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(54) **Title**: METHOD FOR THE REDUCTION OF HOST CELL PROTEINS IN AFFINITY CHROMATOGRAPHY

(57) **Abstract**: The current invention reports a method for purifying an antibody by reducing the content of a host cell protein. The method employs a wash step with a low conductivity aqueous solution in an affinity chromatography.

Method for the reduction of host cell proteins in affinity chromatography

The present invention relates to the field of purification of polypeptides. The present invention in particular relates to the reduction of host cell proteins like phospholipase B-like 2 (PLBL2) or Clusterin in solutions containing antibodies.

Background of the Invention

Proteins and especially immunoglobulins play an important role in today's medical portfolio. For human application every therapeutic protein has to meet distinct criteria. To ensure the safety of biopharmaceutical agents to humans by-products accumulating during the production process have to be removed especially. To fulfill the regulatory specifications one or more purification steps have to follow the manufacturing process. Among other things, purity, throughput, and yield play an important role in determining an appropriate purification process.

Different methods are well established and widespread used for protein purification, such as affinity chromatography (e.g. protein A or protein G affinity chromatography, single chain Fv ligand affinity chromatography), ion exchange chromatography (e.g. cation exchange (sulfopropyl or carboxymethyl resins), anion exchange (amino ethyl resins) and mixed-mode ion exchange), thiophilic adsorption (e.g. with beta-mercaptoethanol and other SH ligands), hydrophobic interaction or aromatic adsorption chromatography (e.g. with phenyl-sepharose, aza-arenophilic resins, or m-aminophenylboronic acid), metal chelate affinity chromatography (e.g. with Ni(II)- and Cu(II)-affinity material), size exclusion chromatography, and electrophoretical methods (such as gel electrophoresis, capillary electrophoresis).

For the purification of recombinantly produced immunoglobulins often a combination of different column chromatography steps is employed. During the purification non-immunoglobulin contaminants such as host cell protein and host cell DNA as well as endotoxins and viruses are depleted. Therefore, generally an affinity chromatography step, like protein A affinity chromatography is followed by one or more additional separation steps. In general, high conductivity buffers are described to be employed in wash steps of affinity chromatrography methods.

In US 6,127,526 a method for purifying proteins by Protein A chromatography is described which comprises the steps of: (a) adsorbing the protein to Protein A

immobilized on a solid phase comprising silica or glass; (b) removing contaminants bound to the solid phase by washing the solid phase with a hydrophobic electrolyte solvent; and (c) recovering the protein from the solid phase.

In WO2011/038894 a protein A chromatography method with a pronounced depletion of host cell protein and DNA by specific wash steps prior to the recovery of the immunoglobulin from the protein A chromatographic material is reported.

In WO2013/177118 compositions and methods for the isolation and purification of antibodies from a sample matrix are reported.

In WO2013/033517 methods for separating a polypeptide of interest (such as an antibody) from a virus are reported.

A method for purifying a protein, including one or more chromatographic processes, in which an amino acid; or a dipeptide, an oligopeptide, or a polyamino acid thereof is included in a buffer solution used in at least one chromatographic process (equilibration buffer, wash buffer, and elution buffer), thereby purifying a high-purity protein with a very small quantity of the impurity (e.g., polymers or host cell proteins) is reported in EP2583973.

Summary of the Invention

Herein is reported a method for the production of an antibody with reduced content of host cell proteins by purifying the antibody with an affinity chromatography step.

In more detail it has been found that by the method of the current invention which uses a low conductivity aqueous solution in a wash step of an affinity chromatography prior to the recovery of an antibody from the chromatographic material, that the content of certain host cell proteins in a solution comprising the antibody can be reduced. Accordingly, it has been found that the content of phospholipases (in particular phospholipase B-like 2 (PLBL2)) can be reduced. It has been found that the PLBL2 content can be reduced 100-fold or more if the antibody is of the IgG4 isotype.

One aspect as reported herein is the use of a low conductivity aqueous solution in a wash step of a protein A chromatography for reducing the content of a host cell protein wherein the protein A chromatography is used to purify a human IgG1 or a human IgG4 isotype antibody.

In one embodiment of this aspect the human IgG4 isotype antibody is an antibody against P-selectin, or an bispecific antibody against factor IXa and factor X, or an antibody against IL-13, or an antibody against amyloid beta. In one embodiment of this aspect the human IgG1 isotype antibody is an antibody against Influenza B, or an antibody against VEGF-A, or an antibody against CD22, or a bispecific antibody against HER3 and EGFR, or an antibody against amyloid beta, or an antibody against Her2, or a bispecific antibody against Ang2 and VEGF-A, or a bispecific antibody against carcinoembryonic antigen (CEA) and CD3.

In one embodiment of this aspect the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less.

In one embodiment of this aspect the host cell protein is phospholipase B-like 2 (PLBL2) or Clusterin.

In one embodiment of this aspect the low conductivity aqueous solution comprises about 0.1 mM to about 8 mM Tris.

In one embodiment of this aspect the low conductivity aqueous solution comprises about 0.05 mM to about 2 mM potassium phosphate.

In one embodiment of this aspect the low conductivity aqueous solution has a pH of about 7 or higher.

In one embodiment of this aspect the low conductivity aqueous solution wash step is preceded or succeeded by a high conductivity aqueous solution wash step.

In one embodiment of this aspect the high conductivity aqueous solution has a conductivity value of about 20 mS/cm or higher.

In one embodiment of this aspect an intermediate wash step is performed with a medium conductivity aqueous solution between the low conductivity aqueous solution wash step and the high conductivity aqueous solution wash step.

In one embodiment of this aspect the medium conductivity aqueous solution has a conductivity value of from more than 0.5 mS/cm to less than 20 mS/cm.

In one embodiment of this aspect the high (or medium) conductivity aqueous solution comprises Histidine.

One aspect as reported herein is a method for producing a human IgG4 or IgG1 isotype antibody comprising the steps of

a) cultivating a cell comprising a nucleic acid encoding a human IgG4 or IgG1 isotype antibody,

b) recovering the human IgG4 or IgG1 isotype antibody from the cell or the cultivation medium,

c) contacting the human IgG4 or IgG1 isotype antibody with a protein A chromatography material,

d) washing the protein A chromatography material with a low conductivity aqueous solution,

e) recovering the human IgG4 or IgG1 isotype antibody from the protein A chromatography material

and thereby producing the human IgG4 or IgG1 isotype antibody.

One aspect as reported herein is method for purifying a human IgG4 or IgG1 isotype antibody from a sample comprising the steps of

a) providing a sample comprising a human IgG4 or IgG1 isotype antibody,

b) purifying the human IgG4 or IgG1 isotype antibody with a protein A chromatography method/step, comprising washing the protein A chromatography material with a low conductivity aqueous solution.

In one embodiment of all aspects the human IgG4 isotype antibody is an antibody against P-selectin or a bispecific antibody against factor IXa and factor X or an antibody against IL-13 or an antibody against amyloid beta. In one embodiment of all aspects the human IgG1 isotype antibody is an antibody against Influenza B or an antibody against VEGF-A or an antibody against CD22 or a bispecific antibody against HER3 and EGFR or an antibody against amyloid beta or an antibody against Her2 or a bispecific antibody against Ang2 and VEGF-A, or a bispecific antibody against carcinoembryonic antigen (CEA) and CD3.

In one embodiment of all aspects the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less.

In one embodiment of all aspects the content of a host cell protein is reduced and the (specific) host cell protein is phospholipase B-like 2 (PLBL2) or Clusterin.

In one embodiment of all aspects the low conductivity aqueous solution comprises about 0.1 mM to about 8 mM Tris.

In one embodiment of all aspects the low conductivity aqueous solution comprises about 0.05 mM to about 2 mM potassium phosphate.

In one embodiment of all aspects the low conductivity aqueous solution has a pH of about 7 or higher.

In one embodiment of all method aspects the method additionally comprises washing the affinity chromatography material with a high conductivity aqueous solution and/or with a medium conductivity aqueous solution before or after washing the protein A chromatography material with low conductivity aqueous solution.

