超滤过滤器”可以互换使用。

[0125] “渗滤”是结合超滤膜从溶液除去盐或其他微溶剂的方法。小分子从溶液中分离，同时较大分子保留在滞留物中。该方法选择性地利用可渗透的(多孔的)膜过滤器，以基于它们的分子大小分离溶液和悬浮液的组分。

[0126] 如本文所用，“滤液”指的是穿过过滤膜的样品部分。

[0127] 如本文所用，“滞留物”指的是基本上由滤膜保留的样品部分。

[0128] 术语“药物制剂”是指一种制剂，其是使得其中包含的活性成分的生物活性是有效的形式，并且不含有对施用该制剂的受试者是不可接受的毒性的其他成分。

[0129] “药学上可接受的载体”是指药物制剂中非活性成分的成分，其对受试者是无毒的。药学上可接受的载体包括但不限于，缓冲剂、赋形剂、稳定剂、或防腐剂。

[0130] 如本文所用，“治疗”(及其语法变形如“治疗(treat)”或“治疗(treating)”)是指临床介入以试图改变被治疗个体的自然进程，并且可以用于预防或临床病理学过程中进行。治疗所期望的效果包括但不限于，预防疾病的发生或复发、缓解症状、减轻疾病的任何直接或间接病理学后果、预防转移、降低疾病进展率、改善或缓解疾病状态、及缓解或改善预后。在一些实施方案中，抗体用于延迟疾病的发展或减缓疾病的进展。

[0131] 文中提及的“约”某值或参数包括(并且描述)涉及该值或参数本身的实施方案。例如，提及“约X”的描述包括“X”的描述。

[0132] 抗IL-13抗体

[0133] 在一些实施方案中，提供结合IL-13的分离的和纯化的抗体。示例性抗IL-13抗体是已知的，包括但不限于，例如，lebrikizumab、IMA-026、IMA-638(也称为anrukinzumab，INN编号910649-32-0;QAX-576)、tralokinumab(也称为CAT-354，CAS编号1044515-88-9)；AER-001、ABT-308(也称为人源化的13C5.5抗体。这种抗IL-13抗体和其他IL13抑制剂的实例公开于，例如WO 2005/062967、WO2008/086395、WO2006/085938、US 7,615,213、US 7,501,121、WO2007/036745、WO2010/073119、WO2007/045477。在一个实施方案中，抗IL-13抗体是人源化的IgG4抗体。在一个实施方案中，抗IL-13抗体是lebrikizumab。在一个实施方案中，抗IL-13抗体包含三个重链CDR，CDR-H1(SEQ ID NO:1)，CDR-H2(SEQ ID NO:2)和CDR-H3(SEQ ID NO:3)。在一个实施方案中，抗IL-13抗体包含三个轻链CDR，CDR-L1(SEQ ID NO:4)，CDR-L2(SEQ ID NO:5)和CDR-L3(SEQ ID NO:6)。在一个实施方案中，抗IL-13抗体包含三个重链CDR和三个轻链CDR，CDR-H1(SEQ ID NO:1)，CDR-H2(SEQ ID NO:2)，CDR-H3(SEQ ID NO:3)，CDR-L1(SEQ ID NO:4)，CDR-L2(SEQ ID NO:5)和CDR-L3(SEQ ID NO:6)。在一个实施方案中，抗IL-13抗体包含具有选自SEQ ID NO:7和8的氨基酸序列的可变重链区，VH。在一个实施方案中，抗IL-13抗体包含具有SEQ ID NO:9的氨基酸序列的可变轻链区，VL。在一个实施方案中，抗IL-13抗体包含具有选自SEQ ID NO:7和8的氨基酸序列的可变重链区VH，和具有SEQ ID NO:9的氨基酸序列的可变轻链区VL。在一个实施方案中，抗IL-13抗体包含具有SEQ ID NO:10或SEQ ID NO:11或SEQ ID NO:12或SEQ ID NO.:13的氨基酸序列的重链。在一个实施方案中，抗IL-13抗体包含具有SEQ ID NO:14的氨基酸序列的轻链。在一个实施方案中，抗IL-13抗体包含具有选自SEQ ID NO:10、SEQ ID NO:11、SEQ ID NO:12和SEQ

ID NO.:13的氨基酸序列的重链，和具有SEQ ID NO:14的氨基酸序列的轻链。

[0134] 在另一方面，抗IL-13抗体包含与氨基酸序列SEQ ID NO:8具有至少90％，91％，92％，93％，94％，95％，96％，97％，98％，99％或100％的序列同一性的重链可变结构域(VH)序列。在某些实施方案，具有至少90％，91％，92％，93％，94％，95％，96％，97％，98％或99％同一性的VH序列包含相对于参考序列的取代(例如，保守取代)、插入或缺失，但包含该序列的抗IL-13抗体保留结合人IL-13的能力。在某些实施方案中，SEQ ID NO:8中总共有1至10个氨基酸被取代、改变地插入和/或缺失。在某些实施方案中，取代、插入或缺失发生在CDR以外的区域(即，在FR内)。任选地，抗IL-13抗体包含SEQ ID NO:8中的VH序列，包括该序列的翻译后修饰。

[0135] 在另一方面，提供抗IL-13抗体，其中该抗体包含与氨基酸序列SEQ ID NO:9具有至少90％，91％，92％，93％，94％，95％，96％，97％，98％，99％或100％序列同一性的轻链可变结构域(VL)。在某些实施方案中，具有至少90％，91％，92％，93％，94％，95％，96％，97％，98％或99％同一性的VL序列包含相对于参考序列的取代(例如，保守取代)、插入或缺失，但包含该序列的抗IL-13抗体保留结合IL-13的能力。在某些实施方案中，SEQ ID NO:9中总共有1至10个氨基酸被取代、插入和/或缺失。在某些实施方案中，取代、插入或缺失发生在CDR以外的区域(即，在FR内)。任选地，抗IL-13抗体包含SEQ ID NO:9中的VL序列，包括该序列的翻译后修饰。

[0136] 在又一实施方案中，抗IL-13抗体包含与氨基酸序列SEQ ID NO:9具有至少90％，91％，92％，93％，94％，95％，96％，97％，98％，99％，或100％的序列同一性的VL区，和与氨基酸序列SEQ ID NO:8具有至少90％，91％，92％，93％，94％，95％，96％，97％，98％，99％或100％的序列同一性的VH区。

[0137] 下面的表格示出了lebrikizumab的CDR-H1，CDR-H2，CDR-H3，CDR-L1，CDR-L2和CDR-L3区的氨基酸序列，以及VH，VL，重链序列和轻链序列。如下面表1所示，VH和重链可以包括N-末端谷氨酰胺，重链还可包含C-末端赖氨酸。如本领域中所熟知的，在制造过程中N-末端谷氨酰胺残基可以形成焦谷氨酸和C末端赖氨酸残基可以被剪除。

[0138] 表1.抗IL-13抗体(lebrikizumab)氨基酸序列。

[0139]

CDR-H1 (SEQ ID NO.:1)	Ala Tyr Ser Val Asn
CDR-H2 (SEQ ID NO.:2)	Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys Ser
CDR-H3 (SEQ ID NO.:3)	Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn
CDR-L1 (SEQ ID NO.:4)	Arg Ala Ser Lys Ser Val Asp Ser Tyr Gly Asn Ser Phe Met His
CDR-L2 (SEQ ID NO.:5)	Leu Ala Ser Asn Leu Glu Ser
CDR-L3 (SEQ ID NO.:6)	Gln Gln Asn Asn Glu Asp Pro Arg Thr
VH (SEQ ID NO.:7)	Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ala Tyr Ser Val Asn Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu Ala Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala Gly Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser
VH (SEQ ID NO.:8)	Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ala Tyr Ser Val Asn Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu Ala Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys

[0140]

	Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala Gly Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser
VL (SEQ ID NO.:9)	Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ser Val Ser Leu Gly Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Lys Ser Val Asp Ser Tyr Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Asn Asn Glu Asp Pro Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
H 链 (SEQ ID NO.:10)	VTLRESGPA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGDGY YPYAMDNWGQ GSLVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTKTYT CNVDHKPSNT KVDKRVESKY GPPCPPCPAP EFLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLG
H 链 (SEQ ID NO.:11)	QVTLRESGPA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGDGY YPYAMDNWGQ GSLVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTKTYT CNVDHKPSNT KVDKRVESKY GPPCPPCPAP EFLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLG
H 链 (SEQ ID NO.:12)	VTLRESGPA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGDGY YPYAMDNWGQ GSLVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTKTYT CNVDHKPSNT KVDKRVESKY GPPCPPCPAP EFLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLGK
H链 (SEQ ID NO.:13)	QVTLRESGPA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGDGY YPYAMDNWGQ GSLVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTKTYT CNVDHKPSNT KVDKRVESKY GPPCPPCPAP EFLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLGK
L链 (SEQ ID NO.: 14)	DIVMTQSPDS LSVSLGERAT INCRASKSVD SYGNSFMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQNNEDPR TFGGGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC

[0141] 其他重组多肽

[0142] 可以根据本文描述的方法纯化CHO细胞中产生的重组多肽，消除或降低仓鼠PLBL2水平，使得仅剩余残留量或检测不到的量。此类多肽包括但不限于，生长因子、细胞因子、免疫球蛋白、抗体、肽体(peptibody)等等。

[0143] 某些示例性的抗体包括Aβ抗体、IL17A/F抗体和CMV抗体。示例性抗Aβ抗体和生产这类抗体的方法之前已被描述于，例如，WO2008011348，WO2007068429，WO2001062801和WO2004071408中。示例性抗IL17A/F抗体和生产这类抗体的方法之前已被描述于，例如，WO2009136286和美国专利号8,715,669。示例性抗CMV抗体，包括抗CMV-MSL，以及生产这种抗体的方法之前已被描述于WO 2012047732。

[0144] 示例性的多肽包括哺乳动物蛋白，诸如，例如，CD4；整联蛋白及其亚基，如β7；生长激素，包括人生长激素和牛生长激素；生长激素释放因子；甲状旁腺激素；促甲状腺激素；脂蛋白；ct-1-抗胰蛋白酶；胰岛素A链；胰岛素B-链；胰岛素原；卵泡刺激素；降钙素；促黄体激素；胰高血糖素；凝血因子如因子VIIIC，因子IX，组织因子和von Willebrands因子；抗凝血因子如蛋白C；心房钠尿因子；肺表面活性剂；纤溶酶原激活物，如尿激酶或组织型纤溶酶原激活物(t-PA，例如，**Activase®，TNKase®，Retevase®**)；bombazine；凝血酶；肿瘤坏死因子α和-β；脑啡肽；RANTES(调节的正常T细胞表达和分泌活化)；人巨噬细胞炎性蛋白(MIP-I-A)；血清白蛋白如人血清白蛋白；苗勒氏抑制物质；小鼠促性腺激素相关肽；DNA酶；抑制素；激活素；血管内皮生长因子(VEGF)；IgE，激素或生长因子的受体；整联蛋白；蛋白A或D；类风湿因子；神经营养因子，如骨衍生的神经营养因子(BDNF)，神经营养蛋白-3，-4，-5或-6(NT-3，NT-4，NT-5或NT-6)，或神经生长因子如NGF-β；血小板衍生的生长因子(PDGF)；成纤维细胞生长因子，如aFGF和bFGF；表皮生长因子(EGF)；转化生长因子(TGF)如TGF-α和TGF-β，包括TGF-β1、TGF-β2、TGF-β3、TGF-β4或TGF-β5；胰岛素样生长因子-I和-II(IGF-I和IGF-II)；des(1-3)-IGF-I(脑IGF-I)；胰岛素样生长因子结合蛋白；其他CD蛋白如CD3，CD8，CD19和CD20；促红细胞生成素(EPO)；血小板生成素(TPO)；骨诱导因子；免疫毒素；骨形态发生蛋白(BMP)；干扰素如干扰素-α，-β，或-γ；集落刺激因子(CSF)，例如M-CSF，GM-CSF和G-CSF；白细胞介素(IL)，例如，IL-1，IL-2，IL-3，IL-4，IL-5，IL-6，IL-7，IL-8，IL-9，IL-10，IL-11IL-12，IL-13，IL-14，IL-15，IL-16，IL-17，IL-18，IL-19，IL-20，IL-21，IL-22，IL-23，IL-24，IL-25，IL-26，IL-27，IL-28，IL-29，IL-30，IL-31，IL-32，IL-33等；超氧化物歧化酶；T细胞受体；表面膜蛋白；衰变加速因子(DAF)；病毒抗原，诸如，例如，HIV包膜的一部分；转运蛋白；归巢受体；地址素；调节蛋白；整联蛋白如CD11a，CD11b，CD11c，CD18，整联蛋白亚组如α4，αE，β7；细胞粘附分子如ICAM，VLA-4和VCAM；肿瘤相关抗原如HER1，(EGFR)，HER2，HER3或HER4受体；Apo2L/TRAIL，和上述列出的多肽的任何片段；和结合上述所列的任何蛋白质的免疫粘附素和抗体；和上述所列的任何蛋白质的生物活性片段或变体。

[0145] 其他的示例性多肽包括脑多肽，包括但不限于β-分泌酶1(BACE1)，Aβ，表皮生长因子受体(EGFR)，人表皮生长因子受体2(HER2)，tau，载脂蛋白E(ApoE)，α-突触核蛋白，CD20，亨廷顿蛋白，朊蛋白(PrP)，富亮氨酸重复激酶2(LRRK2)，帕金蛋白，早老蛋白1，早老蛋白2，γ分泌酶，死亡受体6(DR6)，淀粉状蛋白前体蛋白(APP)，p75神经营养因子受体(p75NTR)，P-选择素，和半胱天冬酶6，和上述列出的任何多肽的片段；和免疫粘附素和结合上述列出的任何蛋白质的抗体；和上述列出的任何蛋白质的生物活性片段或变体。

[0146] 其他示例性多肽包括治疗性抗体和免疫粘附素，包括但不限于，以下一种或多种抗原的抗体，包括抗体片段：HER1(EGFR)，HER2(如，曲妥单抗，帕妥珠单抗)，HER3，HER4，VEGF(如贝伐单抗，兰尼单抗)，MET(如onartuzumab)，CD20(例如，利妥昔单抗，obinutuzumab，ocrelizumab)，CD22，CD11a，CD11b，CD11c，CD18，ICAM，VLA-4，VCAM，IL-17A

和/或F，IgE(例如奥马珠单抗)，DR5，CD40，Apo2L/TRAIL，EGFL7(例如，parsatuzumab)，NRP1，整联蛋白β7(例如，etrolizumab)，IL-13(例如，lebrikizumab)Aβ(例如，crenezumab，gantenerumab)，P-选择素(例如，inclacumab)，IL-6R(例如，tociluzumab)，IFNα(例如，rontalizumab)，M1prime(例如，quilizumab)，促分裂原活化蛋白激酶(MAPK)，OX40L，TSLP，因子D(例如，lampalizumab)和受体，例如：IL-9受体，IL-5受体，IL-4受体α，IL-13受体α1和IL-13受体α2，OX40，TSLP-R，IL-7Rα(TSLP的共受体)，IL17RB(IL-25的受体)，ST2(IL-33的受体)，CCR3，CCR4，CRTH2，FcepsilonRI和FcepsilonRII/CD23(IgE的受体)。其他示例性的抗体包括但不限于选自以下的那些，抗雌激素受体抗体，抗孕酮受体抗体，抗p53抗体，抗组织蛋白酶D抗体，抗Bcl-2抗体，抗E-钙粘蛋白抗体，抗CA125抗体，抗CA15-3抗体，抗CA19-9抗体，抗c-erbB-2抗体，抗P-糖蛋白抗体，抗CEA抗体，抗视网膜母细胞瘤蛋白抗体，抗ras癌蛋白抗体，抗Lewis X抗体，抗Ki-67抗体，抗PCNA抗体，抗CD3抗体，抗CD4抗体，抗CD5抗体，抗CD7抗体，抗-CD8抗体，抗CD9/p24的抗体，抗CD10抗体，抗CD11c抗体，抗CD13抗体，抗CD14抗体，抗CD15抗体，抗CD19抗体，抗CD23抗体，抗CD30抗体，抗CD31抗体，抗CD33抗体，抗CD34抗体，抗CD35抗体，抗CD38抗体，抗CD41抗体，抗LCA/CD45抗体，抗CD45RO抗体，抗CD45RA抗体，抗CD39抗体，抗CD100抗体，抗CD95/Fas抗体，抗CD99抗体，抗CD106抗体，抗泛素抗体，抗CD71抗体，抗c-myc抗体，抗细胞角蛋白抗体，抗波性蛋白抗体，抗HPV蛋白抗体，抗κ轻链抗体，抗λ轻链抗体，抗黑素体抗体，抗前列腺特异性抗原抗体，抗S-100抗体，抗tau抗原抗体，抗血纤蛋白抗体，抗角蛋白抗体和抗Tn抗原抗体。

[0147] 某些纯化方法

[0148] 使用本文描述的方法来纯化的蛋白质一般是使用重组技术生产的。用于生产重组蛋白的方法描述于，例如，美国专利编号5,534,615和4,816,567，在此具体地并入作参考。在某些实施方案中，目的蛋白质在CHO细胞中生产(参见，例如WO 94/11026)。蛋白质的实例包括抗IL-13单克隆抗体(抗IL-13MAb)，其可以使用本文上面已经描述的方法进行纯化。

[0149] 当使用重组技术时，蛋白质可以在细胞内生产、在周质空间生产、或直接分泌到培养基中。如果蛋白质在细胞内生产，作为第一步骤，例如，通过离心或超滤去除宿主细胞或裂解片段的微粒碎片。在蛋白质被分泌到培养基的情况中，例如，可以通过切向流过滤从细胞培养基中分离重组宿主细胞。

[0150] 固定在固相的蛋白A被用于纯化抗IL-13Mab制剂。在某些实施方案中，固相是包含用于固定蛋白A的玻璃、二氧化硅，琼脂糖或聚苯乙烯表面的柱。在某些实施方案中，所述固相是可控孔径玻璃柱(controlled pore glass column)或硅酸柱。有时，柱已包被有试剂，如甘油，力图防止非特异性粘附到柱。可购自Bioprocessing Limited的PROSEP A™柱，是包被有甘油的蛋白A可控孔径玻璃柱的一个例子。本文涉及的柱的其他实例包括**POROS®** 50ATM(聚苯乙烯)柱或rProtein A SEPHAROSE FAST FLOW™(琼脂糖)柱或MABSELECT SURE™(琼脂糖)柱，其可购自GE Healthcare Life Sciences(琼脂糖)。

[0151] 用于蛋白A色谱法的固相用合适的缓冲液平衡。例如，该平衡缓冲液可以是25mM Tris，25mM NaCl，pH7.70+0.20。

[0152] 来自重组宿主细胞且含有杂质和/或污染物的制剂在使用上样缓冲液平衡的固相上上样，上样缓冲液可以与平衡缓冲液相同。当含杂质/污染物的制剂流经固相时，蛋白质被吸附到固定的蛋白A，其他杂质/污染物(在CHO细胞中生产蛋白质的情况下，诸如中国仓