In one embodiment of all aspects the high conductivity aqueous solution has a conductivity value of about 20 mS/cm or higher.

In one embodiment of all aspects the medium conductivity aqueous solution has a conductivity value of from more than 0.5 mS/cm to less than 20 mS/cm.

In one embodiment of all aspects the high or medium conductivity aqueous solution comprises Histidine.

Detailed Description of the Invention

Herein is reported an improved affinity chromatography method and use comprising the washing of the affinity chromatography material with a low conductivity aqueous solution.

It has been found that specific host cell proteins can be reduced with a wash step with a low conductivity aqueous solution, when this wash step is used in an affinity chromatography step, e.g. a protein A chromatrography step. The affinity chromatography step is used in a purification or production method for antibodies. The low conductivity aqueous solution wash step is particularly effective to reduce the content of phospholipase B-like 2 (PLBL2).

One aspect as reported herein is the use of a low conductivity aqueous solution in a wash step of an affinity chromatography for reducing the content of a (specific) host cell protein.

One aspect as reported herein is a method for producing a human IgG isotype antibody comprising

a) cultivating a cell comprising a nucleic acid encoding the human IgG isotype antibody,

b) recovering the human IgG isotype antibody from the cell or the cultivation medium,

c) contacting (a solution comprising) the human IgG isotype antibody with an affinity chromatography material,

d) washing the affinity chromatography material with a low conductivity aqueous solution, while at least 90% the bispecific antibody remains bound to the affinity chromatography material,

e) recovering the human IgG isotype antibody from affinity chromatography material

and thereby producing the human IgG isotype antibody.

One aspect as reported herein is a method for purifying a human IgG isotype antibody from a sample comprising the steps of

a) providing a (buffered aqueous) sample comprising a human IgG isotype antibody,

b) purifying the human IgG isotype antibody with a affinity chromatography method/step, comprising washing the affinity chromatography material with low conductivity aqueous solution.

Recombinant polypeptides produced in CHO cells may be purified according to the methods described herein to remove or reduce levels of a host cell proteins.

Exemplary recombinant 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, CDlla, CDllb, CDllc, CD18, an ICAM, VLA-4, VCAM, IL-17A and/or F, IgE (e.g., omalizumab), DRS, 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), IFNa (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-7R alpha (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, antiBcl-2 antibody, anti-E-cadherin antibody, anti-CA125 antibody, anti- CA15-3 antibody, antiCA19-9 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, antiLCA/CD45 antibody, anti-CD45RO antibody, anti-CD45RA antibody, anti-CD39 antibody, anti-CD100 antibody, anti-CD95/Fas antibody, anti-CD99 antibody, anti-CD106 antibody, antiubiquitin antibody, anti-CD71 antibody, anti-c-myc antibody, anti-cytokeratins antibody, antivimentins antibody, anti-HPV proteins antibody, anti-kappa light chains antibody, anti-lambda light chains antibody, anti-melanosomes antibody, anti-prostate specific antigen antibody, antiS-100 antibody, anti-tau antigen antibody, anti-fibrin antibody, anti-keratins antibody and antiTn- antigen antibody.

In some embodiments, exemplary antibodies include antibodies to Abeta, antibodies to IL17 A/F and antibodies to CMV. Exemplary anti-Abeta antibodies and methods of producing such antibodies have been described previously, for example, in W02008011348, W02007068429, W02001062801, and W02004071408. 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.

In some embodiments the affinity chromatography is used to purify a human IgG isotype antibody. In some embodiments the affinity chromatography is used to purify an IgG4 antibody. In one embodiment the IgG4 isotype antibody is an antibody against P-selectin or a (bispecific) antibody against factor IXa and factor X or an antibody against IL-13 or an antibody against amyloid beta. In some embodiments the affinity chromatography is used to purify an IgG1 isotype antibody. In one embodiment the IgG1 isotype antibody is an antibody against Influenza B or an antibody against VEGF-A or an antibody against CD22 or an (bispecific) antibody against HER3 and EGFR or an antibody against amyloid beta or an antibody against Her2 or a bispecific antibody against Ang2 and VEGF-A or a bispecific antibody against carcinoembryonic antigen (CEA) and CD3.

One aspect as reported herein is a method for producing a human IgG4 isotype antibody (containing solution) comprising

a) cultivating a cell comprising a nucleic acid encoding a human IgG4 isotype antibody,

b) recovering the human IgG4 isotype antibody from the cell or the cultivation medium,

c) contacting the human IgG4 isotype antibody with an affinity chromatography material,

d) washing the affinity chromatography material with a low conductivity aqueous solution,

e) recovering the human IgG4 isotype antibody from the affinity chromatography material

and thereby producing the human IgG4 isotype antibody.

One aspect as reported herein is a method for producing an IgG4 isotype antibody (containing solution) comprising

a) cultivating a cell comprising a nucleic acid encoding an IgG4 isotype antibody,

b) recovering the IgG4 isotype antibody from the cell or the cultivation medium,

c) contacting the IgG4 isotype antibody with an affinity chromatography material,

d) washing the affinity chromatography material with a low conductivity aqueous solution,

e) recovering the IgG4 isotype antibody from the affinity chromatography material

and thereby producing the IgG4 isotype antibody.

One aspect as reported herein is a method for purifying a human IgG4 isotype antibody from a sample comprising the steps of

a) providing a sample comprising a human IgG4 isotype antibody,

b) purifying the human IgG4 isotype antibody with a affinity chromatography method/step, comprising washing the affinity chromatography material with a low conductivity aqueous solution.

One aspect as reported herein is a method for purifying an IgG4 isotype antibody from a sample comprising the steps of

a) providing a sample comprising an IgG4 isotype antibody,

b) purifying the IgG4 isotype antibody with a affinity chromatography method/step, comprising washing the affinity chromatography material with a low conductivity aqueous solution.

It has been found that the content of a host cell protein can be reduced if the conductivity of the aqueous solution used in the wash step is low i.e a low conductivity aqueous solution is used for washing. In one embodiment of all aspects the low conductivity aqueous solution has a conductivity value of about 1 mS/cm or less. In one preferred embodiment of all aspects the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less. In one embodiment the low conductivity aqueous solution has a conductivity value of from about 0.03 µS/cm to about 0.5 mS/cm. In one embodiment the low conductivity aqueous solution has a conductivity value of from about 0.05 µS/cm to about 0.35 mS/cm. In one embodiment of all aspects the low conductivity

aqueous solution is deionized water. For some applications deionized water is not suitable to be used in a wash step. In some embodiments the low conductivity aqueous solution is not deionized water.

It has been found that a protein A affinity chromatography can be used for the purposes as reported herein. In one preferred embodiment of all aspects the affinity chromatography is a protein A affinity chromatography. In one embodiment the protein A affinity chromatography is selected from the group comprising MabSelectSure affinity chromatography, ProSep vA affinity chromatography, Poros Mab Capture A affinity chromatography, ProSep Ultra Plus affinity chromatography, MabSelect SuRe LX, MabSelect, Eshmuno A, Toyopearl AF-rProtein A-650F; Toyopearl AF-rProtein A HC-650HF). In one embodiment the affinity chromatography is a protein G affinity chromatography. In one embodiment the affinity chromatography is an affinity chromatography that uses a recombinant protein as a ligand, that means that the affinity chromatography is a recombinant protein ligand affinity chromatography. In one embodiment the affinity chromatography is an affinity chromatography that uses a single chain Fv as a ligand, that means that the affinity chromatography is a single chain Fv ligand affinity chromatography. In one embodiment the affinity chromatography comprises a mutated Protein A coupled to a chromatography matrix or a fragment of Protein A coupled to a chromatography matrix.

It has been found that the content of (specific) host cell proteins can be reduced. It has been found that especially the content of phospholipase B-like 2 (PLBL2) can be reduced. In one embodiment the (specific) host cell protein is a Chinese hamster ovary (CHO) host cell protein. In one preferred embodiment of all aspects the (specific) host cell protein is phospholipase B-like 2 (PLBL2) or Clusterin. In one embodiment the (specific) host cell protein is phospholipase B-like 2 (PLBL2).

It has been found that low conductivity aqueous solution may comprise certain buffering substances e.g. Tris or potassium phosphate in low amounts. In one embodiment the low conductivity aqueous solution contains tris(hydroxymethyl)aminomethane (Tris). In one embodiment the low conductivity aqueous solution comprises about 0.1 mM to about 10 mM Tris. In one embodiment the low conductivity aqueous solution comprises about 0.5 mM to about 6.5 mM Tris. In one embodiment the low conductivity aqueous solution comprises about 2 mM Tris. In one embodiment the low conductivity aqueous solution contains potassium phosphate. In one embodiment the low conductivity

aqueous solution comprises about 0.05 mM to about 5 mM potassium phosphate. In one embodiment the low conductivity aqueous solution comprises about 0.05 mM to about 2 mM potassium phosphate. In one embodiment the low conductivity aqueous solution comprises about 0.5 mM potassium phosphate.

It has been found that the effect of reducing the content of a host cell protein is pronounced if the low conductivity aqueous solution has a certain pH. In one embodiment the low conductivity aqueous solution has a pH of about 7 or higher. In one embodiment the low conductivity aqueous solution has a pH of about 7.5 or higher. In one embodiment the low conductivity aqueous solution has a pH of from about 7 to about 9.5. In one embodiment the low conductivity aqueous solution has a pH of from about 7.5 to about 8.5. In one embodiment the low conductivity aqueous solution has a pH of about 8. In one embodiment the low conductivity aqueous solution has a pH of about 9.

It has been found that the effect of reducing the content of a host cell protein can also be achieved if the pH of the low conductivity aqueous solution is about 8.5 or higher and the low conductivity aqueous solution has a conductivity value of about 1.2 mS/cm or less. In one embodiment the low conductivity aqueous solution has a pH of about 8.5 or higher and the low conductivity aqueous solution has a conductivity value of about 1.2 mS/cm or less. In one embodiment the low conductivity aqueous solution has a pH of about 8.5 or higher and the low conductivity aqueous solution has a conductivity value of about 1 mS/cm or less. In one embodiment low conductivity aqueous solution has a pH of about 8.5 or higher and the low conductivity aqueous solution comprises about 55 mM Tris or less. In one embodiment low conductivity aqueous solution has a pH of about 8.5 or higher and the low conductivity aqueous solution comprises about 30 mM Tris or less.

In one embodiment the low conductivity aqueous solution is in the pH range of from pH 7 to less than pH 8.5 and has a conductivity value of about 0.5 mS/cm or less and at a pH value of 8.5 or more a conductivity value of about 1.2 mS/cm or less.

It has been found that by the uses and the methods as reported herein the content of host cell proteins like PLBL2 can be reduced to a certain level, e.g. when compared to the load amount of PLBL2 prior to a purification step like an affinity chromatoghraphy step. In one embodiment the content of PLBL2 is reduced at least 20-fold. In one embodiment the content of PLBL2 is reduced at least 40-fold. In

one embodiment the content of PLBL2 is reduced at least 50-fold. In one embodiment the content of PLBL2 is reduced at least 90-fold. In one embodiment the content of PLBL2 is reduced at least 100-fold. In some cases the level of reduction is even higher. In some embodiments the content of PLBL2 is reduced at least 200-fold. In some embodiments the content of PLBL2 is reduced at least 250-fold. In some embodiments the content of PLBL2 is reduced at least 300-fold. In some embodiments the content of PLBL2 is reduced at least 400-fold. In some embodiments the content of PLBL2 is reduced at least1000-fold.In one embodiment the content of PLBL2 is reduced at least by 50%. In one embodiment the content of PLBL2 is reduced at least by 66%. In one embodiment the content of PLBL2 is reduced at least by 80%. In one embodiment the content of PLBL2 is reduced at least by 90%. In one embodiment the content of PLBL2 is reduced at least by 95%. In some embodiments the content of PLBL2 is reduced to below 10 ng per mg of antibody. In some embodiments the content of PLBL2 is reduced to below 5 ng per mg of antibody. In some embodiments the content of PLBL2 is reduced to below 2 ng per mg of antibody.

In the methods and the uses as reported herein further wash steps can be employed with medium and/or high conductivity aqueous solutions. In one embodiment the low conductivity aqueous solution wash step is preceded or succeeded by a high conductivity aqueous solution wash step. In one embodiment the high conductivity aqueous solution has a conductivity value of about 20 mS/cm or higher. In one embodiment the high conductivity aqueous solution has a conductivity value of from about 20 mS/cm to about 100 mS/cm. In one embodiment an intermediate wash step is performed with a medium conductivity aqueous solution between the low conductivity aqueous solution wash step and the high conductivity aqueous solution wash step. In one embodiment the medium conductivity aqueous solution has a conductivity value of from more than 0.5mS/cm to less than 20 mS/cm.

It has been found that the host cell protein reducing effect can be improved when the high or medium conductivity aqueous solution further comprises an amino acid. In one embodiment the high or medium conductivity aqueous solution comprises an amino acid. In one embodiment the high or medium conductivity aqueous solution comprises Histidine or Arginine. In one embodiment the high or medium conductivity aqueous solution comprises Histidine. In one embodiment the high or medium conductivity aqueous solution comprises Histidine and Tris.

The methods and the uses as reported herein may include one or more further chromatography steps. In one embodiment at least one additional chromatography method/step is performed. In one embodiment an additional ion exchange chromatography method/step is performed. In one embodiment an additional anion exchange chromatography method/step is performed. In one embodiment an additional anion exchange chromatography method/step and an additional cation exchange chromatography method/step are performed.

It has been found that the use of a hydrophobic interaction chromatography step may be omitted. In one embodiment the use or the methods is without an hydrophobic interaction chromatography method/step.

One aspect as reported herein is the use of a low conductivity aqueous solution in a wash step of a protein A chromatography for reducing the content of PLBL2 or Clusterin wherein the protein A chromatography is used to purify an IgG4 or IgG1 isotype, e.g., a human IgG4 or IgG1, antibody and wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less and a pH of about 7 or higher.

One aspect is the use of a low conductivity aqueous solution in a wash step of a protein A chromatography for reducing the content of PLBL2 or Clusterin wherein the protein A chromatography is used to purify a human IgG4 or IgG1 isotype antibody and wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less and a pH of about 7 or higher. In some embodiments, the antibody is an IgG4 isotype antibody, e.g., an antibody against P-selectin, or a bispecific antibody against factor IXa and factor X, or an antibody against IL-13, or an antibody against amyloid beta. In some embodiments, the antibody is a IgG1 isotype antibody, e.g., an antibody against Influenza B, or an antibody against VEGF-A, or an antibody against CD22, or a bispecific antibody against HER3 and EGFR, or an antibody against amyloid beta, or an antibody against Her2, or a bispecific antibody against Ang2 and VEGF-A, or a bispecific antibody against carcinoembryonic antigen (CEA) and CD3.

In an aspect, the present disclosure provides a method for producing a human IgG4 or IgG1 isotype antibody comprising

a) cultivating a cell comprising a nucleic acid encoding the human IgG4 or IgG1 isotype antibody,

b) recovering the human IgG4 or IgG1 isotype antibody from the cell or the cultivation medium,

c) contacting the human IgG4 or IgG1 isotype antibody with a protein A affinity chromatography material,

d) washing the protein A affinity chromatography material with a low conductivity aqueous solution,

e) recovering the human IgG4 or IgG1 isotype antibody from affinity chromatography material

and thereby producing the human IgG4 or IgG1 isotype antibody,

wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less and a pH of about 7 or higher.