鼠卵巢蛋白,CHOP)可以非特异性地结合到固相。

[0153] 依次进行的下一步骤需要通过在中间洗涤步骤中洗涤固相,除去结合于固相、抗体和/或蛋白质A的杂质/污染物。上样后,可以在开始中间洗涤步骤之前用平衡缓冲液平衡固相。

[0154] 中间洗涤缓冲液可以包括盐和任选其他化合物,例如(a)去污剂(例如,聚山梨酯,例如聚山梨酯20或聚山梨酯80);(b)溶剂(如己二醇);和(c)聚合物(如聚乙二醇{PEG])。

[0155] 所使用的盐可以基于目的蛋白质选择。示例性的盐包括但不限于,乙酸钠、柠檬酸钠、和磷酸钾。

[0156] 组合物中盐和其他化合物(如果有的话)的量为洗脱杂质/污染物、而基本上不除去目的蛋白质的组合量。这样的洗涤缓冲液中示例性盐浓度是约0.1至约2M、或约0.2M至约0.6M。有用的去污剂浓度为约0.01至约5%、或约0.1%至1%、或约0.5%,例如,其中去污剂是聚山梨醇酯时。示例性溶剂的浓度为约1%至40%、或约5至约25%。其中其他化合物是聚合物(例如PEG 400或PEG 8000)时,其浓度可以是,例如,约1%至约20%,或约5%至约15%。

[0157] 中间洗涤缓冲液的pH通常为约4至约8、或约4.5至约5.5、或约5.0。在一个实施方案中,pH为7.00±0.10。

[0158] 在上述中间洗涤步骤之后,从柱回收目的蛋白质。这通常通过使用合适的洗脱缓冲液实现。该蛋白质可以是,例如,使用具有低pH(亦称为酸性条件)的洗脱缓冲液从柱洗脱,例如pH在约2至约5的范围,或在约2.5至约3.5的范围。用于此目的洗脱缓冲液的实例包括柠檬酸盐或乙酸盐缓冲液。

[0159] 洗脱的蛋白质制剂可在蛋白A色谱法步骤之前或之后经过额外的纯化步骤。示例性进一步的纯化步骤包括羟磷灰石色谱法;透析;使用抗体捕获蛋白的亲和色谱法;疏水相互作用色谱法(HIC);硫酸铵沉淀;阴离子或阳离子交换色谱;乙醇沉淀;反相HPLC;在二氧化硅上的色谱法;聚焦色谱法;超滤渗滤(UFDF),和凝胶过滤。在文中的例子中,蛋白A色谱法步骤之后是下游阴离子交换(例如,Q-Sepharose-Fast Flow)或多模式(例如,混合模式)离子交换(例如,CAPTO™Adhere)和HIC(例如,PHENYL SEPHAROSE™6fast flow-high sub)纯化步骤。

[0160] 由此回收的蛋白质可配制在药学上可接受的载体中,用于对这些分子已知的各种诊断、治疗或其他用途。

[0161] 在本文所述任何方法的一些实施方案中,色谱法材料为离子交换色谱法材料;例如,阴离子交换色谱法材料。在一些实施方案中,阴离子交换色谱法材料是带正电的固相,具有游离的阴离子与流经或流过固相的水性溶液中的阴离子交换。在本文所述任何方法的一些实施方案中,阴离子交换材料可以是膜、整料、或树脂。在一个实施方案中,阴离子交换材料可以是树脂。在一些实施方案中,阴离子交换材料可包括伯胺、仲胺、叔胺或季铵离子官能团,多胺官能团或二乙氨乙基(diethylaminoaethyl)官能团。在上述一些实施方案中,阴离子交换色谱法材料为阴离子交换色谱法柱。在上述一些实施方案中,阴离子交换色谱法材料为阴离子交换色谱法膜。

[0162] 在本文所述任何方法的一些实施方案中,离子交换材料可利用常规的色谱法材料或对流色谱法材料。常规的色谱法材料包括,例如,散发(perfusive)材料(例如,聚(苯乙

烯-二乙烯基苯)树脂)和扩散(diffusive)材料(例如,交联的琼脂糖树脂)。在一些实施方案中,聚(苯乙烯-二乙烯基苯)树脂可以是**POROS®**树脂。在一些实施方案中,交联的琼脂糖树脂可以是sulphopropyl-Sepharose Fast Flow("SPSFF")树脂。对流色谱法材料可以是膜(例如,聚醚砜)或整料材料(例如交联的聚合物)。聚醚砜膜可以是Mustang。交联的聚合物整料材料可以是交联的聚(甲基丙烯酸缩水甘油酯-共-乙二醇二甲基丙烯酸酯)。

[0163] 阴离子交换材料的例子包括,但不限于,**POROS®**HQ 50,**POROS®**PI 50,**POROS®**D,Mustang Q,Q SEPHAROSE™FF和DEAE Sepharose。

[0164] 在一些方面,色谱法材料为疏水相互作用色谱法材料。疏水相互作用色谱法(HIC)是一种液体色谱法技术,其根据疏水性分离生物分子。HIC色谱法材料的例子包括,但不限于,Toyopearl hexyl 650,Toyopearl butyl 650,Toyopearl phenyl 650,Toyopearl ether 650,Source,Resource,Sepharose Hi-Trap,Octyl sepharose,PHENYL SEPHAROSE™high performance,PHENYL SEPHAROSE™6fast flow(low sub)和PHENYL SEPHAROSE™6fast flow(high sub)。在上述的一些实施方案中,HIC色谱法材料是HIC色谱法柱。在上述的一些实施方案中,HIC色谱法材料是HIC色谱法膜。

[0165] 在一些方面,色谱法材料是亲和色谱法材料。亲和色谱法材料的实例包括,但不限于,与蛋白A或蛋白G衍生的色谱法材料。亲和色谱法材料的实例包括,但不限于,Prosep-VA,Prosep-VA Ultra Plus,Protein A sepharose fast flow,Tyopearl Protein A,MAbSelect,MABSELECT SURE™和MABSELECT SURE™LX。在上述的一些实施方案中,亲和色谱法材料是亲和色谱法柱。在上述一些实施方案中,亲和色谱法材料是亲和色谱法膜。

[0166] 可根据,例如,期望的缓冲液pH值、期望的缓冲液导电性、目的蛋白质的特性、和纯化方法使用各种缓冲液。在本文所述任何方法的一些实施方案中,方法包括使用缓冲液。缓冲液可以是上样缓冲液、平衡缓冲液、或洗涤缓冲液。在一些实施方案中,一种或多种上样缓冲液、平衡缓冲液和/或洗涤缓冲液是相同的。在一些实施方案中,上样缓冲液、平衡缓冲液和/或洗涤缓冲液是不同的。在本文所述任何方法的一些实施方案中,缓冲液包含盐。上样缓冲液可以包含氯化钠、乙酸钠或其混合物。在一些实施方案中,上样缓冲液为氯化钠缓冲液。在一些实施方案中,上样缓冲液为乙酸钠缓冲液。

[0167] 如本文所使用的上样物(load),是上样到色谱法材料上的组合物。上样缓冲液是用于将包含目的产物的组合物上样到色谱法材料上的缓冲液。可以在上样待纯化的组合物之前用平衡缓冲液平衡色谱法材料。在一些实施例中,在组合物上样到色谱法材料之后、以及在目的多肽从固相洗脱之前、使用洗涤缓冲液。然而,一些目的产物,例如多肽,可以被洗涤缓冲液(例如,流出模式)从色谱法材料移除。

[0168] 如本文所使用的洗脱,是从色谱法材料除去产物,例如多肽。洗脱缓冲液是用于从色谱法材料洗脱目的多肽或其他产物的缓冲液。在许多情况下,洗脱缓冲液具有不同于上样缓冲液的物理特征。例如,洗脱缓冲液可具有不同于上样缓冲液的导电性或不同于上样缓冲液的pH。在一些实施方案中,洗脱缓冲液具有低于上样缓冲液的导电性。在一些实施方案中,洗脱缓冲液具有高于上样缓冲液的导电性。在一些实施方案中,洗脱缓冲液具有低于上样缓冲液的pH。在一些实施方案中,洗脱缓冲液具有高于上样缓冲液的pH。在一些实施方案中,洗脱缓冲液具有与上样缓冲液不同的导电性和不同的pH。洗脱缓冲液可以具有更高或更低的导电性和更高或更低的pH的任何组合。

[0169] 导电性指水性溶液在两个电极之间传导电流的能力。在溶液中，电流通过离子传输。因此，随着水性溶液中存在的离子量的增加，该水性溶液将具有更高的导电性。测量导电性的基本单位是Siemen(或mho)、mho(mS/cm)，并且可以使用电导仪测量，如Orion电导仪的各种型号。由于电解质的导电性是溶液中离子携带电流的能力，溶液的导电性可以通过改变其中的离子浓度而改变。例如，可以改变溶液中缓冲剂的浓度和/或盐(例如氯化钠、乙酸钠、或氯化钾)的浓度，以获得所期望的导电性。优选地，改变各种缓冲液的盐浓度，以获得期望的导电性。

[0170] 在本文所述任何方法的一些实施方案中，流率小于约50CV/hr、40CV/hr或30CV/hr中的任何。流速可以为约5CV/hr和50CV/hr之间、10CV/hr和40CV/hr之间、或18CV/hr和36CV/hr之间的任何。在一些实施方案中，流速为约9CV/hr、18CV/hr、25CV/hr、30CV/hr、36CV/hr、或40CV/hr的任何。在本文所述任何方法的一些实施方案中，流率小于约100cm/hr、75cm/hr、或50cm/hr的任何。流速可以是约25cm/hr和150cm/hr之间、25cm/hr和100cm/hr之间、50cm/hr和100cm/hr之间、或65cm/hr和85cm/hr之间、或50cm/hr和250cm/hr之间、或100cm/hr和250cm/hr之间、或150cm/hr和250cm/hr之间的任何。

[0171] 床高度是使用的色谱法材料的高度。在本文所述任何方法的一些实施方案中，床高度高于约3cm、10cm、或15cm中的任何。床高度可以是约3cm和35cm之间、5cm和15cm之间、3cm和10cm之间、或5cm和8cm之间中的任何。在一些实施方案中，床高度为约3cm、5cm、10cm、或15cm中的任何。在一些实施方案中，根据上样中的多肽或污染物的量来确定床高度。

[0172] 在一些实施方案中，色谱法在容器的柱中，容器的体积大于约1mL，2mL，3mL，4mL，5mL，6mL，7mL，8mL，9mL，10mL，15mL，20mL，25mL，30mL，40mL，50mL，75mL，100mL，200mL，300mL，400mL，500mL，600mL，700mL，800mL，900mL，1L，2L，3L，4L，5L，6L，7L，8L，9L，10L，25L，50L，100L，200L，400L，或450L。

[0173] 在一些实施方案中，从色谱法收集级分。在一些实施方案中，收集的级分大于约0.01CV，0.02CV，0.03CV，0.04CV，0.05CV，0.06CV，0.07CV，0.08CV，0.09CV，0.1CV，0.2CV，0.3CV，0.4CV，0.5CV，0.6CV，0.7CV，0.8CV，0.9CV，1.0CV，2.0CV，3.0CV，4.0CV，5.0CV。在一些实施方案中，汇集含有产物(例如多肽)的级分。在一些实施方案中，汇集含有来自上样级分和洗脱级分的多肽的级分。级分中多肽的量可以由本领域技术人员确定；例如，级分中多肽的量可通过UV光谱法来确定。在一些实施方案中，汇集含有可检测的多肽片段的级分。

[0174] 在本文所述任何方法的一些实施方案中，至少一种杂质或污染物是宿主细胞材料，如CHOP；过滤的蛋白A；核酸；所期望多肽的变体、片段、聚集体或衍生物；另一多肽；内毒素；病毒污染物；细胞培养基组分，庆大霉素的等任何一种或多种。在一些实施例中，杂质或污染物可以是宿主细胞蛋白(HCP)，其来自例如但不限于，细菌细胞如大肠杆菌细胞、昆虫细胞、原核细胞、真核细胞、酵母细胞、哺乳动物细胞、禽类细胞、真菌细胞。

[0175] 宿主细胞蛋白(HCP)是来自在其中产生多肽的细胞的蛋白质。例如，CHOP是来自宿主细胞的蛋白质，即，中国仓鼠卵巢蛋白。CHOP的量可以通过酶联免疫吸附测定法(“ELISA”)或质谱法来测量。在本文所述任何方法的一些实施方案中，HCP(例如CHOP)的量减少大于约10%，20%，30%，40%，50%，60%，70%，80%，90%，或95%中的任何。HCP的量可以减少约10%和99%之间，30%和95%之间，30%和99%之间，50%和95%之间，50%和99%之间，75%和99%之间，或85%和99%之间中的任何。在一些实施方案中，HCP的量减少

约10%,20%,30%,40%,50%,60%,70%,80%,85%,90%,95%或98%中的任何。在一些实施方案中,通过比较从纯化步骤回收的组合物中HCP的量与纯化步骤之前组合物中HCP的量来确定减少。

[0176] 在本文所述任何方法的一些实施方案中,所述方法进一步包括回收纯化的多肽。在一些实施方案中,纯化的多肽从本文所述的纯化步骤中的任何步骤回收。色谱法步骤可以是阴离子交换色谱法、HIC或蛋白A色谱法。在一些实施方案中,第一色谱法步骤是蛋白A,接着是阴离子交换或多模式离子交换,接着是HIC。

[0177] 在一些实施方案中,色谱法之后多肽进一步通过病毒过滤纯化。病毒过滤是除去多肽纯化进料流中的病毒杂质。病毒过滤的例子包括超滤和微滤。在一些实施方案中,多肽使用细小病毒过滤器纯化。

[0178] 在一些实施方案中,在色谱法后浓缩多肽。浓缩方法的实例在本领域中是已知的,包括但不限于超滤和渗滤。

[0179] 在本文所述任何方法的一些实施方案中,方法进一步包括组合纯化方法中的纯化多肽与药学上可接受的载体。

[0180] 单克隆抗体

[0181] 在一些实施方案中,根据本发明的方法纯化的抗体是单克隆抗体。单克隆抗体从一群基本上同质的抗体获得,即,构成群的各个抗体是相同的和/或结合相同的表位,除了生产单克隆抗体的过程中出现的可能的变体,这种变体通常少量存在。因此,修饰语"单克隆"表示抗体特征,其不是不同的抗体的混合物或多克隆抗体。

[0182] 例如,可通过Kohler等人,Nature 256:495(1975)最初描述的杂交瘤方法制备单克隆抗体,或者可通过重组DNA方法制备(美国专利号4,816,567)。

[0183] 在杂交瘤方法中,如本文所述免疫小鼠或其他合适的宿主动物,如仓鼠,以诱发生成或能够生成抗体的淋巴细胞,所述抗体将特异性结合用于免疫的多肽。或者,可在体外免疫淋巴细胞。然后使用合适的融合剂如聚乙二醇,使淋巴细胞与骨髓瘤细胞融合,以形成杂交瘤细胞(Goding,Monoclonal Antibodies:Principles and Practice,pp.59-103(Academic Press,1986))。

[0184] 将如此制备的杂交瘤细胞接种并生长于合适的培养基,优选含有一种或多种抑制未融合的亲本骨髓瘤细胞生长或存活的物质。例如,如果亲本骨髓瘤细胞缺少酶次黄嘌呤鸟嘌呤磷酸核糖转移酶(HGPRT或HPRT),那么用于杂交瘤的培养基通常包含次黄嘌呤、氨基喋呤和胸苷(HAT培养基),这些物质阻止HGPRT缺陷的细胞生长。

[0185] 在一些实施方案中,骨髓瘤细胞是高效融合、支持所选择的抗体生成细胞稳定高水平地生成抗体、并且对培养基(如HAT培养基)敏感的那些。在这些中,在一些实施方案中,骨髓瘤细胞系是鼠骨髓瘤系,如可从Salk Institute Cell Distribution Center,San Diego,California USA获得的源自MOPC-21和MPC-11小鼠肿瘤的那些,和可从美国典型培养物保藏中心,Rockville,Maryland USA获得的SP-2或X63-Ag8-653细胞。也描述了人骨髓瘤和小鼠-人杂合骨髓瘤细胞系用于生产人单克隆抗体(Kozbor,J.Immunol.133:3001(1984);Brodeur等人,Monoclonal Antibody Production Techniques and Applications pp.51-63(Marcel Dekker,Inc.,New York,1987))。

[0186] 针对生产抗抗原的单克隆抗体而检测杂交瘤细胞生长的培养基。在一些实施方案

中，由杂交瘤细胞生成的单克隆抗体的结合特异性通过免疫沉淀或通过体外结合测定法来确定，如放射免疫测定法(RIA)或酶联免疫吸附测定法(ELISA)。

[0187] 单克隆抗体的结合亲和力，例如，可以通过Munson等人，Anal.Biochem.107:220(1980)的Scatchard分析来确定。

[0188] 鉴定得到生成具有所期望的特异性、亲和力和/或活性的抗体的杂交瘤细胞之后，可将该克隆通过有限稀释步骤亚克隆并通过标准方法生长(Goding，Monoclonal Antibodies:Principles and Practice pp.59-103(Academic Press,1986))。用于此目的的合适的培养基包括例如D-MEM或RPMI-1640培养基。另外，杂交瘤细胞可在体内作为动物腹水肿瘤生长。

[0189] 通过常规免疫球蛋白纯化方法适当地从培养基、腹水或血清中分离由亚克隆分泌的单克隆抗体，如，例如，多肽A-琼脂糖、羟磷灰石色谱法、凝胶电泳、透析或亲和色谱法。

[0190] 使用常规步骤很容易地分离和测序编码单克隆抗体的DNA(例如使用寡核苷酸探针，其能够特异性结合编码鼠抗体重链和轻链的基因)。在一些实施方案中，杂交瘤细胞作为此类DNA的来源。一旦分离，可将DNA置于表达载体中，然后将其转染到否则不产生免疫球蛋白多肽的宿主细胞，如大肠杆菌细胞、猿猴COS细胞、中国仓鼠卵巢(CHO)细胞、或骨髓瘤细胞，以在重组宿主细胞中获得单克隆抗体的合成。在细菌中重组表达编码抗体的DNA的综述性文章包括Skerra等人，Curr.Opinion in Immunol.5:256-262(1993)和Plückthun，Immunol.Revs.,130:151-188(1992)。

[0191] 在进一步的实施方案中，可从使用McCafferty等人，Nature 348:552-554(1990)中描述的技术生产的抗体噬菌体文库中分离抗体或抗体片段。Clackson等人，Nature 352:624-628(1991)和Marks等人，J.Mol.Biol.222:581-597(1991)分别描述了使用噬菌体文库的鼠和人抗体的分离。后续出版物描述了通过链改组生产高亲和力(nM范围)人抗体(Marks等人，Bio/Technology 10:779-783(1992))，以及作为构建非常大的噬菌体文库的策略的组合感染和体内重组(Waterhouse等人，Nuc.Acids.Res.21:2265-2266(1993))。因此，这些技术是可替代传统单克隆抗体杂交瘤技术用于分离单克隆抗体。