In an aspect, the present disclosure provides a method for producing a human IgG4 or IgG1 isotype antibody comprising

a) cultivating a cell comprising a nucleic acid encoding the human IgG4 or IgG1 isotype antibody,

b) recovering the human IgG4 or IgG1 isotype antibody from the cell or the cultivation medium,

c) contacting the human IgG4 or IgG1 isotype antibody with a protein A affinity chromatography material,

d) washing the protein A affinity chromatography material with a low conductivity aqueous solution,

e) recovering the human IgG4 or IgG1 isotype antibody from affinity chromatography material

and thereby producing the human IgG4 or IgG1 isotype antibody,

wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less and a pH of about 7 or higher,

and wherein the human IgG4 isotype antibody is an antibody against P-selectin, or a bispecific antibody against factor IXa and factor X, or an antibody against IL-13,

or an antibody against amyloid beta and wherein the human IgG1 isotype antibody is an antibody against Influenza B, or an antibody against VEGF-A, or an antibody against CD22, or a bispecific antibody against HER3 and EGFR, or an antibody against amyloid beta, or an antibody against Her2, or a bispecific antibody against Ang2 and VEGF-A, or a bispecific antibody against carcinoembryonic antigen (CEA) and CD3.

In an aspect, the present disclosure provides a method for purifying a human IgG4 or IgG1 isotype antibody from a sample comprising the steps of

a) providing a sample comprising a human IgG4 or IgG1 isotype antibody,

b) purifying the human IgG4 or IgG1 isotype antibody with a protein A affinity chromatography method/step, comprising washing the protein A affinity chromatography material with low conductivity aqueous solution,

wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less and a pH of about 7 or higher.

In an aspect, the present disclosure provides a method for purifying a human IgG4 or IgG1 isotype antibody from a sample comprising the steps of

a) providing a sample comprising a human IgG4 or IgG1 isotype antibody,

b) purifying the human IgG4 or IgG1 isotype antibody with a protein A affinity chromatography method/step, comprising washing the protein A affinity chromatography material with low conductivity aqueous solution,

wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less and a pH of about 7 or higher,

and wherein the human IgG4 isotype antibody is an antibody against P-selectin, or an antibody against factor IXa and factor X, or an antibody against IL-13, or an antibody against amyloid beta and wherein the human IgG1 isotype antibody is an antibody against Influenza B, or an antibody against VEGF-A, or an antibody against CD22, or a antibody against HER3 and EGFR, or an antibody against amyloid beta, or an antibody against Her2, or a bispecific antibody against Ang2 and VEGF-A, or a bispecific antibody against carcinoembryonic antigen (CEA) and CD3.

In an aspect, the present disclosure provides a method for producing a human IgG4 isotype antibody comprising

a) cultivating a cell comprising a nucleic acid encoding the human IgG4 isotype antibody,

b) recovering the human IgG4 isotype antibody from the cell or the cultivation medium,

c) contacting the human IgG4 isotype antibody with a protein A affinity chromatography material,

d) washing the protein A affinity chromatography material with a low conductivity aqueous solution,

e) recovering the human IgG4 isotype antibody from affinity chromatography material

and thereby producing the human IgG4 isotype antibody,

wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less and a pH of about 7 or higher,

and wherein the human IgG4 isotype antibody is antibody against factor IXa and factor X.

In an aspect, the present disclosure provides a method for purifying a human IgG4 isotype antibody from a sample comprising the steps of

a) providing a sample comprising a human IgG4 isotype antibody,

b) purifying the human IgG4 isotype antibody with a protein A affinity chromatography method/step, comprising washing the protein A affinity chromatography material with low conductivity aqueous solution,

wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less and a pH of about 7 or higher,

and wherein the human IgG4 isotype antibody is an antibody against factor IXa and factor X.

The terms "anti-P-selectin antibody" and "an antibody that binds to P-selectin" or "antibody against P-selectin"refer to an antibody that is capable of binding P-selectin with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting P-selectin. In one embodiment, the extent of binding of an anti-P-selectin antibody to an unrelated, non- P-selectin protein is less than about 10% of the binding of the antibody to P-selectin as measured, e.g., by ELISA or surface plasmon resonance. In certain embodiments, an anti- P-selectin antibody binds to an epitope of P-selectin that is conserved among P-selectin from different species. The above also holds for the terms "antibody against factor IXa and factor X" or "antibody against IL-13" or "antibody against amyloid beta" or the like.

In some embodiments, the antibody against P-selectin is inclacumab (IgG4 isotype) as described in WO 2005/100402 or SEQ ID NO: 07 to 12. In some embodiments, the antibody is a bispecific antibody against factor IXa and factor X, e.g., anti-FIXa/X antibody (IgG4 isotype) as described in WO 2012/067176. In some embodiments, the antibody is an antibody against Her2, e.g., trastuzumab (IgG1 isotype) as described in WO 1992/022653. In some embodiments, the antibody is a bispecific antibody against angiopoietin 2 (Ang2) and vascular endothelial growth factor A (VEGF-A), e.g., vanucizumab (IgG1 isotype) as described in WO 2011/117329 or SEQ ID NO: 01 to 04. In some embodiments, the antibody is an antibody against amyloid beta, e.g., gantenerumab (IgG1 isotype) as described in WO 2003/070760 or SEQ ID NO: 05 to 06, or crenezumab (IgG4 isotype). In some embodiments, the antibody is an antibody against CD22, an antibody against IL13 (e.g., lebrikizumab), a bispecific antibody against Her3 and EGFR (e.g., duligotuzumab), an antibody against VEGF-A (e.g., bevacizumab), and an antibody against Influenza B. The terms VEGF or VEGF-A can be used interchangeably herein.

As used herein, the term "binding" or "specifically binding" refers to the binding of the antibody to an epitope of the antigen in an in-vitro assay, preferably in a surface plasmon resonance assay (SPR, BIAcore, GE-Healthcare Uppsala, Sweden). The affinity of the binding is defined by the terms ka (rate constant for the association of the antibody from the antibody/antigen complex), k_d (dissociation constant), and K_D (k_d/k_a). Binding or specifically binding means a binding affinity (K_D) of 10^{-7} mol/L or less.

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), and antibody fragments so long as they exhibit the desired antigen-binding activity.

An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, $F(ab')_2$; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments. A Fab fragement is an antibody fragment obtained by a papain digestion of a (full length/complete) antibody.

Bispecific antibodies" are antibodies which have two different antigen-binding specificities. The term "bispecific" antibody as used herein denotes an antibody that has at least two binding sites each of which bind to different epitopes.

The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

The term "human IgG isotype antibody" denotes an antibody that comprises a constant region that is derived from a human wild-type IgG isotype, i.e. for example it may comprise a constant region derived from a human IgG isotype with a mutation, e.g. an P329G mutation (numbering according to Kabat).

The term "human IgG4 isotype antibody" denotes an antibody that comprises a constant region that is derived from a human wild-type IgG4 isotype, i.e. for example it may comprise a constant region derived from a human IgG4 isotype with a mutation, e.g. an an P329G mutation and/or S228P, L235E mutation (numbering according to Kabat).

The term “Fc-region” herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc-regions and variant Fc-regions. In one embodiment, a human IgG heavy chain Fc-region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) or the C-terminal glycyl-lysine dipeptide (Gly446Lys447) of the Fc-region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc-region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat, E.A. et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991), NIH Publication 91-3242.

"Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

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. The term „cell“ includes cells which are used for the expression of nucleic acids. In one embodiment the host cell is a CHO cell (e.g. CHO K1, CHO DG44), or a BHK cell, or a NS0 cell, or a SP2/0 cell, or a HEK 293 cell, or a HEK 293 EBNA cell, or a PER.C6® cell, or a COS cells. In another embodiment the cell is a CHO cell, or a BHK cell, or a PER.C6® cell. As used herein, the expression "cell" includes the subject cell and its progeny.

The term “washing” denotes the applying of a solution to an affinity chromatography material in order to remove non specifically bound polypeptides and non-polypeptide compounds from the chromatography material, especially to

remove host cell protein and host cell DNA. The term "washing" does not encompass the elution of bound material from an affinity chromatography material.

Different methods are well established and widespread used for protein recovery and purification, such as affinity chromatography with microbial proteins (e.g. protein A or protein G affinity chromatography) affinity chromatographie with a recombinant protein as ligand (e.g. single chain Fv as ligand, e.g. Kappa select), ion exchange chromatography (e.g. cation exchange (carboxymethyl resins), anion exchange (amino ethyl resins) and mixed-mode exchange), thiophilic adsorption (e.g. with beta-mercaptoethanol and other SH ligands), hydrophobic interaction or aromatic adsorption chromatography (e.g. with phenyl-sepharose, aza-arenophilic resins, or m-aminophenylboronic acid), metal chelate affinity chromatography (e.g. with Ni(II)- and Cu(II)-affinity material), size exclusion chromatography, and electrophoretical methods (such as gel electrophoresis, capillary electrophoresis). These methods can be combined independently in different embodiments as reported herein.

The term "protein A" denotes a protein A polypeptide either obtained from a natural source or produced synthetically.

The term "protein A chromatography material" denotes an inert solid phase to which a protein A is covalently linked.

In one embodiment the protein A chromatography material is selected from MabSelectSure, ProSep vA, Mab Capture A, ProSep Ultra Plus, Mab Select, Mab Select Xtra, Poros A, or ProSep A.

The term "high conductivity aquaeous solution"denotes an aquaeous solution with a high conductivity value. The conductivity value may be about 20 mS/cm or higher.

The term "medium conductivity aquaeous solution"denotes an aquaeous solution with a medium conductivity value. The conductivity value may be more than 0.5 mS/cm to less than 20 mS/cm.

The term "low conductivity aquaeous solution"denotes an aquaeous solution with a low conductivity value. The conductivity value may be about 0.5 mS/cm or less. The conductivity value may be about 1.2 mS/cm or less, if the pH is about 8.5 or

higher. The conductivity values can be determined by standard methods known to the person skilled in the art.

The following examples and sequences are provided to aid the understanding of the present invention, the true scope of which is set forth in the appended claims. It is understood that modifications can be made in the procedures set forth without departing from the spirit of the invention.

Specific embodiments of the invention

1. Use of a low conductivity aqueous solution in a wash step of an affinity chromatography for reducing the content of a host cell protein.
2. Use according to embodiment 1, wherein the affinity chromatography is used to purify a human IgG isotype antibody.
3. Use according to embodiment 2, wherein the affinity chromatography is used to purify a human IgG4 isotype antibody or a human IgG1 isotype antibody.
4. Use according to embodiment 3, wherein the affinity chromatography is used to purify a human IgG4 isotype antibody or a human IgG1 isotype antibody without a glycosylated glycosylation site in its Fab fragment/with exactly one glycosylation site (at position Asn 297 numbering according to Kabat).
5. Use according to embodiment 4, wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less.
6. Use according to embodiment 5, wherein the low conductivity aqueous solution has a conductivity value of from about 0.03 µS/cm to about 0.5 mS/cm.
7. Use according to embodiment 5, wherein the low conductivity aqueous solution has a conductivity value of from about 0.05 µS/cm to about 0.35 mS/cm.
8. Use according to any of embodiments 5 to 7, wherein the low conductivity aqueous solution is not deionized water.
9. Use according to any of the previous embodiments, wherein the affinity chromatography is a protein A affinity chromatography or a Protein G affinity chromatography or a single chain Fv ligand (KappaSelect) affinity chromatography.
10. Use according to embodiment 9, wherein the affinity chromatography is a protein A affinity chromatography.
11. Use according to embodiment 10, wherein the protein A affinity chromatography is selected from the group comprising MabSelectSure affinity

chromatography, ProSep vA affinity chromatography, Poros Mab Capture A affinity chromatography, ProSep Ultra Plus affinity chromatography, MabSelect SuRe LX, MabSelect, Eshmuno A, Toyopearl AF-rProtein A-650F; Toyopearl AF-rProtein A HC-650HF).

12. Use according to any one of the previous embodiments, wherein said host cell protein is a Chinese hamster ovary (CHO) host cell protein.
13. Use according to embodiment 12, wherein the host cell protein is a phospholipase.
14. Use according to embodiment 13, wherein the host cell protein is a phospholipase A, phospholipase B, phospholipase C or phospholipase D.
15. Use according to embodiments 12, 13 or 14, wherein the host cell protein is phospholipase B-like 2 (PLBL2)
16. Use according to embodiment 12, wherein the host cell protein is phospholipase B-like 2 (PLBL2) or Clusterin.
17. Use according to any of the preceding embodiments, wherein the low conductivity aqueous solution contains tris(hydroxymethyl)aminomethane (Tris).
18. Use according to embodiment 17, wherein the low conductivity aqueous solution comprises about 0.1 mM to about 10 mM Tris.
19. Use according to embodiment 18, wherein the low conductivity aqueous solution comprises about 0.1 mM to about 8 mM Tris.
20. Use according to embodiment 19, wherein the low conductivity aqueous solution comprises about 0.5 mM to about 6.5 mM Tris.
21. Use according to embodiment 20, wherein the low conductivity aqueous solution comprises about 2 mM Tris.
22. Use according to any one of embodiments 17 to 21, wherein the low conductivity aqueous solution contains potassium phosphate.
23. Use according to embodiment 22, wherein the low conductivity aqueous solution comprises about 0.05 mM to about 5 mM potassium phosphate.
24. Use according to embodiment 23, wherein the low conductivity aqueous solution comprises about 0.05 mM to about 2 mM potassium phosphate.
25. Use according to embodiment 24, wherein the low conductivity aqueous solution comprises about 0.5 mM potassium phosphate.
26. Use according to any of the preceeding embodiments, wherein the low conductivity aqueous solution has a pH of about 7 or higher.
27. Use according to embodiment 26, wherein the low conductivity aqueous solution has a pH of about 7.5 or higher.

28. Use according to embodiment 27, wherein the low conductivity aqueous solution has a pH of from about 7 to about 9.5.
29. Use according to embodiment 28, wherein the low conductivity aqueous solution has a pH of from about 7.5 to about 8.5.
30. Use according to embodiment 29, wherein the low conductivity aqueous solution has a pH of about 8.
31. Use according to any one of the preceeding embodiments, wherein the low conductivity aqueous solution wash step is preceded or succeeded by a high conductivity aqueous solution wash step.
32. Use according to embodiment 31, wherein the low conductivity aqueous solution wash step is preceded by a high conductivity aqueous solution wash step.
33. Use according to embodiment 31, wherein the high conductivity aqueous solution has a conductivity value of about 20 mS/cm or higher.
34. Use according to embodiment 33, wherein the high conductivity aqueous solution has a conductivity value of from about 20 mS/cm to about 100 mS/cm.
35. Use according to embodiment 31, wherein an intermediate wash step is performed with a medium conductivity aqueous solution between the low conductivity aqueous solution wash step and the high conductivity aqueous solution wash step.
36. Use according to embodiment 35, wherein the medium conductivity aqueous solution has a conductivity value of from more than 0.5mS/cm to less than 20 mS/cm.
37. Use according to any one of embodiments 33 to 36, wherein the high or medium conductivity aqueous solution comprises an amino acid.
38. Use according to embodiment 37, wherein the high or medium conductivity aqueous solution comprises Histidine.
39. Use according to embodiment 37, wherein the high or medium conductivity aqueous solution comprises Histidine and Tris.
40. Use according to any of the preceeding embodiments, wherein at least one additional chromatography method/step is performed.
41. Use according to embodiment 40, wherein an additional ion exchange chromatography method/step is performed.
42. Use according to embodiment 41, wherein an additional anion exchange chromatography method/step is performed.