[0192] 也可以改变DNA，例如，通过用人重链和轻链恒定结构域的编码序列取代同源鼠序列(美国专利号4,816,567;Morrison等人，Proc.Natl Acad.Sci.USA 81:6851(1984))，或者通过共价连接免疫球蛋白编码序列的全部或部分编码序列与非免疫球蛋白多肽。

[0193] 典型地，这种非免疫球蛋白多肽取代抗体的恒定结构域，或者其取代抗体的一个抗原结合位点的可变结构域，以创建嵌合二价抗体，其包含一个对抗原具有特异性的抗原结合位点和另一个对不同抗原具有特异性的抗原结合位点。

[0194] 在本文所述任何方法的一些实施方案中，抗体是IgA，IgD，IgE，IgG或IgM抗体。在一些实施方案中，抗体是IgG单克隆抗体。

[0195] 人源化抗体

[0196] 在一些实施方案中，抗体是人源化抗体。用于人源化非人抗体的方法在本领域中已有描述。在一些实施方案中，人源化抗体具有引入其中的一个或多个来自非人来源的氨基酸残基。这些非人氨基酸残基常常称作“输入”残基，其通常来自“输入”可变结构域。人源化可基本上根据Winter和合作者的(Jones等人，Nature 321:522-525(1986)；Riechmann等人，Nature 332:323-327(1988)；Verhoeyen等人，Science 239:1534-1536(1988))的方法，

通过用高变区序列取代相应的人抗体序列进行。因此，这样的“人源化”抗体是嵌合抗体(美国专利号4,816,567)，其中基本上不完整的人可变结构域已经被来自非人物种的相应序列取代。在实践中，人源化抗体通常是人抗体，其中一些高变区残基和可能的一些FR残基被来自啮齿类抗体中类似位点的残基取代。

[0197] 在制备人源化抗体中使用的人可变结构域(包括轻链和重链)的选择对于减少抗原性是非常重要的。根据所谓的“最佳拟合”方法，针对已知的人可变结构域序列的整个文库筛选啮齿类抗体的可变结构域序列。然后将最接近啮齿类序列的人序列作为人框架区(FR)用于人源化抗体(Sims等人，J.Immunol.151:2296(1993)；Chothia等人，J.Mol.Biol.196:901(1987))。另一种方法使用源自轻链或重链可变区特定亚组的所有人抗体的共有序列的特定框架区。相同框架可用于数种不同的人源化抗体(Carter等人，Proc.Natl.Acad.Sci.USA 89:4285(1992)；Presta等人，J.Immunol.151:2623(1993))。

[0198] 更重要的是被人源化的抗体保留对抗原的高亲和力和其他有利的生物学特性。为了实现这一目标，在该方法的某些实施方案中，通过使用亲本和人源化序列的三维模型分析亲本序列和各种概念性人源化产物的方法制备人源化抗体。三维免疫球蛋白模型通常是可获得的并且是本领域技术人员熟悉的。可获得一些说明和显示选择的候选免疫球蛋白序列可能的三维构象结构的计算机程序。检查这些显示能够分析在候选免疫球蛋白序列中发挥功能的残基的可能作用，即，分析影响候选免疫球蛋白结合其抗原能力的残基。以这种方式，可以从接受(序列)和输入序列选择和组合FR残基，从而实现所期望的抗体特征，如增加的对靶抗原的亲和力。一般而言，高变区残基直接和最实质地参与影响抗原结合。

[0199] 人抗体

[0200] 在一些实施方案中，抗体是人抗体。作为人源化的替代，可以生成人抗体。例如，现在有可能生成转基因动物(例如小鼠)，其免疫后能够产生人抗体的所有组成成分，而没有内源性免疫球蛋白生成。例如，已经描述了嵌合和种系突变小鼠中抗体重链连接区(J_H)基因的纯合缺失，导致内源性抗体生成的完全抑制。在此类种系突变小鼠中转移人种系免疫球蛋白基因阵列将导致在抗原攻击时产生人抗体。见，例如，Jakobovits等人，Proc.Natl.Acad.Sci.USA 90:2551(1993)；Jakobovits等人，Nature 362:255-258(1993)；Bruggermann等人，Year in Immuno.7:33(1993)；和美国专利号5,591,669；5,589,369；和5,545,807。

[0201] 可替代地，可使用噬菌体展示技术(McCafferty等人，Nature 348:552-553(1990))在体外从未免疫供体的免疫球蛋白可变(V)结构域基因所有组成成分生产人抗体和抗体片段。根据该技术，抗体V结构域基因在框内(in-frame)克隆至丝状噬菌体(如M13或fd)的主要或次要外壳多肽基因，并作为功能性抗体片段展示在噬菌体颗粒的表面。因为丝状颗粒包含噬菌体基因组的单链DNA拷贝，基于抗体功能特性的选择也导致编码展示那些特性的抗体的基因的选择。因此，噬菌体模仿了B细胞的一些特性。噬菌体展示可以各种形式进行；有关综述参见，例如，Johnson,Kevin S.和Chiswell,David J.,Current Opinion in Structural Biology 3:564-571(1993)。V基因区段的数种来源可用于噬菌体展示。Clackson等人，Nature 352:624-628(1991)从源自免疫小鼠脾小的V基因随机组合文库分离了多样化的抗恶唑酮抗体。可以构建来自未经免疫的人供体的V基因组集，并基本上根据Marks等人，J.Mol.Biol.222:581-597(1991)，或Griffith等人，EMBO J.12:725-734(1993)

所描述的技术分离多样化抗原(包括自抗原)的抗体。还参见,美国专利号5,565,332和5,573,905。

[0202] 也可以通过体外活化的B细胞来产生人抗体(参见美国专利5,567,610和5,229,275)。

[0203] 抗体片段

[0204] 在一些实施方案中,抗体是抗体片段。已经开发了各种技术用于生成抗体片段。传统上,这些片段经由蛋白水解消化完整的抗体衍生(见,例如,Morimoto等人,Journal of Biochemical and Biophysical Methods 24:107-117(1992)和Brennan等人,Science 229:81(1985))。然而,这些片段现在可直接由重组宿主细胞产生。例如,抗体片段可以从上文讨论的抗体噬菌体文库中分离。可替代地,Fab'-SH片段可以直接从大肠杆菌回收并化学偶联以形成$F(ab')_2$片段(Carter等人,Bio/Technology10:163-167(1992))。根据另一方法,$F(ab')_2$片段可以直接从重组宿主细胞培养物分离。用于生成抗体片段的其他技术是本领域技术人员显而易见的。在其他实施方案中,选择的抗体是单链Fv片段(scFv)。参见WO93/16185;美国专利号5,571,894;和美国专利号5,587,458。抗体片段还可以是"线性抗体",例如,如在美国专利5,641,870中描述的。此类线性抗体片段可以是单特异性的或双特异性的。

[0205] 在一些实施方案中,提供了本文所述的抗体片段。在一些实施方案中,抗体片段是抗原结合片段。在一些实施方案中,抗原结合片段选自Fab片段、Fab'片段、$F(ab')_2$片段、scFv、Fv和双抗体。

[0206] 嵌合多肽

[0207] 本文描述的多肽可以以一种方式改变,以形成包含融合另一异源多肽或氨基酸序列的多肽的嵌合分子。在一些实施方案中,嵌合分子包括多肽与标签多肽融合,所述标签多肽提供了抗标签抗体可选择性地结合的表位。表位标签通常位于多肽的氨基或羧基末端。可以使用抗标签多肽的抗体来检测多肽的此类表位标签形式的存在。另外,提供表位标签使得能够容易地使用抗标签抗体或其他类型的结合表位标签的亲和基质通过亲和纯化纯化多肽。

[0208] 其他

[0209] 多肽共价修饰的另一种类型包括将多肽连接至多种非蛋白质性质的聚合物之一,例如聚乙二醇,聚丙二醇,聚氧化烯(polyoxyalkylenes),或聚乙二醇和聚丙二醇的共聚物。多肽还可以包裹在例如,通过凝聚技术或通过界面聚合(例如,分别为羟甲基纤维素或明胶微胶囊和聚(甲基丙烯酸甲酯)微胶囊)制备的微胶囊中、在胶状药物递送系统(例如脂质体,白蛋白微球,微乳剂,纳米颗粒和纳米胶囊)中,或在大乳剂中。这种技术公开于Remington's Pharmaceutical Sciences,第18版,Gennaro,A.R.,Ed.,(1990)。

[0210] 获得多肽

[0211] 本文所描述的纯化方法中使用的多肽可以使用本领域中熟知的方法获得,包括重组方法。下面的部分提供了有关这些方法的指导。

[0212] 多核苷酸

[0213] 如本文中可互换使用的"多核苷酸"或"核酸",指任何长度核苷酸的聚合物,包括DNA和RNA。

[0214] 编码多肽的多核苷酸可以从任何来源获得，包括但不限于，从认为具有多肽mRNA且以可检测水平表达的组织制备的cDNA文库。因此，编码多肽的多核苷酸可以方便地从人组织制备的cDNA文库获得。编码多肽的基因也可从基因组文库获得，或通过已知的合成步骤(例如自动化核酸合成)获得。

[0215] 例如，多核苷酸可编码整个免疫球蛋白分子链，如轻链或重链。完整的重链不仅包含重链可变区(V_H)，还包含重链恒定区(C_H)，其通常包含三个恒定结构域：C_H1，C_H2和C_H3；和“铰链”区。在某些情况下，恒定区的存在是期望的。

[0216] 可以由多核苷酸编码的其他多肽包括抗体的抗原结合片段，如单结构域抗体(“dAb”)，Fv，scFv，Fab'和$F(ab')_2$和“微抗体”。微抗体(通常情况下)是C_H1和C_K或C_L结构域已经被从其上切除的二价抗体片段。由于微抗体比常规抗体更小，其在临床/诊断应用中应实现更好的组织穿透，但作为二价其应该保留比单价抗体片段(例如dAb)更高的结合亲和力。相应地，除非上下文另有规定，文中所使用的术语“抗体”在本文中不仅包括整个抗体分子，还包括上面讨论的类型的抗体的抗原结合片段。优选存在于所编码多肽中的各构架区相对于相应的人受体构架区包含至少一个氨基酸取代。因此，例如，框架区相对于受体构架区可以总共包含，三，四，五，六，七，八，九，十，十一，十二，十三，十四，或十五个氨基酸取代。

[0217] 合适地，本文描述的多核苷酸可以被分离和/或纯化。在一些实施方案中，多核苷酸是分离的多核苷酸。

[0218] 术语“分离的多核苷酸”意图指分子从其正常或天然环境中被移除或分离，或以不存在于正常或天然环境的方式生成。在一些实施方案中，多核苷酸是纯化的多核苷酸。术语纯化的意图指至少一些污染分子或物质被除去。

[0219] 合适地，多核苷酸基本上是纯化的，使得相关的多核苷酸构成存在于组合物中占主导地位的(即，最丰富的)多核苷酸。

[0220] 多核苷酸的表达

[0221] 下面的描述主要涉及通过培养含有编码多肽的多核苷酸的载体转化或转染的细胞生产多肽。当然，预期在本领域中公知的替代性方法，可以用来制备多肽。例如，可以使用固相技术通过直接肽合成生产适当的氨基酸序列，或其部分(见，例如，Stewart等人，Solid-Phase Peptide Synthesis W.H.Freeman Co.,San Francisco,Calif.(1969)；Merrifield,J.Am.Chem.Soc.85:2149-2154(1963))。体外蛋白合成可以使用手动技术或通过自动化来进行。自动合成可通过，例如，使用Applied Biosystems Peptide Synthesizer (Foster City,Calif.)，使用制造商的说明书实现。可以独立地化学合成多肽的各个部分，并使用化学或酶促方法组合各部分，来生产所期望的的多肽。

[0222] 如本文所述的多核苷酸被插入到表达载体用于生产多肽。术语“控制序列”是指在特定宿主生物体中表达所必需的DNA序列，其可操作地连接编码序列。该控制序列包括但不限于，启动子(例如，天然相关的或异源启动子)、信号序列、增强子元件和转录终止序列。

[0223] 当其被置于与另一多核苷酸序列的功能关系中时，多核苷酸被“可操作地连接”。例如，如果多肽作为参与多肽分泌的前蛋白表达时，前序列或分泌前导的核酸可操作地连接到多肽的核酸；如果其影响序列的转录，则启动子或增强子可操作地连接到编码序列；或者如果其被定位以促进翻译，则核糖体结合位点可操作地连接到编码序列。一般地，“可操

作地连接”是指被连接的核酸序列是连续的，并且，在分泌前导的情况下，是连续的并在阅读框中。然而，增强子不必是连续的。通过在方便的限制性位点的连接完成连接。如果不存在此类位点，根据常规实践使用合成的寡核苷酸衔接头或接头。

[0224] 对于抗体，轻链和重链可以克隆到相同或不同的表达载体。编码免疫球蛋白链的核酸区段可操作地连接到表达载体中的控制序列，以确保免疫球蛋白多肽的表达。

[0225] 含有多核苷酸序列(例如，可变重链和/或可变轻链编码序列和任选的表达控制序列)的载体可通过公知的方法转移到宿主细胞中，这取决于细胞宿主的类型。例如，氯化钙转染通常用于原核细胞，而磷酸钙处理、电穿孔、脂质转染、基因枪法或基于病毒的转染可用于其他细胞宿主。(一般参见Sambrook等人，Molecular Cloning:A Laboratory Manual (Cold Spring Harbor Press,2nd ed.,1989))。用于转化哺乳动物细胞的其他方法包括使用聚凝胺、原生质体融合、脂质体、电穿孔和显微注射。为了生产转基因动物，转基因可显微注射到受精卵母细胞，或可掺入到胚胎干细胞的基因组中，并且这种细胞的细胞核转移到去核卵母细胞中。

[0226] 载体

[0227] 术语“载体”包括表达载体和转化载体和穿梭载体。

[0228] 术语“表达载体”是指能够在体内或体外表达的构建体。

[0229] 术语“转化载体”是指能够从一个实体转移到另一个实体的构建体-实体可以是相同物种的实体或可以是不同物种的实体。如果构建体能够被从一个物种转移到另一个物种-例如从大肠杆菌质粒转移到细菌，如芽孢杆菌属，那么转化载体有时称为“穿梭载体”。它甚至可以是能够从大肠杆菌质粒转移到土壤杆菌到植物中的构建体。

[0230] 如下所述，载体可以转化到合适的宿主细胞以提供多肽的表达。各种载体是公众可获得的。载体，例如，可以是质粒、粘粒、病毒颗粒或噬菌体的形式。合适的核酸序列可以通过各种步骤插入载体中。在一般情况下，使用本领域中已知的技术将DNA插入到适当的限制性内切酶位点。采用本领域技术人员已知的标准连接技术构建含有这些组分中的一种或多种的合适的载体。

[0231] 载体可以是例如，质粒、病毒或噬菌体载体，其具有复制起点，任选具有用于表达所述多核苷酸的启动子和任选具有启动子的调节子。载体可含有本领域中公知的一种或多种选择性标记基因。

[0232] 这些表达载体通常在宿主生物体内或者作为附加体或者作为宿主染色体DNA的整合部分，是可复制的。

[0233] 宿主细胞

[0234] 宿主细胞例如，可以是细菌、酵母或其他真菌细胞、昆虫细胞、植物细胞、或哺乳动物细胞。

[0235] 可使用已经被遗传工程操作的转基因多细胞宿主生物体生产多肽。该生物体可以是，例如，转基因哺乳动物生物体(例如，转基因山羊或小鼠系)。

[0236] 合适的原核生物包括但不限于真细菌，如革兰氏阴性或革兰氏阳性生物体，例如肠杆菌科，如大肠杆菌。多种大肠杆菌菌株是公众可获得的，如大肠杆菌K12菌株MM294 (ATCC 31,446)；大肠杆菌X1776(ATCC 31,537)；大肠杆菌菌株W3110(ATCC 27,325)和K5 772(ATCC 53,635)。其他合适的原核宿主细胞包括肠杆菌科如埃希氏菌属(Escherichia)，

例如，大肠杆菌，肠杆菌属(Enterobacter)、欧文氏菌属(Erwinia)，克雷伯氏菌属(Klebsiella)，变形杆菌属(Proteus)，沙门氏菌属(Salmonella)，例如，鼠伤寒沙门氏菌(Salmonella typhimurium)，沙雷氏菌属(Serratia)，例如，(Serratia marcescans)，和志贺氏菌属(Shigella)，以及杆菌(Bacilli)，如枯草芽孢杆菌(B.subtilis)和地衣芽孢杆菌(B.licheniformis)(例如，地衣芽孢杆菌41P(B.licheniformis 41P))，假单胞菌属(Pseudomonas)，如铜绿假单胞菌(P.aeruginosa)，和链霉菌属(Streptomyces)。这些实例是说明性的而不是限制性的。菌株W3110是一个特别优选的宿主或亲本宿主，因为它是重组多核苷酸产物发酵的常用宿主菌株。优选地，宿主细胞分泌最小量的蛋白质水解酶。例如，可以修饰菌株W3110，以实现编码宿主内源性多肽的基因中的基因突变，此类宿主的例子包括大肠杆菌W3110菌株1A2，其具有完整的基因型tonA；大肠杆菌W3110菌株9E4，其具有完整的基因型tonA ptr3；大肠杆菌W3110菌株27C7(ATCC 55,244)，其具有完整的基因型tonA ptr3 phoA E15(argF-lac)169degP ompT kan'；大肠杆菌菌株W3110 37D6，其具有完整的基因型tonA ptr3 phoA E15(argF-lac)169degP ompT rbs7 ilvG kan'；大肠杆菌W3110菌株40B4，其是具有非卡那霉素抗性degP缺失突变的菌株37D6；和具有突变的周质蛋白酶的大肠杆菌菌株。或者，体外克隆方法，例如PCR或其他核酸聚合酶反应，也是合适的。

[0237] 可以在这些原核宿主中制备表达载体，其通常包含与宿主细胞相容的表达控制序列(例如，复制起点)。此外，将存在任何数量的各种众所周知的启动子，如乳糖启动子系统、色氨酸(trp)启动子系统、β-内酰胺酶启动子系统、或来自λ噬菌体的启动子系统。启动子通常控制表达，任选具有操纵子序列，并具有核糖体结合位点序列等，用于起和完成转录和翻译。