43. Use according to embodiment 40, wherein an additional cation exchange chromatography method/step is performed.
44. Use according to embodiment 40, wherein an additional anion exchange chromatography method/step and an additional cation exchange chromatography method/step are performed.
45. Use according to embodiment 40, wherein the use is without a hydrophobic interaction chromatography method/step.
46. Use according to any of the preceeding embodiments, wherein the human IgG4 isotype antibody is an antibody against P-selectin or an antibody against factor IXa and factor X or an antibody against IL-13 or an antibody against amyloid beta.
47. Use according to embodiments 1 to 45, wherein the human IgG1 isotype antibody is an antibody against Influenza B or an antibody against VEGF-A or an antibody against CD22 or an antibody against HER3 and EGFR or an antibody against amyloid beta or an antibody against Her2 or an antibody against Ang2 and VEGF-A or an antibody against carcinoembryonic antigen (CEA) and CD3.
48. Method for producing a human IgG isotype antibody comprising
    a) cultivating a cell comprising a nucleic acid encoding the human IgG isotype antibody,
    b) recovering the human IgG isotype antibody from the cell or the cultivation medium,
    c) contacting the human IgG isotype antibody with an affinity chromatography material,
    d) washing the affinity chromatography material with a low conductivity aqueous solution
    e) recovering the human IgG isotype antibody from affinity chromatography material

    and thereby producing the human IgG isotype antibody.
49. Method for producing a human IgG4 isotype antibody comprising
    a) cultivating a cell comprising a nucleic acid encoding a human IgG4 isotype antibody,
    b) recovering the human IgG4 isotype antibody from the cell or the cultivation medium,
    c) contacting the human IgG4 isotype antibody with an affinity chromatography material,

d) washing the affinity chromatography material with a low conductivity aqueous solution

e) recovering the human IgG4 isotype antibody from the affinity chromatography material

and thereby producing the human IgG4 isotype antibody.

50. Method according to embodiment 48, wherein the human IgG isotype antibody is a human IgG4 isotype antibody or a human IgG1 isotype antibody.

51. Method according to embodiment 48, wherein the human IgG isotype antibody is a human IgG4 isotype antibody or a human IgG1 isotype antibody without a glycosylated glycosylation site in its Fab fragment/with exactly one glycosylation site (at position Asn 297 numbering according to Kabat).

52. Method according to embodiment 48, wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less.

53. Method according to embodiment 52, wherein the low conductivity aqueous solution has a conductivity value of from about 0.03 µS/cm to about 0.5 mS/cm.

54. Method according to embodiment 53, wherein the low conductivity aqueous solution has a conductivity value of from about 0.05 µS/cm to about 0.35 mS/cm.

55. Method according to embodiment 54, wherein the low conductivity aqueous solution is not deionized water.

56. Method according to any one of embodiments 48 to 55, wherein the affinity chromatography is a protein A affinity chromatography or a Protein G affinity chromatography or a single chain Fv ligand (KappaSelect) affinity chromatography.

57. Method according to embodiment 56, wherein the affinity chromatography is a protein A affinity chromatography.

58. Method according to embodiment 56, wherein the protein A affinity chromatography is selected from the group comprising MabSelectSure affinity chromatography, ProSep vA affinity chromatography, Poros Mab Capture A affinity chromatography, ProSep Ultra Plus affinity chromatography, MabSelect SuRe LX, MabSelect, Eshmuno A, Toyopearl AF-rProtein A-650F; Toyopearl AF-rProtein A HC-650HF).

59. Method according to embodiment 48 or 49, wherein the content of a host cell protein is reduced.

60. Method according to any one of embodiments 59, wherein said host cell protein is a Chinese hamster ovary (CHO) host cell protein.

61. Method according to embodiment 60, wherein the host cell protein is a phospholipase.
62. Method according to embodiment 61, wherein the host cell protein is a phospholipase A, phospholipase B, phospholipase C or phospholipase D.
63. Method according to any one of embodiments 62, wherein the host cell protein is phospholipase B-like 2 (PLBL2).
64. Method according to embodiment 60, wherein the host cell protein is phospholipase B-like 2 (PLBL2) or Clusterin.
65. Method according to any of embodiments 48 to 64, wherein the low conductivity aqueous solution contains tris(hydroxymethyl)aminomethane (Tris).
66. Method according to embodiment 65, wherein the low conductivity aqueous solution comprises about 0.1 mM to about 10 mM Tris.
67. Method according to embodiment 66, wherein the low conductivity aqueous solution comprises about 0.1 mM to about 8 mM Tris.
68. Method according to embodiment 67, wherein the low conductivity aqueous solution comprises about 0.5 mM to about 6.5 mM Tris.
69. Method according to embodiment 68, wherein the low conductivity aqueous solution comprises about 2 mM Tris.
70. Method according to embodiment 65 to 69, wherein the low conductivity aqueous solution contains potassium phosphate.
71. Method according to embodiment 70, wherein the low conductivity aqueous solution comprises about 0.2 mM to about 5 mM potassium phosphate.
72. Method according to embodiment 71, wherein the low conductivity aqueous solution comprises about 0.05 mM to about 2 mM potassium phosphate.
73. Method according to embodiment 72, wherein the low conductivity aqueous solution comprises about 0.5 mM potassium phosphate.
74. Method according to any of embodiments 48 to 73, wherein the low conductivity aqueous solution has a pH of about 7 or higher.
75. Method according to embodiment 74, wherein the low conductivity aqueous solution has a pH of about 7.5 or higher.
76. Method according to embodiment 75, wherein the low conductivity aqueous solution has a pH of from about 7 to about 9.5.
77. Method according to embodiment 76, wherein the low conductivity aqueous solution has a pH of from about 7.5 to about 8.5.
78. Method according to embodiment 77, wherein the low conductivity aqueous solution has a pH of about 8.

79. Method according to embodiment 48 or 49, wherein the method additionally comprises washing the affinity chromatography material with a high conductivity aqueous solution before or after washing the affinity chromatography material with low conductivity aqueous solution.
80. Method according to embodiment 79, wherein the method additionally comprises washing the affinity chromatography material with a high conductivity aqueous solution before washing the affinity chromatography material with low conductivity aqueous solution.
81. Method according to embodiment 79, wherein the method additionally comprises washing the affinity chromatography material with a high conductivity aqueous solution and/or with a medium conductivity aqueous solution before or after washing the affinity chromatography material with low conductivity aqueous solution.
82. Method according to embodiment 79, wherein the method additionally comprises washing the affinity chromatography material with a high conductivity aqueous solution and/or with a medium conductivity aqueous solution before washing the affinity chromatography material with low conductivity aqueous solution.
83. Method according to any one of embodiments 79 to 82, wherein the high conductivity aqueous solution has a conductivity value of about 20 mS/cm or higher.
84. Method according to embodiment 83, wherein the high conductivity aqueous solution has a conductivity value of from about 20 mS/cm to about 100 mS/cm.
85. Method according to any one of embodiments 81 to 82, wherein the medium conductivity aqueous solution has a conductivity value of from more than 0.5 mS/cm to less than 20 mS/cm.
86. Method according to any one of embodiments 79 to 85, wherein the high or medium conductivity aqueous solution comprises an amino acid.
87. Method according to embodiment 86, wherein the high or medium conductivity aqueous solution comprises Histidine.
88. Method according to embodiment 86 or 87, wherein the high or medium conductivity aqueous solution comprises Histidine and Tris.
89. Method according to embodiment 48 or 49, wherein at least one additional chromatography method/step is performed after step e).
90. Method according to embodiment 89, wherein an additional ion exchange chromatography method/step is performed after step e).