[0238] 真核微生物可用于表达。真核微生物如丝状真菌或酵母是用于编码多肽的载体的合适的克隆或表达宿主。酿酒酵母(Saccharomyces cerevisiae)是常用的低等真核宿主微生物。其他包括裂殖酵母(Schizosaccharomyces pombe)；克鲁维酵母宿主(Kluyveromyces hosts)如，例如，乳酸克鲁维酵母(K.lactis)(MW98-8C,CBS683,CBS4574)，脆弱克鲁维酵母(K.fragilis)(ATCC 12,424)，保加利亚克鲁维酵母(K.bulgaricus)(ATCC 16045)，威克克鲁维酵母(K.wickeramii)(ATCC 24178)，K waltii(ATCC 56,500)，果蝇克鲁维酵母(K.drosophilarum)(ATCC 36906)，K thermotolerans，和马克斯克鲁维(K.marxianus)；耶氏酵母(yarrowia)(EP 402226)；毕赤酵母(Pichia pastoris)；假丝酵母菌属(Candida)；里氏木霉(Trichoderma reesia)；粗糙脉胞霉(Neurospora crassa)；许旺酵母(Schwanniomyces)如西方许旺酵母(Schwanniomyces occidentalis)；和丝状真菌如，例如脉孢菌(Neurospora)，青霉属(Penicillium)，弯颈霉属(Tolypocladium)和曲霉(Aspergillus)宿主，如构巢曲霉(A.nidulans)和黑曲霉(A.niger)。甲基营养型酵母在这里是合适的，包括但不限于，能够在甲醇生长的酵母，其选自汉逊酵母(Hansenula)，念珠菌(Candida)，Kloeckera，毕赤酵母属(Pichia)，酵母(Saccharomyces)，球拟酵母属(Torulopsis)和红酵母属(Rhodotorula)。酵母(Saccharomyces)是优选的酵母宿主，其带有合适的载体，所述载体具有根据需要的表达控制序列(例如，启动子)、复制起点、终止序列等。典型的启动子包括3-磷酸甘油酸激酶和其他糖酵解酶。诱导型酵母启动子除其他外，包括来自醇脱氢酶、异细胞色素C、负责麦芽糖和半乳糖利用的酶的启动子。

[0239] 除了微生物，哺乳动物组织细胞培养物也可以用于表达和生产如本文所述的多

肽，并且在某些情况下是优选的(参见Winnacker,From Genes to Clones VCH Publishers,N.Y.,N.Y.(1987)。对于一些实施方案，真核细胞可以是优选的，因为在本领域中开发了许多能够分泌异源多肽(例如，完整的免疫球蛋白)的适宜宿主细胞系，包括CHO细胞系、各种Cos细胞系、HeLa细胞、优选，骨髓瘤细胞系、或转化的B细胞或杂交瘤。在一些实施方案中，哺乳动物宿主细胞是CHO细胞。

[0240] 在一些实施方案中，宿主细胞是脊椎动物宿主细胞。有用的哺乳动物宿主细胞系的实例是用SV40转化的猴肾CV1系(COS-7,ATCC CRL 1651)；人胚肾系(293或293细胞亚克隆用于在悬浮培养中生长)；幼仓鼠肾细胞(BHK,ATCC CCL 10)；中国仓鼠卵巢细胞/-DHFR(CHO或CHO-DP-12系)；小鼠睾丸支持细胞；猴肾细胞(CV1 ATCC CCL 70)；非洲绿猴肾细胞(VERO-76,ATCC CRL-1587)；人宫颈癌细胞(HELA,ATCC CCL 2)；犬肾细胞(MDCK,ATCC CCL 34)；布法罗大鼠肝细胞(BRL 3A,ATCC CRL 1442)；人肺细胞(W138,ATCC CCL 75)；人肝细胞病毒(Hep G2,HB 8065)；小鼠乳腺肿瘤(MMT 060562,ATCC CCL51)；TRI细胞；MRC 5细胞；FS4细胞；和人肝细胞瘤系(Hep G2细胞)。

[0241] 制剂及制备制剂的方法

[0242] 本文提供了制剂和制备制剂的方法，所述制剂包含用本文所描述的方法纯化的多肽(例如，抗体)。例如，纯化的多肽可以与药学上可接受的载体组合。

[0243] 为了存储，在一些实施方案中的多肽制剂可通过混合具有期望纯度的多肽和任选的药学上可接受的载体、赋形剂或稳定剂来制备(Remington's Pharmaceutical Sciences，第16版，Osol,A.Ed.(1980))，以冻干制剂或水溶液的形式。

[0244] 本文所用的“载体”包括药学上可接受的载体、赋形剂或稳定剂，其在所采用的剂量和浓度上对暴露于其的细胞或哺乳动物是无毒的。通常生理上可接受的载体是pH缓冲水溶液。

[0245] 可接受的载体、赋形剂、或稳定剂在使用的剂量和浓度下对接受者是无毒的，并且包括缓冲液如磷酸盐，柠檬酸盐和其他有机酸；抗氧化剂包括抗坏血酸和甲硫氨酸；防腐剂(如十八烷基二甲基苯甲基氯化铵；氯化六甲双铵；苯扎氯铵，苄索氯铵；苯酚，丁基或苯甲醇；烷基对羟基苯甲酸酯如甲基或丙基对羟基苯甲酸酯；儿茶酚；间苯二酚；环己醇；3-戊醇；和间甲酚)；低分子量(少于约10个残基)多肽；蛋白质，如血清白蛋白，明胶，或免疫球蛋白；亲水性聚合物如聚乙烯吡咯烷酮；氨基酸如甘氨酸，谷氨酰胺，天冬酰胺，组氨酸，精氨酸或赖氨酸；单糖，二糖和其他碳水化合物，包括葡萄糖，甘露糖，或糊精；螯合剂如EDTA；糖，如蔗糖，甘露醇，海藻糖或山梨醇；形成盐的抗衡离子如钠；金属络合物(例如Zn-蛋白复合物)；和/或非离子表面活性剂例如TWEEN™，PLURONICS™或聚乙二醇(PEG)。

[0246] 在一些实施方案中，多肽制剂中的多肽保持功能活性。

[0247] 用于体内施用的制剂必须是无菌的。这可容易通过无菌滤膜过滤来实现。

[0248] 本文的制剂也可以含有一种以上的对所治疗的具体适应症所必需的活性化合物，优选具有互补活性且不会不利地相互影响的那些。例如，除了多肽，可能期望在一个制剂中包含另外的多肽(例如，抗体)。可替代地，或另外地，组合物还可包含化疗剂、细胞毒剂、细胞因子、生长抑制剂、抗激素试剂、和/或心脏保护剂。此类分子适合以有效用于预期目的的量存在于组合中。

[0249] 本文所述的抗IL-13抗体的示例性制剂在国际专利公开号WO 2013/066866中提

供。

[0250] 产品

[0251] 本文中所描述的方法纯化的多肽和/或包含本文中所描述的方法纯化的多肽的制剂可以包含在产品中。产品可包含含有多肽和/或多肽制剂的容器。在某些实施方案中，所述产品包含：(a)容器，在容器中包含含有本文所述的多肽和/或多肽制剂的组合物；和(b)说明书，具有向受试者施用制剂的说明。

[0252] 产品包含容器和插在容器上或与容器相关联的标签或说明书。合适的容器包括，例如，瓶子、小瓶、注射器等。容器可以由各种材料，如玻璃或塑料制成。容器装有或含有制剂，并且可以具有无菌存取口(例如容器可以是静脉溶液袋或具有可被皮下注射针刺穿的塞子的小瓶)。在组合物中的至少一种活性剂是多肽。标签或说明书指示受试者中组合物的使用，配有关于给药量和提供的多肽和其他药物的间隔的具体指导。产品可进一步包括从商业和用户观点来看需要的其他物质，包括其他缓冲剂、稀释剂、滤器、针头和注射器。在一些实施方案中，容器是注射器。在一些实施例中，注射器还包含在注射装置内。在一些实施方案中，注射装置是自动注射器。

[0253] “说明书”用于指通常包含在治疗产品的商业包装中的说明书，其含有关于适应症、用法、剂量、施用、禁忌症、与包装产物组合的其他治疗产物的信息，和/或关于使用此类治疗产物的警告。

[0254] 含有本文描述的抗IL-13抗体的制剂的示例性的产品提供于国际专利公开WO 2013/066866中。

[0255] 本发明的进一步细节通过以下非限制性实施例说明。在本说明书中所有引用的公开内容通过引用明确地并入本文。

实施例

[0256] 如下面的实施例与本文其他地方使用的，“PLB2”和“PLBL2”和“PLBD2”可互换使用并且是指酶“磷脂酶B样2”或其同义词，“磷脂酶B结构域样2”。

[0257] 实施例1-通用方法

[0258] 除非在实施例另有说明，所有实施例的材料和方法如下所述。

[0259] MAb原料

[0260] 用于所有实施例的MAb原料选自Genentech(South San Francisco,CA,U.S.A.)的工业的、中试规模或小规模细胞培养批次。细胞培养发酵一段时期后，将细胞分离，在某些情况下，通过蛋白A色谱法和一种或多种另外的色谱法步骤和过滤步骤纯化澄清的液体(收获的细胞培养液，HCCF)，如以下的实施例所示。

[0261] MAb定量

[0262] 使用UV可见分光光度计(8453模型G1103A;Agilent Technologies;Santa Clara,CA,U.S.A.)或NanoDrop 1000型ND-1000(Thermo Fisher Scientific;Waltham,MA,USA)在280和320nm的吸光度测定抗体浓度。抗体以外的其他种类(即杂质)浓度太低，对UV吸光度没有明显的作用。根据需要，将样品用合适的非干扰稀释剂稀释在0.1-1.0吸光度单位的范围内。样品制备和UV测量一式两份进行，将其平均值记录下来。mAb吸收系数范围为1.42～1.645/mg·ml·cm。

[0263] 总CHO宿主细胞蛋白(CHOP)定量

[0264] 用ELISA定量称为CHOP的总宿主细胞蛋白的水平。用于检测产物中CHO蛋白的ELISA基于夹心ELISA模式。亲和纯化的CHOP的多克隆抗体包被在96孔微量板上。然后将标准、对照和样品一式两份上样到单独的孔中。如果样品中存在CHOP,其将结合到包被抗体(多克隆抗CHOP)。孵育步骤后,向平板中加入缀合辣根过氧化物酶(HRP)的抗CHOP多克隆抗体。在最后的洗涤步骤后,通过加入四甲基联苯胺(TMB)溶液定量CHOP,该溶液也可作为SUREBLUE RESERVE™,从KPL,Kirkegaard&Perry Laboratories,Inc.,Gaithersburg,MD,商品号53-00-03获得,其中,当HRP酶作用时产生色度信号。测量每个孔中450nm处的光密度(OD)。五参数曲线拟合程序(**SOFTMAX®**Pro,Molecular Devices,Sunnyvale,CA)用于产生标准曲线,从标准曲线计算样品CHOP浓度。总CHOP ELISA的测定范围是从5至320ng/ml。以ng/mL计的CHOP浓度,是指使用CHOP标准作为校准的样品中的CHOP的量。CHOP比(以ng/mg或ppm计)指计算的CHOP浓度与产物浓度的比率,在某些情况下,是该测试方法的报告值。总CHOP ELISA可以用于定量样品中总的CHOP水平,但不定量单个蛋白质的浓度。

[0265] 鼠单克隆抗仓鼠PLBL2 ELISA测定法

[0266] 小鼠抗仓鼠PLBL2单克隆抗体的产生和使用这种抗体的用于检测和定量重组多肽制剂中PLBL2的ELISA测定法的开发描述在美国临时专利申请号61/877,503和61/991,228中。简言之,该测定法如下进行。

[0267] 鼠单克隆抗体19C10以在碳酸盐缓冲液(0.05M碳酸钠,pH9.6)中0.5μg/mL的浓度包被于96孔微量板的半区,在2-8℃过夜。包被后,将板用封闭缓冲液(0.15M NaCl,0.1M磷酸钠,0.1%鱼明胶,0.05%聚山梨酯20,0.05%**Proclin®**300[Sigma-Aldrich];也称为测定稀释液)封闭,以防止蛋白质的非特异性粘附。标准、对照和样品在测定稀释液(0.15M NaCl,0.1M磷酸钠,0.1%鱼明胶,0.05%聚山梨酯20,0.05%**Proclin®**300[Sigma-Aldrich])中稀释,然后一式两份上样到单独的孔中,并在室温(22-27℃)孵育2小时。如果样品中存在PLBL2,PLBL2将结合到包被(在本文中也称捕获)抗体。上述孵育步骤之后,未结合的物质被洗涤缓冲液(0.05%聚山梨酯20/PBS[Corning cellgro目录号99-717-CM])洗掉,缀合生物素的15G11抗PLBL2鼠单克隆抗体在测定稀释液中稀释至浓度0.03125μg/mL,并加入到微量板的孔中。

[0268] 生物素缀合如下进行。生物素化试剂盒从Pierce Thermo Scientific购买,(P/N 20217,E-Z Link NHS-Biotin),以及链霉亲和素HRP(SA-HRP)从Jackson Immuno Cat.No.016-030-084购买。遵循Pierce试剂盒中的说明。简言之,将IgG透析到PBS,pH7.4中,将生物素加入到蛋白质中,在室温下混合1小时。然后将标记的抗体对PBS,pH7.4渗析以除去过量的生物素,过滤,蛋白质浓度由A280测定。

[0269] 与生物素化15G11在室温下孵育2小时的步骤后,链霉亲和素HRP(在测定稀释液中1:200,000稀释)加入到微量板的孔中。用洗涤缓冲液(如上所述)最后洗涤的步骤后,加入TMB溶液(50μl/孔)(SUREBLUE RESERVE™,购自KPL,Kirkegaard&Perry Laboratories,Inc.,Gaithersburg,MD,目录号53-00-03),随后在室温下孵育10-20分钟发色(对于PLBL2定量)。使用Molecular Devices SpectraMax M5e在450nm处评估每孔中的光密度(OD)进行检测。四参数曲线拟合程序(SoftMax Pro v5.2rev C)用于生成标准曲线,从标准曲线的线性范围计算样品的PLBL2浓度。在标准曲线线性范围内的值被用于计算标称PLBL2(ng/mg或

ppm)。线性范围为约EC_{10}-EC_{85}或1.5-40ng/mL，由于板与板间的范围略微变化。使用该ELISA得到的PLBL2值与通过其他方法(例如，LC-MS/MS，多克隆PLBL2ELISA或总CHOP ELISA，稀释到测定的LOQ时)获得的估算是可比的。

[0270] 兔多克隆抗仓鼠PLBL2ELISA测定法

[0271] 兔抗仓鼠PLBL2多克隆抗体的生成和使用这种抗体用于检测和定量重组多肽制剂中PLBL2的ELISA测定法的开发描述在美国临时专利申请号61/877,503和61/991,228中。简言之，该测定如下进行。

[0272] 亲和纯化的兔多克隆抗体以在碳酸盐缓冲液(0.05M碳酸钠，pH9.6)中0.5μg/mL的浓度包被于96孔微量板的半区，在2-8℃过夜。包被后，将板用封闭缓冲液(0.15M NaCl，0.1M磷酸钠，0.1％鱼明胶，0.05％聚山梨酯20，0.05％**Proclin®**300[Sigma-Aldrich])封闭，以防止蛋白质的非特异性粘附。标准、对照和样品在测定稀释液(0.15M NaCl，0.1M磷酸钠，0.1％鱼明胶，0.05％聚山梨酯20，0.05％**Proclin®**300[Sigma-Aldrich])中稀释，然后一式两份上样到单独的孔中，并在室温(22-27℃)孵育2小时。如果样品中存在PLBL2，PLBL2将结合到包被(在本文中也称捕获)抗体上。上述孵育步骤之后，未结合的物质被洗涤缓冲液(0.05％聚山梨酯20/PBS[Corning cellgro目录号99-717-CM])洗掉，缀合辣根过氧化物酶(HRP)的亲和纯化的兔多克隆抗体在测定稀释液中稀释至浓度40ng/mL，并加入到微量板的孔中。

[0273] HRP缀合如下进行。HRP缀合试剂盒从Pierce Thermo Scientific，(P/N 31489，EZ-连接并活化的过氧化物酶和试剂盒(E-Z Link Plus Activated Peroxidase and Kit))购买。遵循Pierce试剂盒的说明书。简言之，将IgG透析入碳酸盐-碳酸氢盐缓冲液，pH9.4，将EZ-连接并活化的过氧化物酶(E-Z Link Plus Activated Peroxidase)加入到蛋白质中，在室温下混合1小时。随后加入氰基硼氢化钠和淬灭缓冲液，以稳定缀合和淬灭反应。然后将标记的抗体对PBS，pH7.4，透析、过滤，蛋白质浓度由A280测定。

[0274] 在与HRP缀合的兔多克隆抗体在室温下孵育2小时的步骤后，用洗涤缓冲液(如上所述)进行最后洗涤步骤。而后，加入TMB溶液(50μl/孔)(SUREBLUE RESERVE™，购自KPL，Kirkegaard&Perry Laboratories，Inc.，Gaithersburg，MD，目录号53-00-03)，随后在室温下孵育10-20分钟发色(对于PLBL2定量)。使用Molecular Devices SpectraMax M5e在450nm处评估每孔中的光密度(OD)进行检测。五参数曲线拟合程序(SoftMax Pro v5.2rev C)用于生成标准曲线，从标准曲线的线性范围计算样品的PLBL2浓度。在标准曲线线性范围内的值被用于计算标称PLBL2(ng/mg或ppm)。测定法的定量范围为0.5-50ng/mL。使用该ELISA得到的PLBL2值与，通过其他方法(例如，鼠单克隆PLBL2ELISA，LC-MS/MS或总CHOP ELISA，稀释到测定的LOQ时)获得的估算是可比的。

[0275] LC-MS/MS测定法

[0276] 对于通过LC-MS/MS的PLBL2定量，使用Waters Acquity H-Class Bio UPLC和AB Sciex TripleTOF 5600+质谱仪。样品和校准标准品(重组PLBL2掺入来自小鼠NS0细胞系的重组人源化单克隆抗体制剂[NS0细胞系不含有仓鼠PLBL2])被胰蛋白酶还原和消化。总共40μg消化的样品注射到UPLC，使用Waters BEH300C18柱，颗粒大小1.7μm。使用线性梯度的乙腈，以流速300μl/min和柱温度60℃洗脱肽。

[0277] 从UPLC洗脱的肽通过电喷射离子化以正电离模式引入到质谱仪。离子源温度设定

在400℃，离子喷射电压5500v，去簇电压(declustering potential)76v。使用设置为32的碰撞能碎片化所选择的肽离子。在多反应监测高分辨率(MRM^{HR})模式操作质谱仪，使用四个特定PLBL2肽和它们的碎片离子转换。由四极质谱仪选择母体离子，质荷比(mass to charge)(M/Z)选择窗口为1.2amu。每个母体离子的碎片离子由飞行时间质谱仪分离并选择用于定量后的数据采集，选择窗口为0.025amu。

[0278] 通过测量四个转换的特定信号响应测定样品中PLBL2的浓度，使用线性拟合从来自2-500ppm范围内的标准物的那些校准。下面的表2示出了通过LC-MS/MS监测的PLBL2肽的列表。

[0279] 表2.通过LC-MS/MS监测的PLBL2肽的列表。

[0280]