91. Method according to embodiment 90, wherein an additional anion exchange chromatography method/step is performed after stepe).
92. Method according to embodiment 90, wherein an additional cation exchange chromatography method/step is performed after step e).
93. Method according to embodiment 90, wherein an additional anion exchange chromatography method/step and an additional cation exchange chromatography method/step are performed after step e).
94. Method according to embodiment 48 or 49, wherein the method is without an hydrophobic interaction chromatography method/step.
95. Method according to any of embodiments 49 to 94, wherein the human IgG4 isotype antibody is an antibody against P-selectin or an antibody against factor IXa and factor X or an antibody against IL-13 or an antibody against amyloid beta.
96. Method according to any one of embodiments 48 or 50 to 94, wherein the human IgG1 isotype antibody is an antibody against Influenza B or an antibody against VEGF-A or an antibody against CD22 or a bispecific antibody against HER3 and EGFR or an antibody against amyloid beta or an antibody against Her2 or a bispecific antibody against Ang2 and VEGF-A or an antibody against carcinoembryonic antigen (CEA) and CD3.
97. Method for purifying a human IgG isotype antibody from a sample comprising the steps of
    a) providing a sample comprising a human IgG isotype antibody,
    b) purifying the human IgG isotype antibody with a affinity chromatography method/step, comprising washing the affinity chromatography material with low conductivity aqueous solution.
98. Method according to embodiment 97, wherein the human IgG isotype antibody is a human IgG4 isotype antibody or a human IgG1 isotype antibody.
99. Method according to embodiment 98, wherein the human IgG isotype antibody is a human IgG4 isotype antibody or a human IgG1 isotype antibody without a glycosylated glycosylation site in its Fab fragment/with exactly one glycosylation site (at position Asn 297 numbering according to Kabat).
100. Method according to any one of embodiments 97 to 99, wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less.
101. Method according to embodiment 100, wherein the low conductivity aqueous solution has a conductivity value of from about 0.03 µS/cm to about 0.5 mS/cm.

102. Method according to embodiment 101, wherein the low conductivity aqueous solution has a conductivity value of from about 0.05 µS/cm to about 0.35 mS/cm.

103. Method according to embodiment 102, wherein the low conductivity aqueous solution is not deionized water.

104. Method according to any one of embodiments 97 to 104, wherein the affinity chromatography is a protein A affinity chromatography or a Protein G affinity chromatography or a single chain Fv ligand (KappaSelect) affinity chromatography.

105. Method according to embodiment 104, wherein the affinity chromatography is a protein A affinity chromatography.

106. Method according to embodiment 105, wherein the protein A affinity chromatography is selected from the group comprising MabSelectSure affinity chromatography, ProSep vA affinity chromatography, Poros Mab Capture A affinity chromatography, ProSep Ultra Plus affinity chromatography, MabSelect SuRe LX, MabSelect, Eshmuno A, Toyopearl AF-rProtein A-650F; Toyopearl AF-rProtein A HC-650HF).

107. Method according to any one of embodiments 97 to 106, wherein the content of a host cell protein is reduced.

108. Method according to embodiment 107, wherein said host cell protein is a Chinese hamster ovary (CHO) host cell protein.

109. Method according to embodiment 108, wherein the host cell protein is a phospholipase.

110. Method according to embodiment 109, wherein the host cell protein is a phospholipase A, phospholipase B, phospholipase C or phospholipase D.

111. Method according to any one of embodiments 110, wherein the host cell protein is phospholipase B-like 2 (PLBL2).

112. Method according to embodiment 107, wherein the host cell protein is phospholipase B-like 2 (PLBL2) or Clusterin.

113. Method according to any one of embodiments 97 to 112, wherein the low conductivity aqueous solution contains tris(hydroxymethyl)aminomethane (Tris).

114. Method according to embodiment 113, wherein the low conductivity aqueous solution comprises about 0.1 mM to about 10 mM Tris.

115. Method according to embodiment 114, wherein the low conductivity aqueous solution comprises about 0.1 mM to about 8 mM Tris.

116. Method according to embodiment 115, wherein the low conductivity aqueous solution comprises about 0.5 mM to about 6.5 mM Tris.
117. Method according to embodiment 116, wherein the low conductivity aqueous solution comprises about 2 mM Tris.
118. Method according to any one of embodiments 113 to 117, wherein the low conductivity aqueous solution contains potassium phosphate.
119. Method according to embodiment 118, wherein the low conductivity aqueous solution comprises about 0.2 mM to about 5 mM potassium phosphate.
120. Method according to embodiment 119, wherein the low conductivity aqueous solution comprises about 0.2 mM to about 2 mM potassium phosphate.
121. Method according to embodiment 120, wherein the low conductivity aqueous solution comprises about 0.5 mM potassium phosphate.
122. Method according to any one of embodiments 97 to 121, wherein the low conductivity aqueous solution has a pH of about 7 or higher.
123. Method according to embodiment 122, wherein the low conductivity aqueous solution has a pH of about 7.5 or higher.
124. Method according to embodiment 123, wherein the low conductivity aqueous solution has a pH of from about 7 to about 9.5.
125. Method according to embodiment 124, wherein the low conductivity aqueous solution has a pH of from about 7.5 to about 8.5.
126. Method according to embodiment 125, wherein the low conductivity aqueous solution has a pH of about 8.
127. Method according to any one of embodiments 97 to 126, wherein the method additionally comprises washing the affinity chromatography material with a high conductivity aqueous solution before or after washing the affinity chromatography material with low conductivity aqueous solution.
128. Method according to embodiment 127, wherein the method additionally comprises washing the affinity chromatography material with a high conductivity aqueous solution before washing the affinity chromatography material with low conductivity aqueous solution.
129. Method according to embodiment 127, wherein the method additionally comprises washing the affinity chromatography material with a high conductivity aqueous solution and/or with a medium conductivity aqueous solution before or after washing the affinity chromatography material with low conductivity aqueous solution.
130. Method according to embodiment 127, wherein the method additionally comprises washing the affinity chromatography material with a high

conductivity aqueous solution and/or with a medium conductivity aqueous solution before washing the affinity chromatography material with low conductivity aqueous solution.

131. Method according to any one of embodiments 127 to 130, wherein the high conductivity aqueous solution has a conductivity value of about 20 mS/cm or higher.

132. Method according to embodiment 131, wherein the high conductivity aqueous solution has a conductivity value of from about 20 mS/cm to about 100 mS/cm.

133. Method according to embodiment 129 or 130, wherein the medium conductivity aqueous solution has a conductivity value of from more than 0.5 mS/cm to less than 20 mS/cm.

134. Method according to any one of embodiments 127 to 133, wherein the high or medium conductivity aqueous solution comprises an amino acid.

135. Method according to embodiment 134, wherein the high or medium conductivity aqueous solution comprises Histidine.

136. Method according to embodiment 134, wherein the high or medium conductivity aqueous solution comprises Histidine and Tris.

137. Method according to embodiment 97 or 98, wherein at least one additional chromatography method/step is performed after step b).

138. Method according to embodiment 137, wherein an additional ion exchange chromatography method/step is performed after step b).

139. Method according to embodiment 138, wherein an additional anion exchange chromatography method/step is performed after step b).

140. Method according to embodiment 138, wherein an additional cation exchange chromatography method/step is performed after step b).

141. Method according to embodiment 138, wherein an additional anion exchange chromatography method/step and an additional cation exchange chromatography method/step are performed after stepb).

142. Method according to any one of embodiments 97 to 141, wherein the method is without an hydrophobic interaction chromatography method/step.

143. Method according to embodiment 97 or 98, wherein the human IgG4 isotype antibody is an antibody against P-selectin or an antibody against factor IXa and factor X or an antibody against IL-13 or an antibody against amyloid beta.

144. Method according to embodiment 97 or 98, wherein the human IgG1 isotype antibody is an antibody against Influenza B or an antibody against VEGF-A or an antibody against CD22 or an (bispecific) antibody against HER3 and EGFR

or an antibody against amyloid beta or an antibody against Her2 or a bispecific antibody against Ang2 and VEGF-A or a bispecific antibody against carcinoembryonic antigen (CEA) and CD3.

145. Use of a low conductivity aqueous solution in a wash step of a protein A chromatography for reducing the content of a host cell protein wherein the protein A chromatography is used to purify a human IgG4 or IgG1 isotype antibody, wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less.

146. Method for producing a human IgG4 or IgG1 isotype antibody comprising the steps of

a) cultivating a cell comprising a nucleic acid encoding a human IgG4 or IgG1 isotype antibody,

b) recovering the human IgG4 or IgG1 isotype antibody from the cell or the cultivation medium,

c) contacting the human IgG4 or IgG1 isotype antibody with a protein A chromatography material,

d) washing the protein A chromatography material with a low conductivity aqueous solution, wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less,

e) recovering the human IgG4 or IgG1 isotype antibody from the protein A chromatography material

and thereby producing the human IgG4 or IgG1 isotype antibody.