三 TOF 5600+ 扫描循环					
扫描#	扫描类型	肽	目的碎片离子	母体 m/z	碎片 m/z
1	TOF MS	N/A	N/A	N/A	N/A
2	产物离子	SVLLDAASGQLR (SEQ ID NO: 31)	+2y8	615.3461	817.4163
3	产物离子	GLEDSYEGR (SEQ ID NO: 32)	+2y7	513.2304	855.3479
4	产物离子	AFIPNGPSPGSR (SEQ ID NO: 33)	+2y9	600.3120	868.4272
5	产物离子	VTSFSLAK (SEQ ID NO: 34)	+2y6	426.7449	652.3665

[0281] 实施例2-为降低仓鼠PLBL2改进的纯化方法

[0282] 建立了CHO生产的抗IL-13MAb(lebrikizumab)的纯化方法，以支持早期阶段的临床试验，在此被称为“初始方法(Initial Process)”。初始方法依次采用以下的色谱法步骤：蛋白A亲和色谱法(MABSELECT SURE^{TM})、而后是阳离子交换(**POROS®**HS)、而后是阴离子交换(Q SEPHAROSE^{TM} Fast Flow)。包括额外的病毒灭活和过滤步骤，以及最终的超滤渗滤(UFDF)步骤。最终产物(药品)配制在20mM组氨酸醋酸盐、6％蔗糖、0.03％聚山梨醇酯20，pH值5.7中，浓度125mg/mL。

[0283] 使用总CHOP ELISA测定法(以上实施例1中描述)，我们发现，根据初始方法纯化的过程中的中间体和药品证实非典型稀释依赖行为，导致标准化系列样品稀释间变异系数＞20％。该稀释依赖行为由表3中呈现的数据示例，其中抗IL-13MAb产物的每一个连续两倍的稀释导致更高水平的CHOP(以ppm表示)，如使用总CHOP ELISA所确定。使用灵敏的分析方法，如LC-MS/MS，我们确定了单一CHOP种类或HCP，是这一非典型稀释依赖行为的原因。特别是，我们确立了在总CHOP ELISA上稀释依赖行为是由于抗原过量。进一步的调查使我们能够确定单一HCP为酶，仓鼠磷脂酶B样2(PLBL2)。通过将产物样品稀释至定量测定法的极限(LOQ)，我们能够估计使用初始方法纯化的lebrikizumab的临床批次中PLBL2的水平，确定存在高达300ppm(300ng/mg)以及以上的水平。

[0284] 表3.产物稀释和CHOP水平。

[0285]

稀释倍数	总CHOP(ppm)
2	0.58

4	1
8	2
16	4
32	7
64	14
128	26
256	49
512	97
1024	147
2048	228
4096	314
8192	346

[0286] 如我们观察到的单一CHOP种类的此杂质水平(＞300ppm)，被认为是用于人临床和/或治疗应用、特别是后期临床试验以及以后的MAb产物中所不希望的。例如，如实施例3中所述，当施用给人受试者时这样的水平可以是免疫原性的。

[0287] 因此，我们研究了各种对初始方法的改进，如以下简要概述。根据这些研究的结果，我们开发了一种在下面详细描述的改进的纯化方法，并且在此将其称为"改进方法"。改进方法的使用导致纯化的抗IL-13MAb(lebrikizumab)产物含有实质上降低的PLBL2水平。

[0288] 改进纯化方法以降低PLBL2的努力包括与初始方法正交的方法，包括：沉淀、测试各种HCCF的添加剂、额外的柱洗涤、疏水相互作用和混合模式色谱法。通过使用实施例1中描述的测定法中的一种或多种进行这些努力，来监测研究的每种改进对降低总CHOP和/或PLBL2水平的有效性。探讨的各种改进如下所述。

[0289] 使用辛酸沉淀HCCF和蛋白A池中的CHOP

[0290] 以前已经描述了辛酸沉淀，包括用于单克隆抗体工业中(Wang等人，BioPharm International；Downstream Processing 2010，p4-10，Oct2009；Brodsky等人，Biotechnology and Bioengineering，109(10)：2589，2012)以选择性地从目的靶蛋白质中沉淀杂质。辛酸，也称为正辛酸，是具有八个碳原子的饱和脂肪酸(式$CH_3(CH_2)_6COOH$)。使用抗IL-13MAb进行研究以确定收获的细胞培养液(HCCF)或蛋白A池的辛酸沉淀是否会导致CHOP降低和/或在总CHOP ELISA中稀释依赖行为的降低。

[0291] 用于这些研究的抗IL13MAb起始材料是从1KL收获的HCCF和蛋白A池。1％(v/v)辛酸加入到HCCF中，不同浓度的辛酸(0％-3％v/v)加入到pH 4.5或pH 5.0的蛋白A池。在环境温度下混合样品，持续≥5小时，0.2μm过滤，并用总CHOP ELISA稀释剂稀释，用于使用总CHOP ELISA的检测和定量。根据本领域中已知的标准方法进行HPLC滴度测定法，用于测定辛酸处理之前和之后的HCCF中抗IL-13MAb的滴度。

[0292] 用1％v/v辛酸处理HCCF降低CHOP约5倍，导致91％的抗IL-13MAb的产率。当用不同浓度的辛酸(范围从0-3％v/v)处理蛋白A池时，我们观察到在pH5.0产率损失>20％，在pH4.5产率没有损失。当我们评估这些辛酸处理的蛋白A池中总CHOP时，我们观察到CHOP降低2倍至3倍(图1A和B)。然而，也如图1A和B所示，在各检测条件下稀释依赖性仍存在，表明辛酸沉淀不能有效地解决总CHOP ELISA中观察到的稀释依赖行为，因此不能有效地降低该

产物中PLBL2的水平。

[0293] HCCF的添加剂

[0294] Sisodiya等人,Biotech J.7:1233(2012)先前的工作证明,向HCCF添加的添加剂如胍或氯化钠可以降低随后纯化的蛋白A池中的CHOP。也表明,当作为蛋白A柱上的洗涤剂使用时,精氨酸降低CHOP(Millipore Technical Bulletin,Lit.No.TB1024EN00,Rev.A,December,2005;Millipore Technical Bulletin,Lit.No.1026EN00,July,2006,可以从网址www.Millipore.com获得),我们将精氨酸作为HCCF的添加剂包括在内。各种盐、离液剂、及辛酸加入到抗IL-13MAb HCCF中,以评估在MABSELECT SURE™(MSS)蛋白A色谱法上捕获产物期间,其每个对降低产物和CHOP相互作用的效力。测试的向HCCF添加的添加剂为:0.6M胍、0.6M精氨酸、0.6M氯化钠、磷酸盐缓冲盐水、和1%辛酸。

[0295] 将用各种HCCF添加剂处理的样品加载至MSS上的蛋白A色谱法上。调节蛋白A池至pH4.9,并使用初始方法条件在**POROS®**HS阳离子交换色谱法步骤上进一步纯化。稀释蛋白A池和**POROS®**HS池,并进行总Chop ELISA。根据本领域中已知的方法在SEC-HPLC上测试调整的蛋白A池,用于评估%聚集体,%变异种类等。

[0296] 对于其中将胍或精氨酸加入HCCF的运行,MABSELECT SURE™上的产率稍低。在测试的所有HCCF的添加剂中,胍和精氨酸最有效地实质上降低CHOP水平(见表4)并且呈现降低蛋白A池上的稀释依赖性(数据未显示)。但是,**POROS®**HS上蛋白A池的进一步的下游处理显示,CHOP ELISA稀释依赖性残留在相应的**POROS®**池中,如图2所示。因此,数据表明,向HCCF加入胍或精氨酸对降低该产物的PLBL2水平无效。

[0297] 表4.HCCF添加剂和对CHOP的效果。

[0298]

添加剂	上样 pH	产率 (%)	总 CHOP (ppm)
对照 (无添加剂)	7.4	101	3417
0.6M 胍	7.6	90	892
0.6M 精氨酸	7.1	88	1237
0.6M NaCl	7.7	99	2619
PBS	7.4	98	2773
1% 辛酸	6	93	3173

[0299] 蛋白A柱的洗涤(MABSELECT SURE™)

[0300] 观察到,在MABSELECT SURE™(MSS)蛋白A色谱法上更多的稀释依赖性CHOP洗脱在早期含有产物的级分中。这表明,在洗脱前MSS上额外的洗涤步骤可能进一步降低CHOP/PLBL2。测试了MSS上一些洗涤降低蛋白A池中CHOP/PLBL2的能力。对于这项研究,使用纯化的抗IL-13MAb UFDF池作为上样物质。稀释该UFDF池至1.7mg/mL(约HCCF滴度),并以29克/L树脂上样到MSS。测试多种洗涤,例如,0.5M精氨酸pH 8.5,有和没有1%聚山梨醇酯20的0.5M精氨酸pH9.5,0.5M TMAC pH7.1,25mM MOPS pH7.1,并与高盐洗涤pH7.0相比较。产物

在酸性条件下(pH2.8)洗脱并汇集,在0.5OD(A 280)开始并持续至2.4个柱体积的总体积。稀释各调节池,并用总CHOP ELISA测定。这些结果的总结是,没有任何洗涤充分降低CHOP/PLBL2或降低总CHOP ELISA中的稀释依赖性。因此,看起来我们不会发现有效降低该抗IL-13MAb产物中PLBL2水平的蛋白A的洗涤条件,因而我们没有进一步调查。

[0301] 阳离子交换柱的洗涤(**POROS®**HS)

[0302] 根据使用抗IL-13MAb和PLBL2杂质氨基酸序列的理论计算,我们估计PLBL2的pI约为6.0,与抗IL-13MAb(pI6.1)的类似。我们还估计,在≤pH4和≥pH10时,抗IL13MAb和PLBL2之间的净电荷将有显著差异。因此,我们测试了初始方法**POROS®**HS阳离子交换步骤上的各种低pH洗涤,以评估其是否会有效地、选择性地降低总CHOP和/或PLBL2和稀释依赖行为。在pH4测试了以下洗涤液:(i)乙酸盐梯度,300mM-1000mM超过20个柱体积(CV);(ii)柠檬酸梯度,100mM-500mM超过20CV;(iii)在260mM柠檬酸盐洗涤步骤;及(iv)精氨酸梯度15mS/cm(导电性测量)超过20CV。

[0303] 结果表明,在pH4乙酸盐梯度高达测试盐浓度1M时,不能洗脱抗IL-13MAb与CHOP。柠檬酸盐或乙酸盐增加的量导致产物的不溶性和沉淀。所有的pH4的洗涤条件导致**POROS®**HS步骤上的低产率,没有洗涤条件显著降低CHOP稀释依赖性。因此,在阳离子交换柱中加入低pH洗涤不能有效地降低该产物中PLBL2的水平。

[0304] 羟磷灰石树脂和CAPTO™Adhere树脂

[0305] 陶瓷羟基磷灰石(CHT)大孔树脂I型,40um(BioRad)由重复的六边形结构的磷酸钙($Ca_5(PO_4)_3OH)_2$组成。有两个不同的结合位点;C位点具有5个钙离子双连体的组,P位点含有一对-OH,各-OH含有三个磷酸根(phosphate triplets)。这种树脂有混合模式特性,并已被证明分离具有挑战性的杂质如聚集体(P.Gagnon,New Biotechnology 25(5):287(2009))。

[0306] 为了鉴定运行CHT柱的初始条件,我们进行CHT树脂I型,40um的高通量机器人筛选,测试的pH范围6.5-8.0,以及不同的氯化钠和磷酸钠浓度用于洗脱。这种高通量机器人筛选先前已被描述,例如,在Wensel等人,Biotechnol.Bioeng.100:839(2008)中。在总CHOP ELISA中测试了来自这些筛选的样品。

[0307] CAPTO™Adhere(GE Healthcare)是即呈现离子特性又呈现疏水性特性的混合模式树脂。基本矩阵是刚性的琼脂糖,配体是N-苄基-N-甲基乙醇胺。首先用高通量筛选研究、然后用随后的柱条件评估了这种树脂降低总CHOP和/或PLBL2的能力。

[0308] 使用与以上描述类似的高通量机器人筛选方法,进行确定运行CAPTO™Adhere柱条件的初始研究,以测试在两个上样密度(5克/L树脂和为40g/L树脂)下抗IL-13MAb与CAPTO™Adhere的结合。还测试了盐和pH的范围;从25mM-200mM乙酸钠,pH4.0-6.5。上样物质是初始方法UFDF池,在总CHOP ELISA的LOQ上,其含有约200ppm的总CHOP。稀释CAPTO™Adhere上未结合(流出)的样品,并使用总CHOP ELISA测定。

[0309] 结果如下。对于CHT色谱法,没有测试条件实质上降低总CHOP或PLBL2或影响测定的稀释依赖行为。另外,收益率差,并且没有实现高分子量种类的清除。对于CAPTO™Adhere色谱法,收益率差,测定物质显示,在总CHOP ELISA中实质上的稀释依赖行为。因此,没有进一步开发CHT和CAPTO™Adhere树脂的使用,因为很明显,我们将不可能发现使用这些树脂有效降低该抗IL-13MAb产物中PLBL2水平的条件。

[0310] 疏水相互作用色谱法树脂和膜

[0311] 我们最初测试的HIC膜吸附称为Sartobind，由Sartorius制造。Sartobind由再生纤维素的基础基质与共价连接的疏水苯基配体基团组成。

[0312] 测试的膜是Sartobind HIC 3mL装置(8mm床高度)。我们将来自初始方法**POROS®**HS池的池调整到0.55M磷酸钾pH7.0，并使用10mL/min的流速。在0.55M磷酸钾pH7.0中洗脱产物(在未结合级分中收集3mL级分)。

[0313] 我们观察到当条件设定到0.55M磷酸钾时抗IL-13MAb变得模糊和浑浊，需要额外的0.2um过滤步骤。结果表明：总CHOP降低，然而，剩余的CHOP在总CHOP ELISA中仍表现出稀释依赖行为。没有进一步评估这种膜的用途，因为似乎不可能确定降低该产物中PLBL2水平的有效条件。

[0314] 接下来，我们采用高通量筛选评估几种不同的HIC树脂。**OCTYL-SEPHAROSE®** Fast Flow(FF)、**BUTYL-SEPHAROSE®**4Fast Flow、PHENYL SEPHAROSE™6Fast Flow(high sub)和PHENYL SEPHAROSE™6Fast Flow(low sub)从GE Healthcare获得。选择这四个树脂，是因为它们代表了广泛变化的疏水性(**OCTYL-SEPHAROSE®**Fast Flow疏水性最低，其次为PHENYL SEPHAROSE™6Fast Flow(low sub)和**BUTYL-SEPHAROSE®**4Fast Flow，PHENYL SEPHAROSE™6Fast Flow(high sub)疏水性最高。我们测试了几个pH和盐浓度的组合的降低抗IL-13MAb制剂中PLBL2的有效性。用于HIC树脂实验的抗IL-13MAb制剂来自使用初始方法运行的UFDF池。抗IL-13MAb浓度为180mg/mL和上样密度为40mg抗体/mL树脂。我们测试了pH5.5(25mM乙酸钠)、pH6.0(25mM MES)、pH 7.0(25mM MOPS)和pH8.0(25mM Tris)和0mM至400mM之间浓度的硫酸钠。对于测试的每个条件，收集、稀释流出样品，并使用总CHOP ELISA测定法进行测试。

[0315] 结果示于图3A-D。随着盐增多，我们观察到各树脂流出液中的总CHOP越少。**OCTYL-SEPHAROSE®**Fast Flow树脂(图3A)显示总CHOP的水平最高，而PHENYL SEPHAROSE™6Fast Flow(high sub)树脂将总CHOP降低到非常低的水平，即使使用较低量的盐(图3D)，PHENYL SEPHAROSE™6Fast Flow(low sub)和**BUTYL-SEPHAROSE®** Fast Flow树脂显示中等水平的总CHOP。有趣的是，pH对使用各树脂去除CHOP也具有最小的影响，除低盐条件的PHENYL SEPHAROSE™6Fast Flow(high sub)以外(图3D)。对于这种树脂，低盐条件、较高的pH导致流出级分中更高的CHOP(图3D)。基于这些结果，PHENYL SEPHAROSE™6Fast Flow(high sub)看起来是有前景的，选择其用于进一步的研究，包括在结合-洗脱或流通模式运行柱。

[0316] 使用PHENYL SEPHAROSE™6Fast Flow(high sub)树脂在结合-洗脱模式操作HIC需要用盐调整抗IL-13MAb上样，使得抗体与树脂结合。增加盐就增加了上样产物与树脂的动态结合能力(每mL树脂抗IL-13的mg)。但随着产物中盐浓度增加，我们观察到增加的浑浊度和高分子量种类(HMW)的形成，特别是组合较低的pH时。

[0317] 如上所述，PHENYL SEPHAROSE™6Fast Flow(high sub)也可以在流出模式操作，该操作需要上样中较少的盐条件。从产物质量和产物稳定性的观点考虑，例如，具有较少混浊度、较少HMW的产物，需要较少的盐条件。因此，我们继续进行流出模式下使用PHENYL SEPHAROSE™6Fast Flow(high sub)树脂的流程优化。

[0318] 为了优化流程，我们调查了许多用于运行HIC柱的参数，包括上样浓度、上样pH、上样盐摩尔浓度、树脂上的上样密度、床高度、流速、温度、平衡缓冲液pH和摩尔浓度。对于这

些实验，我们使用总CHOP ELISA监测总CHOP，并还通过LC-MS/MS监测PLBL2。某些示例性数据示于表5。表5中的数据表明，在流出模式的指定条件下运行HIC柱有效地从蛋白A池检测到的高水平实质上地降低了PLBL2水平。与蛋白A池的水平相比，HIC后PLBL2水平降低几百倍。

[0319] 表5.在不同HIC柱条件下总CHOP和PLBL2水平。

[0320]

样品 (床高度，流速)	% 产率	总 CHOP (ppm 由ELISA在LOQ测定)	PLBL2 (ppm 由LC-MS/MS测定)
蛋白A池(HIC柱的上样)		3324	957
15 cm, 150 cm/hr	88	43	4
25 cm, 150 cm/hr	92	44	2
15 cm, 100 cm/hr	90	67	5
25 cm, 100 cm/hr	92	63	3
15 cm, 200 cm/hr	93	62	6
25 cm, 200 cm/hr	90	72	4
15 cm, 150 cm/hr	54	76	2

[0321] 使用PLBL2LC-MS/MS测定法和其他典型的产物质量测定法(如，SE-HPLC、CE-SDS、iCIEF)指导流程参数选择，我们确定下列条件作为运行HIC柱的期望条件，如产物质量特性和PLBL2的降低所评估的：平衡和洗涤缓冲液：50mM乙酸钠，pH 5.0；目标上样密度：100g/L，流速：150cm/hr，22℃±3℃。这些条件的某些小的变化也可能是希望的，例如，25℃±3℃或27℃±3℃。光密度(OD)用在280nm的吸光度(A280)监测，在0.5OD至1.5OD之间或在洗涤8个柱体积后收集池(即，流出液)。

[0322] 如上所提及，初始方法是：蛋白A亲和色谱法(MABSELECT SURE™)，随后是阳离子交换(**POROS®**HS)，随后是阴离子交换(Q SEPHAROSE™Fast Flow)。在如上所述开发降低PLBL2水平的方法后，我们接下来试图以方便的方式实现方法的变化。因此，我们研究向初始方法增加HIC柱，从而产生一个四柱方法，以及用HIC柱替代CEX柱或AEX柱，最后我们探索了柱的顺序。我们发现三柱方法，蛋白A亲和色谱法(MABSELECT SURE™)，随后是阴离子交换(Q SEPHAROSE™Fast Flow)，随后是在流出模式操作HIC(PHENYL SEPHAROSE™6Fast Flow (high sub))提供了最方便的方法，其最有效降低最终药品中的PLBL2。此三柱方法将在下面详细描述。

[0323] 第一亲和色谱法步骤是使用MABSELECT SURE™树脂的结合-和-洗脱过程。柱平衡(25mM氯化钠，25mM Tris pH7.7)后，将HCCF上样到柱上，并用平衡缓冲液和高盐pH7.0的洗涤缓冲液洗涤。抗IL-13MAb在酸性条件下(pH2.8)下从柱洗脱。

[0324] 第二阴离子交换色谱法步骤使用Q SEPHAROSE™Fast Flow(QSFF)树脂在结合-洗脱模式运行。柱平衡(50mM Tris,pH8.0)后,调整来自MABSELECT SURE™柱的抗IL13池至pH8.05,并上样到柱上。洗涤该柱(50mM Tris,pH8.0),用85mM氯化钠、50mM Tris pH8.0从柱洗脱抗IL-13MAb。

[0325] 第三和最后的疏水相互作用色谱法步骤使用PHENYL SEPHAROSE™6Fast Flow (High Sub)树脂在流出模式下操作。柱平衡(50mM乙酸钠pH5.0)后,调整来自QSFF柱的抗IL13池至pH5.0并上样到柱上。该抗IL-13MAb流出,并用平衡缓冲液(50mM乙酸钠pH5.0)洗涤柱。基于A280起始和终止该抗IL-13MAb池,在0.5至1.50D之间进行汇集或汇集最多8个柱体积。

[0326] 如同初始方法,包括其他病毒灭活和过滤步骤,以及最终的超滤渗滤(UFDF)步骤。最终产物(药品)配制在20mM组氨酸醋酸盐,6%蔗糖,0.03%聚山梨醇酯20,pH5.7中,浓度125mg/mL。

[0327] 如分别由总CHOP ELISA和单克隆PLBL2ELISA所测量的,初始方法与改进方法在总CHOP和PLBL2方面的比较,提供在表6(初始方法)和7(改进方法)中。表6中的数据清楚地表明,初始方法导致纯化的产物(UFDF池)含有高水平的总CHOP(在三个不同的运行中179,310和189ng/mg)和高水平的PLBL2(在三个不同的运行中242,328,和273ng/mg),而表7中的数据清楚地表明,改进方法相当有效地生产具有实质上降低水平的总CHOP(在四个不同的运行中1.1,<0.9,2.8和3.4ng/mg)和实质上降低水平的PLBL2(在四个不同的运行中0.21,0.42,0.35,和0.24ng/mg)的纯化产物。与上面给出的数据一致,表7中的数据表明,在上述条件下运行HIC柱特别有效地降低抗IL-13MAb制剂中的总CHOP和PLBL2水平。

[0328] 表6.使用初始方法在纯化抗IL-13MAb的各个阶段中总CHOP和PLBL2水平。

[0329]

方法过程中的样品	总 CHOP (ng/mg 由ELISA在LOQ测定)			PLBL2 (ng/mg 由ELISA测定)		
运行号	1	2	3	1	2	3
HCCF	620920	541072	608789	1895	3669	2535
ProA 池	2892	2855	3505	587	769	478
CEX 池	136	310	138	345	439	287
AEX 池	104	163	93	291	304	261
UFDF 池	179	310	189	242	328	273

[0330] 表7使用改进方法在纯化抗IL-13MAb的各个阶段中总CHOP和PLBL2水平。

[0331]

方法过程中的样品	总 CHOP (ng/mg 由ELISA在LOQ 测定)				PLBL2 (ng/mg 由ELISA测定)			
运行号	1	2	3	4	1	2	3	4
HCCF	332132	399157	540134	644549	4084	3770	3077	2986
ProA 池	2318	2768	3552	3797	1354	1995	1027	975
AEX 池	495	653	414	377	723	933	677	616
HIC 池	< 2.1	< 1.9	5.0	7.7	< 0.6	< 0.6	< 0.6	< 0.6
UFDF 池	1.1	< 0.9	2.8	3.4	0.21	0.42	0.35	0.24

[0332] 总之,面对纯化的抗IL-13MAb制剂中单一CHOP种类的高水平导致的测定法非线性稀释行为的问题,我们首先确定了CHOP种类为仓鼠PLBL2,其是先前未被描述的由CHO细胞

生产的重组蛋白制剂中的杂质。接下来我们确定了有效地降低抗IL-13MAb制剂中PLBL2水平的纯化条件。最后，我们综合这些纯化条件形成导致现有抗IL-13MAb纯化方法的改进的整体纯化方法。这个改进的方法采用在流出模式运行的HIC柱，以降低PLBL2水平，其组合亲和色谱法步骤和阴离子交换色谱法步骤运行。我们表明，改进的方法稳健和有效地实质上降低抗IL-13MAb制剂中仓鼠PLBL2水平。我们表明，改进的方法与初始方法相比，可重复地降低PLBL2水平约1000倍。PLBL2水平的这种降低对于生产适用于晚期临床试验及以后的患者治疗用途的纯化抗IL13MAb产物是重要的。

[0333] 降低抗Aβ抗体制剂中仓鼠PLBL2的纯化方法

[0334] 接下来，我们设法评估上述纯化方法，特别是使用HIC柱用于最终色谱法步骤，是否同样有效地用于降低其他抗体制剂中的PLBL2水平。对于该实验，我们选择了抗Aβ抗体，其是在CHO细胞中产生。示例性抗Aβ抗体和生产这类抗体的方法先前已被描述，例如，在WO2008011348、WO2007068429、WO2001062801和WO2004071408中。这些特定的实验中使用被称为crenezumab的抗Aβ抗体。如对抗IL-13MAb所述，我们探索了用于蛋白A亲和柱之后的第二柱的各种树脂和缓冲液，以及我们探索了用于HIC柱的各种缓冲液和运行条件，以确定对抗Aβ的产物质量和稳定性特性以及对于去除仓鼠PLBL2最佳的那些条件。

[0335] 我们发现，三柱方法，蛋白A亲和色谱法(MABSELECT SURE™)、接着使用混合模式树脂(CAPTO™Adhere)、接着是流出模式下操作HIC(PHENYL SEPHAROSE™6Fast Flow(high sub))方便和有效地降低最终药品中的PLBL2。此三柱方法将在下面详细描述。

[0336] 第一亲和色谱法步骤是使用类似于以上描述的用于抗IL-13 MAb的MABSELECT SURE™树脂的结合-洗脱方法。

[0337] 第二混合模式色谱法步骤使用CAPTO™Adhere树脂在流出模式操作。柱平衡(20mM MES，150mM乙酸钠，pH6.25)之后，调节来自MABSELECT SURE™柱的抗Aβ池的pH值至6.25并上样到柱上。在上样期间汇集开始在0.5OD。完成上样后，用5个柱体积(CV)的平衡缓冲液(20mM MES，150mM乙酸钠，pH6.25)洗涤柱并且还收集整个5个CV。

[0338] 第三和最后的疏水相互作用色谱法步骤使用PHENYL SEPHAROSE™6Fast Flow(High Sub)树脂在流出模式操作。柱平衡(150mM乙酸钠pH5.0)之后，调节来自CAPTO™Adhere柱的抗Aβ池的pH值至5.0并上样到柱上。抗AβMAb流出，并且还用平衡缓冲液(150mM乙酸钠pH5.0)洗涤柱。在上样期间开始抗Aβ MAb池，基于A280汇集开始于0.5OD。用10CV的平衡缓冲液(150mM乙酸钠pH5.0)洗涤柱，并且还收集了整个10CV。如抗IL-13MAb，还包括了另外的病毒灭活和过滤步骤，以及最终的超滤渗滤(UFDF)步骤。

[0339] 在四种不同的纯化运行中使用上述方法的结果示于下表8。

[0340] 表8.在使用HIC纯化抗AβMAb的各个阶段的PLBL2水平。

[0341]

方法过程中的样品	PLBL2 (ng/mg 由ELISA)			
运行编号	1	2	3	4
HCCF	622	564	1264	553
CpA 池	7	8	9	2.5
HIC 池 (300 g/L 上样密度)	0.7	0.6	0.3	0.3
HIC 池 (100 g/L 上样密度)	< 0.2	< 0.2	< 0.2	未检测

[0342] 在表8中所示的结果表明，使用HIC树脂作为最终色谱法步骤有效地将抗Aβ MAb制剂中残留的PLBL2水平降低至类似于抗IL-13 MAb中看到的量。尽管从产物回收和PLBL2降低的观点看，上样密度300g/L产生了所希望的结果，然而将HIC柱的上样密度从300g/L减少至100g/L观察到残留PLBL2进一步减少。

[0343] 我们还研究了HIC色谱法步骤的其他两个条件，上样pH值和上样硫酸盐摩尔浓度。对于这些实验，我们用含有51ng/mg PLBL2(如通过ELISA测量的)、15mM乙酸钠pH5.5的CAPTO™Adhere池开始。我们使用不同pH值的0mM硫酸钠或800mM硫酸钠储备溶液，调整上样pH和上样硫酸盐摩尔浓度至下表9所示的值。我们测试了在低硫酸盐摩尔浓度的条件下(0mM)和高硫酸盐摩尔浓度的条件下(240mM)下表9中所示的各上样pH值。在上样密度60g/L下测试各条件。如表9呈现的结果所示，降低上样pH至pH4或pH5或增加上样硫酸盐的摩尔浓度(至240mM硫酸盐)分别有效地降低在最终HIC池中的PLBL2水平。上样中pH4.0和240mM硫酸盐的组合对最小化HIC池中残留的PLBL2量是特别有效的。

[0344] 表9.在一定范围的上样pH和硫酸盐摩尔浓度下观察的HIC池中PLBL2水平。

[0345]

上样 pH	PLBL2 (ng/mg 由 ELISA)	
	低硫酸盐摩尔浓度 (0 mM)	高硫酸盐摩尔浓度 (240 mM)
4	4	1
5	10	3
6	27	5
7	64	6

[0346] 因此，使用HIC树脂作为纯化CHO产生的多肽(如本文所述的抗IL-13MAb和抗Aβ MAb)的最终色谱法步骤，有效地降低仓鼠PLBL2的残留量至很低的水平，例如在HIC池中1ng/mg或以下。

[0347] 降低IgG1抗体制剂中仓鼠PLBL2的纯化方法

[0348] 接下来，我们评估了描述的用于纯化抗IL-13和抗Aβ IgG4抗体制剂的方法(特别是使用HIC柱用于最终色谱法步骤)是否同样有效地降低IgG1抗体制剂中的PLBL2水平。对于这些实验，我们首先选择抗IL17A/F抗体，其是IgG1抗体且在CHO细胞中产生。示例性的抗IL17A/F抗体和产生这类抗体的方法先前已被描述，例如WO 2009136286和美国专利号8,715,669。如对抗IL-13和抗Aβ MAb所描述的，我们探索了各种树脂(具体地，PHENYL SEPHAROSE™ FF[low sub]和PHENYL SEPHAROSE™FF[high sub]和缓冲液条件(具体地，50mM乙酸钠，pH5.5和50mM Tris，85mM乙酸钠，pH8.0)用于HIC柱，以确定那些用于抗IL17A/F的产物质量和稳定性特性以及用于去除仓鼠PLBL2的最佳条件。

[0349] 我们发现，三柱方法，蛋白A亲和色谱法(MABSELECT SURE™)、接着使用结合-洗脱模式下操作的阳离子交换色谱法(**POROS®** 50HS)、和流出模式下操作的HIC(PHENYL SEPHAROSE™6Fast Flow(high sub))方便和有效地降低最终药品中的PLBL2。此三柱方法将在下面详细描述。

[0350] 第一亲和色谱法步骤是类似于以上描述的用于抗IL13和抗AβMAb的使用MABSELECT SURE™树脂的结合-洗脱方法。第二阳离子交换色谱法步骤使用**POROS®** 50HS树脂，在结合-洗脱模式下操作。柱平衡(40mM乙酸钠，pH5.5)后，将调过pH的抗IL17A/F MABSELECT SURE™池(pH为5.0)上样到柱上。洗涤柱(40mM乙酸钠，pH5.5)，然后用40和400mM乙酸钠，pH5.5创建的导电性梯度将抗IL17A/F抗体从柱上洗脱。在梯度洗脱期间基于A280汇集，≥0.50D开始以及≤2.00D结束。

[0351] 第三和最后的疏水相互作用色谱法步骤使用PHENYL SEPHAROSE™6Fast Flow (High Sub)树脂在流出模式操作。柱平衡(50mM乙酸钠pH5.5)之后，来自**POROS®** 50HS柱的抗IL17 A/F池直接上样到柱上，不调节pH。抗IL17 A/F MAb流出。在上样期间基于A280汇集抗IL17 A/F MAb，开始于≥0.50D。用10CV的平衡缓冲液(50mM乙酸钠，pH5.5)洗涤柱，在洗涤期间在≤1.00D结束汇集。

[0352] 在一个纯化运行期间使用上述方法的结果示于下表10。

[0353] 表10使用HIC纯化抗IL17 A/F MAb的各个阶段的PLBL2水平。

[0354]

方法过程中的样品	**PLBL2 (ng/mg由ELISA)**
运行编号	**1**
HCCF	**713**
MABSELECT SURE™ 池	**151**
POROS® 50HS 池	**47**
HIC 池 (100 g/L 上样密度)	**<0.7**

[0355] 表10所示的结果表明，使用HIC树脂作为最终色谱法步骤有效地降低抗IL17A/F MAb(IgG1)制剂中残留的PLBL2水平，降低的量与抗IL13和抗Aβ MAb(IgG4)观察到的类似。

[0356] 抗CMV抗体

[0357] 除了测试抗IL17A/F，我们测试了另一个IgG1MAb，抗CMV-MSL抗体，其也在CHO细胞

中产生。示例性抗CMV抗体，包括抗CMV-MSL，以及生产这种抗体的方法之前已被描述，例如WO 2012047732。

[0358] 再次，我们发现，三柱方法，蛋白A亲和色谱法(MABSELECT SURE™)，接着是在结合-洗脱模式操作的阳离子交换色谱法(**POROS®**50HS)，和流出模式下操作的HIC(PHENYL SEPHAROSE™6Fast Flow(high sub))方便和有效地降低最终药品中的PLBL2。此三柱方法在下面详细描述。

[0359] 第一亲和色谱法步骤是使用类似于上述的用于抗IL-13、抗Aβ和抗IL17A/F MAb的MABSELECT SURE™树脂的结合-洗脱方法。第二阳离子交换色谱法步骤中使用**POROS®** 50HS树脂并在结合-洗脱模式下操作。柱平衡(40mM乙酸钠，pH5.5)后，将pH调整过的CMV-MSL MABSELECT SURE™池(pH5.0)上样到柱上。洗涤该柱(40mM乙酸钠，pH5.5)，然后用40和400mM乙酸钠，pH5.5创建的导电性梯度从柱上洗脱CMV-MSL抗体。在梯度洗脱期间基于A280汇集，在≥0.50D开始并在≤1.00D结束。

[0360] 在此具体的运行中，在阳离子交换和疏水相互作用色谱法步骤之间使用Viresolve Pro作为病毒过滤和Fluorodyne UEDF过滤器作为预过滤进行在病毒过滤步骤。

[0361] 第三和最后的疏水相互作用色谱法步骤使用PHENYL SEPHAROSE™6Fast Flow (High Sub)树脂在流出模式操作。柱平衡(50mM乙酸钠pH5.5)之后，来自**POROS®** 50HS柱的抗CMV-MSL池直接上样到柱上，不调节pH。抗CMV-MSL MAb流出。在上样期间基于A280汇集抗CMV-MSL MAb，开始于≥0.50D。用10CV的平衡缓冲液(50mM乙酸钠，pH5.5)洗涤柱，在洗涤期间在≤0.50D结束汇集。

[0362] 在一个纯化运行期间使用上述方法的结果示于下表11。

[0363] 表11.使用HIC纯化抗CMV-MSL MAb的各个阶段中PLBL2水平。

[0364]

方法过程中的样品	**PLBL2 (ng/mg由ELISA)**
运行编号	**1**
HCCF	**2608**
MABSELECT SURE™ 池	**319**
POROS® 50HS 池	**33**
Viresolve Pro 池	**32**
HIC 池 (60 g/L 上样密度)	**<0.6**

[0365] 表11所示的结果表明，使用HIC树脂作为最终色谱法步骤有效地降低抗CMV-MSL MAb制剂中残留的PLBL2水平，降低量类似于在抗IL-13、抗Aβ和抗IL17A/F MAb中看到的。因此，在纯化CHO-产生的多肽(如抗IL-13MAb和本文描述的其他MAb)中使用HIC树脂作为最终色谱法步骤，有效地将仓鼠PLBL2的残留量降低至非常低的水平，例如，在HIC池中少于1ng/mg。因此，我们表明使用如本文中描述的HIC色谱法步骤来降低PLBL2水平对IgG1MAb和IgG4MAb是有效的，示出了该方法用于降低重组多肽制剂中仓鼠PLBL2水平的普遍适用性。

[0366] 实施例3-在施用含不同量仓鼠PLBL2的抗IL13MAb组合物的患者中评估人抗仓鼠

PLBL2反应

[0367] 为了评估CHO PLBL2杂质的潜在影响，我们开发了ELISA测定法(桥连ELISA测定法)来检测接受抗IL-13MAb(lebrikizumab)的人受试者中抗仓鼠PLBL2的抗体。分析参加lebrikizumab各种临床研究的患者以及接受安慰剂受试者的血清样品的给药前和给药后的抗仓鼠PLBL2抗体的证据。临床研究的细节先前已被描述(WO 2012/083132,Corren等人，N Engl J Med 365:1088-98(2011))，在下面仅提供这些研究中最相关的细节。

[0368] 开发并验证了检测人血清中抗仓鼠PLBL2抗体的抗体桥接ELISA测定法，使用两个缀合试剂来捕获针对仓鼠PLBL2抗体的所有同种型：纯化的缀合生物素的仓鼠PLBL2(生物素-PLBL2)和纯化的缀合地高辛的仓鼠PLBL2(DIG-PLBL2)。使用本领域技术人员公知的标准方法进行仓鼠PLBL2的生产和纯化，其也公开在美国临时申请号61/877,503和61/991,228中，使用本领域技术人员公知的标准方法进行与生物素或地高辛的缀合。在该半同质抗体桥接ELISA测定法中，在测定稀释剂中(PBS/0.5％BSA/0.05％聚山梨醇酯20/0.05％Proclin300，pH值7.4±0.1)的75μL/孔的缀合溶液含有Biotin-PLBL2和DIG-PLBL2各3μg/mL，在环境温度下与75μL/孔的测定稀释液中1:20稀释的血清样品和对照样品，在聚丙烯微管(National Scientific Supply Co.;Claremont,CA)中共同孵育过夜(16-24小时)。孵育后，100μL/孔混合物从微管转移到链亲和素包被的96孔微量板(StreptaWell™ High Bind;Roche Diagnostics;Indianapolis,IN)，该微量板在自动洗板仪器(BioTek ELx405)中用400μL/孔的洗涤缓冲液(PBS/0.05％聚山梨醇酯20)洗涤3次，并在环境温度下孵育2小时±10分钟。将板在洗板仪中用400μL/孔洗涤缓冲液洗涤4次，随后，以100μL/孔加入400ng/mL缀合辣根过氧化物酶(HRP)的小鼠抗地高辛抗体(Jackson ImmunoResearch Cat.200-032-156)，并在环境温度下孵育2小时±10分钟，进行检测。板在洗板仪中用400μL/孔洗涤缓冲液洗涤4次以后，以100μL/孔加入过氧化物酶底物(四甲基联苯胺)(0.4g/L TMB)和过氧化物酶溶液B(0.02％过氧化氢)(KPL Cat.50-76-03)的等量混合溶液，在环境温度下孵育18-28分钟用于显色，通过加入100μL/孔的1M磷酸停止反应。在450nm读取板以检测吸光度和在630nm读取参考吸光度。用于该测定法的阳性对照是一种单克隆抗体构建体，其由人IgG1框架上的鼠抗仓鼠PLBL2特异的互补决定区(CDR)组成。使用该抗体的测定法的相对灵敏度被确定为25ng/mL。使用该抗体的测定药物耐受性的实验表明，血清中高达50μg/mL的lebrikizumab或1μg/mL的仓鼠PLBL2在测定法中没有造成干扰或交叉反应性。

[0369] 为了进行测定法，首先在测定法中以1/20的最小稀释筛选血清样品。然后使用竞争验证性测定法确认筛选的阳性样品的仓鼠PLBL2特异性。如果样品被确认为阳性，则系列稀释该样品得到滴度值。以滴度单位报告阳性反应，其是稀释因子的log10，在该值样品信号等于测定法截点(用于确定阳性的阈值)的信号。

[0370] 使用如上所述的抗仓鼠PLBL2ELISA分析患者样品的四个临床研究如下简要描述。研究1是随机、双盲、安慰剂对照、概念验证研究的II期，以评估哮喘患者中lebrikizumab的作用，用吸入皮质类固醇(ICS)的长期治疗过程中对该哮喘疾病控制不佳。总共219名患者随机(分组)，其中106名接收至少一次皮下(SC)250mg lebrikizumab的剂量，92名接收六个月的剂量。

[0371] 研究2是对哮喘患者的随机、双盲、安慰剂对照、剂量范围研究的II期，其中哮喘患者没有接受ICS治疗。患者由SC施用接受lebrikizumab三个剂量(500,250,或125mg)之一或

安慰剂。在12周的治疗期间施用研究药物四次。共有158名患者暴露于至少一个lebrikizumab剂量，145名患者接受所有四个剂量。

[0372] 研究3是在健康的日本和高加索志愿者中lebrikizumab的PK研究的I期。20名健康日本和高加索受试者(每个种族组中10名受试者)的三个离散组以7:3的比率随机分为lebrikizumab(125，250，和375mg SC)和安慰剂组。受试者第1天接受一次剂量和随后监控120天。共有42受试者各接受一次lebrikizumab。

[0373] 在研究1-3中，共306受试者，其中264是哮喘患者，每位接受至少一次剂量的含仓鼠PLBL2的物质。对仓鼠PLBL2的暴露是可变的，取决于接受的lebrikizumab剂量。

[0374] 研究4是随机、双盲、安慰剂对照研究的IIb期，以评估在不受控制的哮喘患者中lebrikizumab的疗效和安全性，该哮喘患者使用ICS和第二控制药物。患者通过SC施用每月接受三个剂量(250，125，或37.5mg)的lebrikizumab中之一或安慰剂。在研究4中，总共463名患者随机(分组)，其中347名接收至少一个剂量的lebrikizumab。对仓鼠PLBL2的暴露是可变的，取决于接受的lebrikizumab剂量。

[0375] 以下表12提供了研究1-4每个的总结，其表明受试者暴露的仓鼠PLBL2的水平范围和lebrikizumab的剂量。

[0376] 表12.在Lebrikizumab临床试验中仓鼠PLBL2暴露。

[0377]

研究	药物PLBL2 (ng/mg)	Lebrikizumab剂量 (mg/月)	PLBL2 (μg/剂量)
1	34-137[a]	250	9-34
2	34-137[a]	125	4-17
		250	9-34
		500	17-69
3	34	125	4
		250	9
		375	13
4	242	37.5	9
	328	125	41
	328	250	82

[0378] [a]来自四个不同批次的临床材料的范围。

[0379] 使用上述抗仓鼠PLBL2抗体测定法进行从研究1所选时间点的回顾分析，检测抗仓鼠PLBL2抗体。分析来自安慰剂和给药受试者的样品，以确定预先存在的反应水平，以及对lebrikizumab给药反应的抗体的发生。有113名安慰剂受试者和106名接受至少一次

lebrikizumab剂量的给药受试者。选择用于分析的时间点是第0、29、85、141、225天和提前终止。在下一次剂量前取样品；因此，第29天的样品在第二次剂量施用前提取。在每个时间点抗仓鼠PLBL2抗体阳性受试者的百分比如下计算：取在每个时间点阳性受试者的数目，除以在每个时间点检测的受试者总数。该数据示于表13。

[0380] 表13.研究1的抗仓鼠PLBL2抗体的结果。

[0381]

	在每个时间点的阳性% (阳性受试者数/评估的受试者总数)					
研究的天:	0	29	85	141	225	提前终止
安慰剂	6 (7/110)	7 (8/107)	9 (9/104)	8 (8/99)	5 (5/97)	25 (2/8)
250 mg 剂量	5 (5/102)	6 (6/100)	89 (90/101)	98 (92/94)	98 (91/93)	100 (8/8)[a]

[0382] [a]在药物研究早期没有继续的8名lebrikizumab受试者中，只有3名报告了作为药物研究停止的原因的不良反应。

[0383] 在第0天给药前是阳性的6名研究1安慰剂受试者在整个研究过程持续阳性。在验证性竞争测定法中证实来自这些受试者的样品为阳性，在第0天具有滴度范围从1.6到2.9滴度单位。在以后的访问中获得的滴度类似于第0天所获得的滴度。其他一些安慰剂受试者在研究期间具有低水平的阳性反应。

[0384] 在该接受lebrikizumab的研究1的受试者中，98%(104/106)给药后具有阳性抗体反应，并贯穿研究结束仍为阳性，其中大多数受试者在接受至少两个剂量的lebrikizumab之后成为阳性。给药后滴度范围从1.35至4.76滴度单位，滴度一般随着时间而增加。抗仓鼠PLBL2抗体发展的临床意义不明。在本研究中没有确定临床上重要的安全性信号，鉴于仓鼠PLBL2抗体的高发率，可以认为其与安全事件无相关性。

[0385] 在研究4收集的样品上还进行了中期分析。分析了来自安慰剂和给药的受试者，以确定预先存在的反应水平以及对lebrikizumab给药反应的抗仓鼠PLBL2抗体的发生。有116名安慰剂受试者和347名接受至少一次lebrikizumab剂量的给药受试者。来自92名安慰剂受试者和268名给药受试者的样品在这数据组中表示。结果示于表14。

[0386] 表14.没有事先暴露于Lebrikizumab的受试者的研究4的抗仓鼠PLBL2抗体结果。

[0387]

	在每个时间点的阳性% (阳性受试者数/评估的受试者总数)					
研究的天	0	29	85	169	253	提前终止
安慰剂	4 (4/89)	4 (3/78)	4 (2/48)	0 (0/13)	NA	0 (0/5)
37.5 mg 剂量	9 (8/88)	9 (7/82)	55 (35/64)	79 (27/34)	66 (2/3)	43 (3/7)[a]
125 mg 剂量	4 (3/81)	11 (8/73)	87 (48/55)	100 (9/9)	NA	0 (0/2)[a]
250 mg 剂量	5 (4/88)	10 (7/72)	96 (49/51)	100 (13/13)	NA	67 (2/3)[a]

[0388] [a]在药物研究早期没有继续的12名lebrikizumab受试者中，只有4名报告了作为研究药物停止的原因的不良反应。

[0389] 在给药前第0天是阳性的四名研究4的安慰剂受试者具有低水平的阳性反应，仅高于测定法的检测极限。在某些随后的时间点上(但不是全部)可检测低水平的反应。

[0390] 在给药前第0天是阳性的15名研究4的接受lebrikizumab的受试者在随后的时间点持续阳性，多次剂量后滴度增加。此外，研究4中有10名受试者之前在研究1中接受lebrikizumab。这些受试者有9名随后在研究4中再给药lebrikizumab，而1名接受安慰剂。所有10名受试者在研究4的给药前第0天是阳性，并在随后的时间点持续阳性。这10名受试者的数据从表14排除，因为他们以前暴露于lebrikizumab。

[0391] 在研究4接受lebrikizumab的受试者中，在剂量组之间似乎存在阳性率的差异。然而，由于这些数据是不完整的，在此时间点不能作出关于这些差异的显著性的结论。与来自研究1的数据相似，大多数受试者在接受至少两个剂量的lebrikizumab后变为阳性。给药后滴度范围从1.68到4.55滴度单位，滴度一般随着时间而增加。由于这是一个不完整的数据组，阳性百分数和滴度范围可以由附加数据的累积而改变。

[0392] 研究4的中期安全性评估显示出类似较早完成的研究的安全特性，其中没有临床显著的安全性信号，包括无过敏反应或严重超敏反应的报告。值得注意的是，在研究1中接受lebrikizumab并随后在研究4中重新给药lebrikizumab的9名患者中的6名在中期分析的时间点没有报告任何不良事件，只有1名患者报告有任何局部注射部位反应。迄今在临床试验中没有确定该抗仓鼠PLBL2抗体反应的临床后遗症。

[0393] 我们还对来自研究2的125mg剂量组进行了评估，那些结果显示在表15中。

[0394] 表15.研究2抗仓鼠PLBL2抗体的结果。

[0395]

	% 在每个时间点的阳性% (阳性受试者数/评估的受试者总数)					
研究的天:	0	29	57	85	141	提前终止
125 mg 剂量	4 (2/51)	21 (11/53)	70 (35/50)	88 (45/51)	86 (43/50)	100 (2/2)[a]

[0396] [a]在药物研究早期没有继续的2名受试者中，均没有报告作为研究药物停止的原因的不良反应。

[0397] 在给药前第0天是阳性的两名研究2的受试者在所有后续的时间点持续阳性，多次剂量后滴度增加。在接受125mg lebrikizumab的研究2受试者中，87%(46/53)给药后具有阳性抗体反应，并在整个研究至结束保持阳性，其中大多数受试者在接受至少两次剂量的lebrikizumab之后变成阳性。给药后滴度范围从1.51～4.09滴度单位，滴度一般随着时间而增加。

[0398] 结论

[0399] 为了评估CHO PLBL2杂质的潜在影响，开发了一种测定法，其检测接受含有显著水平的仓鼠PLBL2的lebrikizumab制剂的受试者中抗仓鼠PLBL2抗体。在研究1的完整数据集和研究2的125mg剂量组和研究4的部分数据组的基础上，在lebrikizumab制剂中存在的仓鼠PLBL2在暴露于仓鼠PLBL2的大多数受试者中产生免疫反应。

[0400] 在抗仓鼠PLBL2抗体测定法中许多安慰剂和lebrikizumab剂量组受试者具有预先存在的免疫反应性。这种预先存在的反应的原因是未知的；先前在正常人血清样品中已表征并证实对CHO宿主细胞蛋白的抗体反应性，该血清无已知的之前暴露于CHO-衍生的生物制品(Xue等人，The AAPS Journal 12(1):98-106(2010))。但是，没有对单一种类CHOP，PLBL2特定的数据公布。

[0401] 对于在抗仓鼠PLBL2抗体测定法中在该研究开始时具有预先存在的免疫反应的受试者，重复给药lebrikizumab后有一个持续上升的抗体滴度。对于在研究开始时抗体阴性的受试者，所有四项研究中的多数受试者在至少两次施用lebrikizumab后变成阳性，且在所有随后的时间点仍然是阳性。

[0402] 抗仓鼠PLBL2抗体发生的临床意义尚不清楚。虽然在研究受试者中仓鼠PLBL2抗体的发生率高，但是可以认为其与安全事件之间没有相关性。重要的是，在这些完全或中期研究中没有鉴定到安全信号，特别是没有报道过敏反应、类过敏反应、或者严重的超敏性反应。尽管如此，仍担心长期暴露于重复给药可能会增加潜在的不期望的效果，例如过敏反应、超敏反应和免疫复合物沉积，特别是在哮喘患者人群和其他过敏或超敏的患者群体。因此，在后期临床研究和之后(其中可能有很长一段时间这样重复给药)向患者给药含有实质上降低仓鼠PLBL2水平的IL-13MAb(例如，lebrikizumab)制剂以便尽可能降低免疫原性是重要的。

[0403] 其他抗体序列提供于下表16。

[0404] 表16.抗IL17A/F抗体氨基酸序列(SEQ ID NOS:15-22)和抗Aβ抗体氨基酸序列

(SEQ ID NOS:23-30)。

[0405]

CDR-H1 (SEQ ID NO.:15)	Asp Tyr Ala Met His
CDR-H2 (SEQ ID NO.:16)	Gly Ile Asn Trp Ser Ser Gly Gly Ile Gly Tyr Ala Asp Ser Val Lys Gly
CDR-H3 (SEQ ID NO.:17)	Asp Ile Gly Gly Phe Gly Glu Phe Tyr Trp Asn Phe Gly Leu
CDR-L1 (SEQ ID NO.:18)	Arg Ala Ser Gln Ser Val Arg Ser Tyr Leu Ala
CDR-L2 (SEQ ID NO.:19)	Asp Ala Ser Asn Arg Ala Thr
CDR-L3 (SEQ ID NO.:20)	Gln Gln Arg Ser Asn Trp Pro Pro Ala Thr
VH (SEQ ID NO.:21)	Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Gly Ile Asn Trp Ser Ser Gly Gly Ile Gly Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg Asp Ile Gly Gly Phe Gly Glu Phe Tyr Trp Asn Phe Gly Leu Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser
VL (SEQ ID NO.:22)	Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Arg Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Pro Ala Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
CDR-H1 (SEQ ID NO.:23)	GFTFSSYGMS
CDR-H2 SEQ ID NO.:24)	SINSNGGSTY YPDSVK
CDR-H3 SEQ ID NO.: 25)	GDY
CDR-L1 (SEQ ID NO.:26)	RSSQSLVYSN GDTYLH

[0406]

CDR-L2 (SEQ ID NO.: 27)	KVSNRFS
CDR-L3 (SEQ ID NO.: 28)	SQSTHVPWT
VH (SEQ ID NO.: 29)	EVQLVESGGG LVQPGGSLRL SCAASGFTFS SYGMSWVRQA PGKGLELVAS INSNGGSTYY PDSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCASGD YWGQGTTVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT KTYTCNVDHK PSNTKVDKRV ESKYGPPCPP CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQ KSLSLSLG
VL (SEQ ID NO.: 30)	DIVMTQSPLS LPVTPGEPAS ISCRSSQSLV YSNGDTYLHW YLQKPGQSPQ LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCSQSTHVP WTFGQGTKVE IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK VQWKVDNALQ SGNSQESVTE QDSKDSTYSL SSTLTLSKAD YEKHKVYACE VTHQGLSSPV TKSFNRGEC

序列表

<110> 豪夫迈·罗氏有限公司

<120>包含纯化的重组多肽的方法和组合物
<130> P5704R1-WO

<140>
<141>

<150> 61/877,517
<151> 2013-09-13

<160> 34

<170> PatentIn version 3.5

<210> 1
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[0001]
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<223> 人工序列的描述：合成的肽

<400> 1
Ala Tyr Ser Val Asn
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<223> 人工序列的描述：合成的肽

<400> 2
Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys Ser
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<211> 10
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<220>
<223> 人工序列的描述：合成的肽

<400> 3
Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn
1 5 10

<210> 4
<211> 15
<212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的肽

<400> 4
Arg Ala Ser Lys Ser Val Asp Ser Tyr Gly Asn Ser Phe Met His
1 5 10 15

[0002]
<210> 5
<211> 7
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<213> 人工序列

<220>
<223> 人工序列的描述：合成的肽

<400> 5
Leu Ala Ser Asn Leu Glu Ser
1 5

<210> 6
<211> 9
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<220>
<223> 人工序列的描述：合成的肽

<400> 6
Gln Gln Asn Asn Glu Asp Pro Arg Thr
1 5

<210> 7
<211> 117
<212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的多肽

<400> 7
Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln Thr
1 5 10 15

Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ala Tyr Ser
20 25 30

Val Asn Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu Ala
35 40 45

Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys Ser
50 55 60

[0003] Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val Val Leu Thr
65 70 75 80

Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala Gly
85 90 95

Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn Trp Gly Gln Gly Ser Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 8
<211> 118
<212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的多肽

<400> 8
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln

1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ala Tyr
20 25 30

Ser Val Asn Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu
35 40 45

Ala Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Gly Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn Trp Gly Gln Gly Ser
100 105 110

Leu Val Thr Val Ser Ser
[0004] 115

<210> 9
<211> 112
<212> PRT
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<220>
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<400> 9
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ser Val Ser Leu Gly
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Lys Ser Val Asp Ser Tyr
20 25 30

Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Asp
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80

Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Asn Asn
85 90 95

Glu Asp Pro Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
100 105 110

<210> 10
<211> 443
<212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的多肽

<400> 10
Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln Thr
1 5 10 15

[0005]
Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ala Tyr Ser
20 25 30

Val Asn Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu Ala
35 40 45

Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys Ser
50 55 60

Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val Val Leu Thr
65 70 75 80

Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala Gly
85 90 95

Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn Trp Gly Gln Gly Ser Leu
100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115 120 125

Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys
130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145 150 155 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165 170 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180 185 190

Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn
195 200 205

Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro
210 215 220

Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe
225 230 235 240

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
245 250 255

[0006] Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe
260 265 270

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
275 280 285

Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
290 295 300

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
305 310 315 320

Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala
325 330 335

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln
340 345 350

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
355 360 365

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro

370 375 380

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
385 390 395 400

Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu
405 410 415

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
420 425 430

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly
435 440

<210> 11
<211> 444
<212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的多肽

[0007]

<400> 11
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ala Tyr
20 25 30

Ser Val Asn Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu
35 40 45

Ala Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Gly Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn Trp Gly Gln Gly Ser
100 105 110

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125

Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly
130 135 140

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190

Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser
195 200 205

Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys
210 215 220

[0008]

Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu
225 230 235 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245 250 255

Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln
260 265 270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275 280 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu
290 295 300

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310 315 320

Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys
325 330 335

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340 345 350

Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
385 390 395 400

Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln
405 410 415

Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly
435 440

<210> 12
<211> 444
<212> PRT
[0009] <213> 人工序列

<220>
<223> 人工序列的描述：合成的多肽

<400> 12
Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln Thr
1 5 10 15

Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ala Tyr Ser
20 25 30

Val Asn Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu Ala
35 40 45

Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys Ser
50 55 60

Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val Val Leu Thr
65 70 75 80

Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala Gly

85 90 95

Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn Trp Gly Gln Gly Ser Leu
100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115 120 125

Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys
130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145 150 155 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165 170 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180 185 190

Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn
[0010] 195 200 205

Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro
210 215 220

Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe
225 230 235 240

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
245 250 255

Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe
260 265 270

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
275 280 285

Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
290 295 300

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
305 310 315 320

Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala
325 330 335

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln
340 345 350

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
355 360 365

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
370 375 380

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
385 390 395 400

Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu
405 410 415

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
420 425 430

[0011] Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
435 440

<210> 13
<211> 445
<212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的多肽

<400> 13
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ala Tyr
20 25 30

Ser Val Asn Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu
35 40 45

Ala Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val Val Leu
65 70 75 80

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Gly Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn Trp Gly Gln Gly Ser
100 105 110

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125

Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly
130 135 140

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175

[0012] Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190

Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser
195 200 205

Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys
210 215 220

Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu
225 230 235 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245 250 255

Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln
260 265 270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275 280 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu

290 295 300

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310 315 320

Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys
325 330 335

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340 345 350

Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
385 390 395 400

Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln
[0013] 405 410 415

Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
435 440 445

<210> 14
<211> 218
<212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的多肽

<400> 14
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ser Val Ser Leu Gly
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Lys Ser Val Asp Ser Tyr
20 25 30

Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Asp
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80

Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Asn Asn
85 90 95

Glu Asp Pro Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140

[0014] Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 15
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<400> 15
Asp Tyr Ala Met His
1 5

<210> 16
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<223> 人工序列的描述：合成的肽

<400> 16
Gly Ile Asn Trp Ser Ser Gly Gly Ile Gly Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

[0015]

<210> 17
<211> 14
<212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的肽

<400> 17
Asp Ile Gly Gly Phe Gly Glu Phe Tyr Trp Asn Phe Gly Leu
1 5 10

<210> 18
<211> 11
<212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的肽

<400> 18
Arg Ala Ser Gln Ser Val Arg Ser Tyr Leu Ala
1 5 10

<210> 19

<211> 7
<212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的肽

<400> 19
Asp Ala Ser Asn Arg Ala Thr
1 5

<210> 20
<211> 10
<212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的肽

<400> 20
Gln Gln Arg Ser Asn Trp Pro Pro Ala Thr
[0016] 1 5 10

<210> 21
<211> 123
<212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的多肽

<400> 21
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Ile Asn Trp Ser Ser Gly Gly Ile Gly Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Asp Ile Gly Gly Phe Gly Glu Phe Tyr Trp Asn Phe Gly Leu
100 105 110

Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 22
<211> 108
<212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的多肽

<400> 22
[0017] Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Arg Ser Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Pro
85 90 95

Ala Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 23
<211> 10

<212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的肽

<400> 23
Gly Phe Thr Phe Ser Ser Tyr Gly Met Ser
1 5 10

<210> 24
<211> 16
<212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的肽

<400> 24
Ser Ile Asn Ser Asn Gly Gly Ser Thr Tyr Tyr Pro Asp Ser Val Lys
1 5 10 15

[0018]
<210> 25
<211> 3
<212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的肽

<400> 25
Gly Asp Tyr
1

<210> 26
<211> 16
<212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的肽

<400> 26
Arg Ser Ser Gln Ser Leu Val Tyr Ser Asn Gly Asp Thr Tyr Leu His

1 5 10 15

<210> 27
<211> 7
<212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的肽

<400> 27
Lys Val Ser Asn Arg Phe Ser
1 5

<210> 28
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的肽

[0019]

<400> 28
Ser Gln Ser Thr His Val Pro Trp Thr
1 5

<210> 29
<211> 438
<212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的多肽

<400> 29
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Leu Val
35 40 45

Ala Ser Ile Asn Ser Asn Gly Gly Ser Thr Tyr Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
100 105 110

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
115 120 125

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
130 135 140

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
145 150 155 160

[0020] Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
165 170 175

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
180 185 190

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
195 200 205

Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro
210 215 220

Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
225 230 235 240

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
245 250 255

Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
260 265 270

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe
275 280 285

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
290 295 300

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
305 310 315 320

Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
325 330 335

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
340 345 350

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
355 360 365

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
370 375 380

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
385 390 395 400

[0021] Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
405 410 415

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
420 425 430

Leu Ser Leu Ser Leu Gly
435

<210> 30
<211> 219
<212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的多肽

<400> 30
Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val Tyr Ser

20 25 30

Asn Gly Asp Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Ser
85 90 95

Thr His Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
115 120 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
130 135 140

[0022]

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145 150 155 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
165 170 175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
180 185 190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
195 200 205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 31
<211> 12
<212> PRT
<213> 人工序列

<220>

<223> 人工序列的描述：合成的肽

<400> 31
Ser Val Leu Leu Asp Ala Ala Ser Gly Gln Leu Arg
1 5 10

<210> 32
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的肽

<400> 32
Gly Leu Glu Asp Ser Tyr Glu Gly Arg
1 5

<210> 33
<211> 12
[0023] <212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的肽

<400> 33
Ala Phe Ile Pro Asn Gly Pro Ser Pro Gly Ser Arg
1 5 10

<210> 34
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 人工序列的描述：合成的肽

<400> 34
Val Thr Ser Phe Ser Leu Ala Lys
1 5

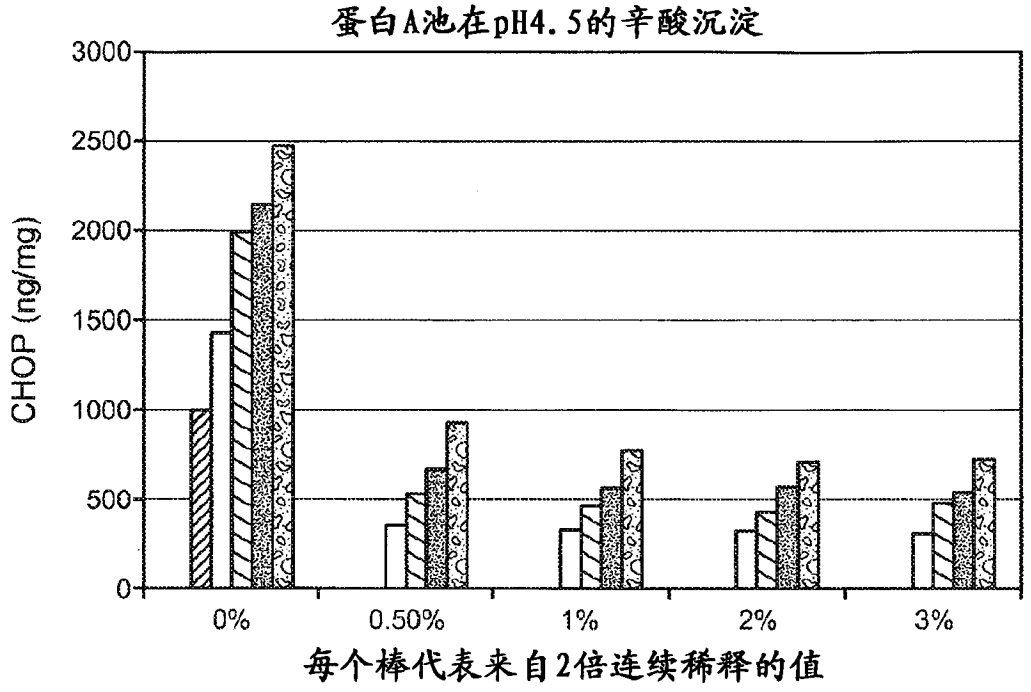
蛋白A池在pH4.5的辛酸沉淀
CHOP (ng/mg)
3000
2500
2000
1500
1000
500
0
0%
0.50%
1%
2%
3%
每个棒代表来自2倍连续稀释的值

图1A

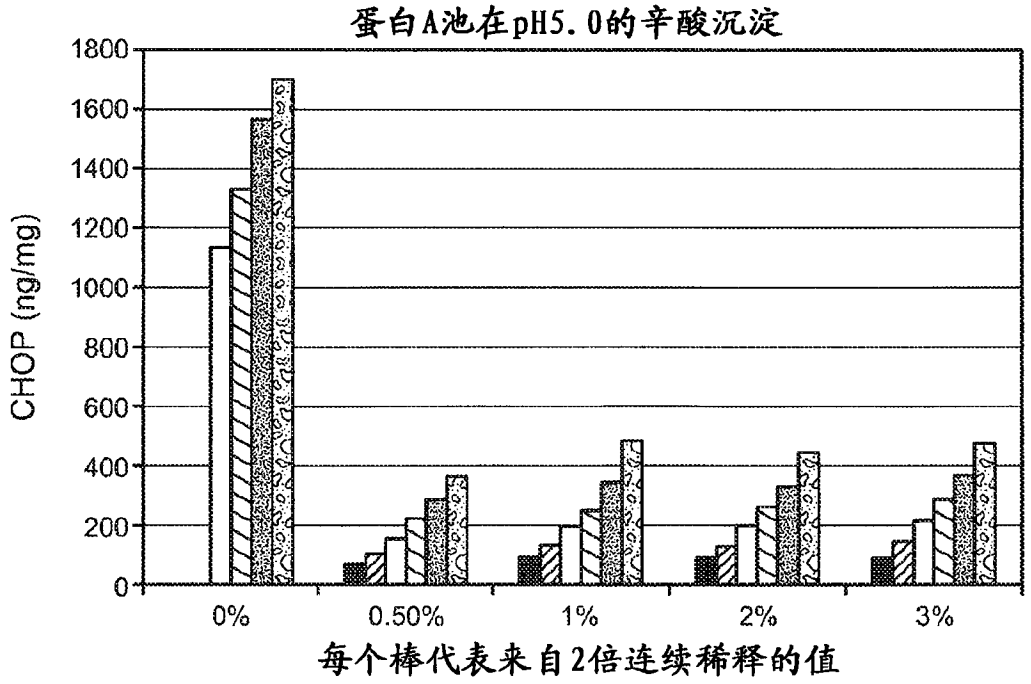
蛋白A池在pH5.0的辛酸沉淀
CHOP (ng/mg)
1800
1600
1400
1200
1000
800
600
400
200
0
0%
0.50%
1%
2%
3%
每个棒代表来自2倍连续稀释的值

图1B

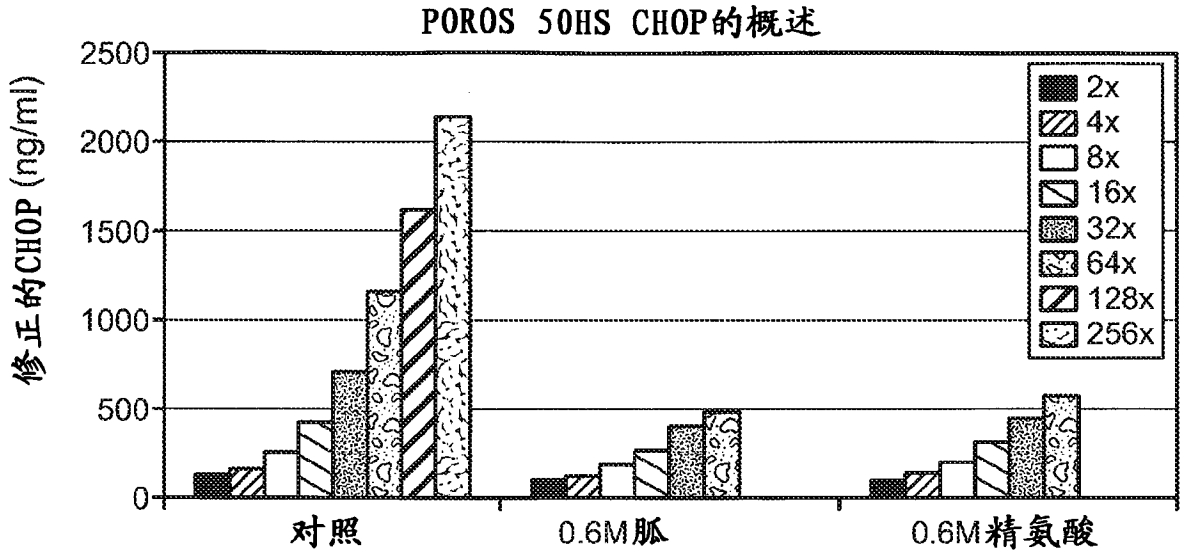
POROS 50HS CHOP的概述
修正的CHOP (ng/ml)
2500
2000
1500
1000
500
0
2x
4x
8x
16x
32x
64x
128x
256x
对照
0.6M胍
0.6M精氨酸

图2

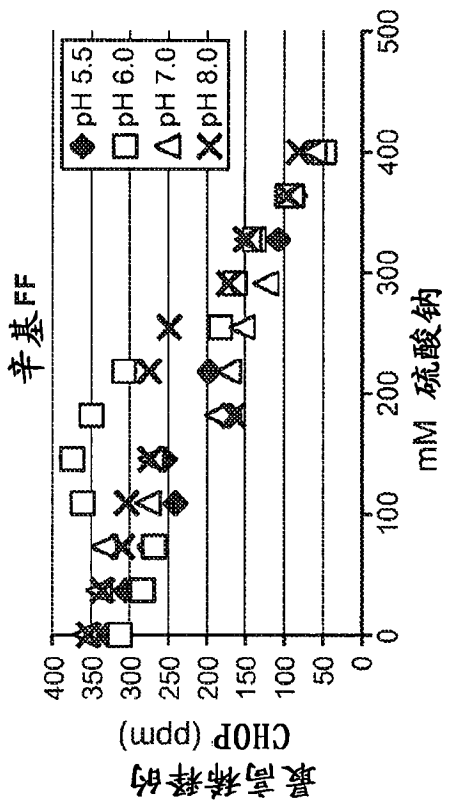
辛基FF
pH 5.5
pH 6.0
pH 7.0
pH 8.0
mM 硫酸钠
0
100
200
300
400
500
最高稀释的
CHOP (ppm)
400
350
300
250
200
150
100
50
0

图3A

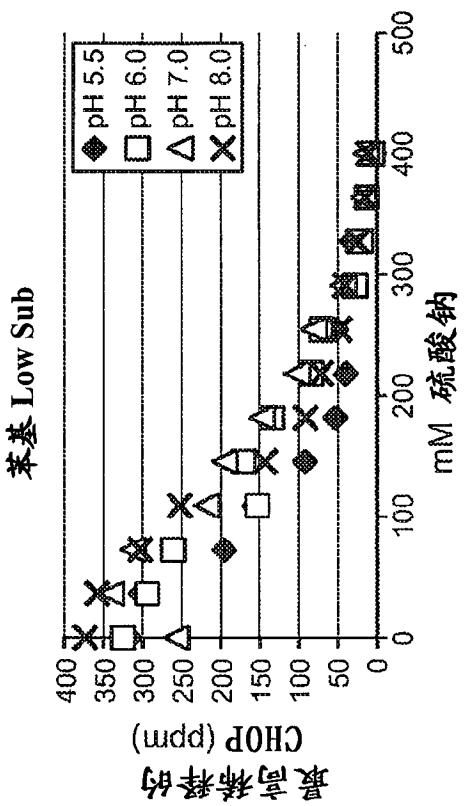
苯基 Low Sub
pH 5.5
pH 6.0
pH 7.0
pH 8.0
mM 硫酸钠
0
100
200
300
400
500
最高稀释的
CHOP (ppm)
400
350
300
250
200
150
100
50
0

图3B

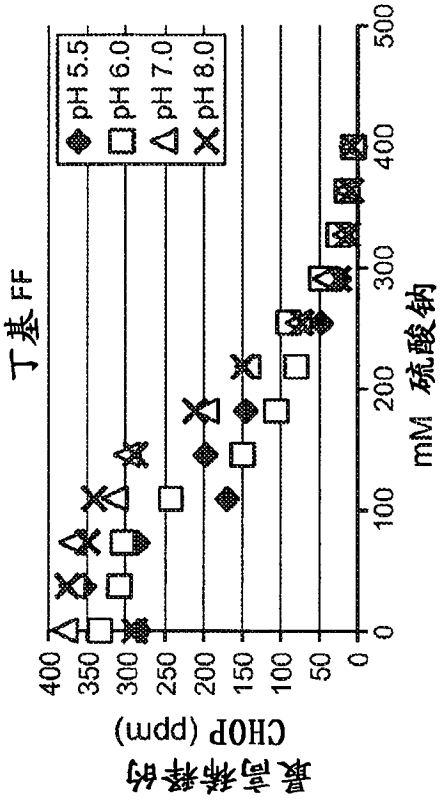
丁基 FF
pH 5.5
pH 6.0
pH 7.0
pH 8.0
0
100
200
300
400
500
mM 硫酸钠
0
50
100
150
200
250
300
350
400
最高稀释的
CHOP (ppm)

图3C

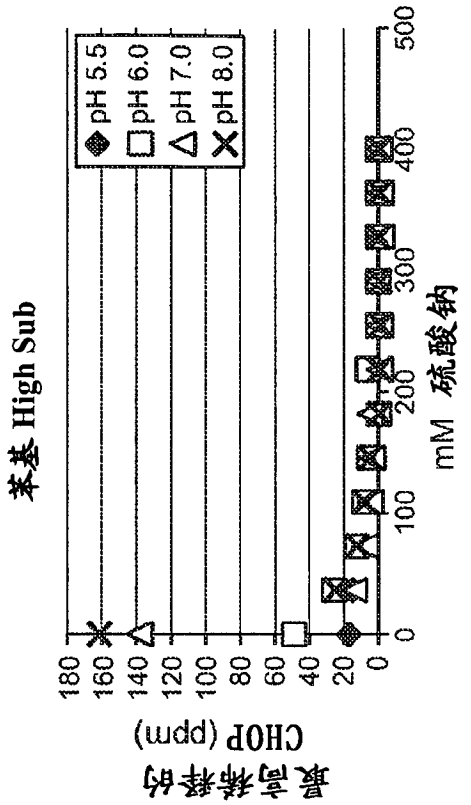
苯基 High Sub
pH 5.5
pH 6.0
pH 7.0
pH 8.0
0
100
200
300
400
500
mM 硫酸钠
0
20
40
60
80
100
120
140
160
180
最高稀释的
CHOP (ppm)

图3D

Abstract

Purified recombinant polypeptides isolated from Chinese hamster ovary host cells, including antibodies, such as therapeutic antibodies, and methods of making and using such polypeptides are provided.