147. Method for purifying a human IgG4 or IgG1 isotype antibody from a sample comprising the steps of

a) providing a sample comprising a human IgG4 or IgG1 isotype antibody,

b) purifying the human IgG4 or IgG1 isotype antibody with a protein A chromatography method/step, comprising washing the protein A chromatography material with a low conductivity aqueous solution, wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less.

148. Use according to any of embodiments 5 to 7, wherein the low conductivity aqueous solution is deionized water.

149. Method according to embodiment 54, wherein the low conductivity aqueous solution is deionized water.

Description of the Sequence Listing

SEQ ID NO: 01	variable heavy chain domain VH of <VEGF>
SEQ ID NO: 02	variable light chain domain VL of <VEGF>
SEQ ID NO: 03	variable heavy chain domain VH of <ANG-2>
SEQ ID NO: 04	variable light chain domain VL of < ANG-2>
SEQ ID NO: 05	variable heavy chain domain VH of anti-amyloid beta antibody (IgG1 isotype)
SEQ ID NO: 06	variable light chain domain VL of anti-amyloid beta antibody (IgG1 isotype)
SEQ ID NO: 07	variable heavy chain domain VH1 of anti-P-selectin antibody
SEQ ID NO: 08	variable heavy chain domain VH2 of anti-P-selectin antibody
SEQ ID NO: 09	variable heavy chain domain VH3 of anti-P-selectin antibody
SEQ ID NO: 10	variable light chain domain VL1 of anti-P-selectin antibody
SEQ ID NO: 11	variable light chain domain VL2 of anti-P-selectin antibody
SEQ ID NO: 12	variable light chain domain VL3 of anti-P-selectin antibody

Example 1

Material and Methods

Antibodies

The current invention is exemplified using a number of exemplary antibodies, including: an antibody against P-selectin (anti-P-selectin antibody; inclacumab; IgG4 isotype) as described in WO 2005/100402 or SEQ ID NO: 07 to SEQ ID NO: 12; a bispecific antibody against factor IXa and factor X (anti-FIXa/X antibody; IgG4 isotype) as described in WO 2012/067176; with an antibody against Her2; a bispecific antibody against Ang2 and VEGF-A (anti-Ang2/VEGF-A antibody; vanucizumab; IgG1 isotype) as described in WO 2011/117329 or SEQ ID NO: 01 to SEQ ID NO: 04; an antibody against amyloid beta (anti-amyloid beta antibody; gantenerumab; IgG1 isotype) as described in WO 2003/070760 or SEQ ID NO: 05 to SEQ ID NO: 06. Also included herein are a number of IgG1 antibodies and IgG4 antibodies, as described in the examples below.

Detection methods for overall host cell protein (HCP), Phospholipase B-like 2 protein (PLBL2) and Clusterin

a) CHO HCP assay

The residual CHO HCP content in process samples is determined by an electrochemiluminescence immunoassay (ECLIA) on cobas e 411 immunoassay analyzer (Roche Diagnostics).

The assay is based on a sandwich principle using polyclonal anti-CHO HCP antibody from sheep.

First incubation: Chinese hamster ovary host cell protein (CHO HCP) from 15 µL sample (neat and/or diluted) and a biotin conjugated polyclonal CHO HCP specific antibody form a sandwich complex, which becomes bound to streptavidin-coated microparticles via interaction of biotin with streptavidin.

Second incubation: After addition of polyclonal CHO HCP-specific antibody labeled with ruthenium complex (Tris(2,2`-bipyridyl)ruthenium(II)-complex) a ternary sandwich complex is formed on the microparticles.

The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed in a washing step. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

The concentration of CHO HCP in the test sample is finally calculated from a CHO HCP standard curve of known concentration.

b) CHO PLBL2 assay

The residual Chinese hamster ovary (CHO) Phospholipase B-like 2 protein (PLBL2) content in process samples is determined by an electrochemiluminescence immunoassay (ECLIA) on cobas e 411 immunoassay analyzer (Roche Diagnostics).

The assay is based on a sandwich principle using monoclonal anti-CHO PLBL2 antibody from mouse.

In a first incubation step, CHO PLBL2 from 30 µL sample (neat and/or diluted), biotin labeled monoclonal CHO PLBL2-specific antibody, and a monoclonal CHO

PLBL2-specific antibody labeled with a ruthenium complex (Tris(2,2`-bipyridyl)ruthenium(II)-complex) form a sandwich complex.

In a second step after addition of streptavidin-coated microparticles, the ternary complex becomes bound to the solid phase via interaction of biotin and streptavidin.

The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed in a washing step. Application of a voltage to the electrode then induces chemiluminescence, which is measured by a photomultiplier.

The concentration of CHO PLBL2 in the test sample is finally calculated from a CHO PLBL2 standard curve of known concentration.

c) Clusterin assay

The residual Clusterin content in process samples is determined by a commercial assay from Merck Millipore (GyroMark HT Kit GYRCLU-37K) which was used according to the manufacturer's instructions.

In brief, this assay is a Sandwich ELISA based, sequentially, on:

1) binding of the rat Clusterin biotinylated capture antibody to the streptavidin coated affinity columns of the Bioaffy 1000nL CD,

2) capture of rat Clusterin molecules from samples to the anti Clusterin antibody,

3) binding of a second dye-labeled anti Clusterin detection antibody to the captured molecules,

4) quantification of the rat Clusterin using the Gyrolab Evaluator.

Example 2

Purification of an anti-P-Selectin antibody (IgG4 isotype) in a protein A chromatography

Antibody: anti-P-Selectin

General chromatography conditions

Column resin: Protein A material "Mab Select SuRe" (GE-Healthcare) Ø 1cm, Height: 20,1 cm, CV: 15,79 ml

Equipment: Äkta Avant 150

Flow rate: 300 cm/h during all steps

A solution containing an anti-P-Selectin antibody, was applied to a Protein A affinity column after equilibration (step 1) of the column. Initial load of PLBL2 determined in solution containing an anti-P-Selectin antibody: 335 ng PLBL2/mg of antibody. Initial load of Clusterin determined in solution containing an anti-P-Selectin antibody: 2874.8 ng Clusterin/mg of antibody. Initial load of CHOP determined in solution containing an anti-P-Selectin antibody: 100971 ng CHOP/mg of antibody.

The chromatographic steps were performed according to the following general scheme:

Step 1: Equilibration:
Step 2: Load of antibody containing solution
Step 3: Wash I
Step 4: Wash II
Step 5: Wash III
Step 6: Wash IV (additional wash)
Step 7: Elution

After Elution from Protein A affinity column the protein was determined by size exclusion chromatography (SEC) and spectrophotometrically (OD) Analytics.

SEC:

Resin:	TSK 3000 (Tosoh)
Column:	300 x 7,8 mm
Flow rate:	0,5 ml/min
Buffer:	200 mM potassium phosphate containing 250 mM potassium chloride, adjusted to pH 7,0

Wavelength: 280 nm

OD:

Specific coefficient: 1,54

Wavelength: 280 nm minus 320 nm

Specific buffer conditions for Protein A chromatography (anti-P-Selectin antibody)

a) Control (wash with equilibration buffer only)

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 4: Wash II:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 5: Wash III:	---
Step 6: Wash IV:	---
Step 7: Elution:	50 mM acetic acid, pH 4,0

b) low conductivity wash (with Tris buffer only)

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 4: Wash II:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 5: Wash III:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 6: Wash IV:	2 mM Tris, pH 8.0
Step 7: Elution:	50 mM acetic acid, pH 4,0

c) low conductivity wash (with potassium phosphate (KP) only)

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 4: Wash II:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 5: Wash III:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 6: Wash IV:	0.5 mM potassium phosphate, pH 8.0
Step 7: Elution:	50 mM acetic acid, pH 4,0

d) high conductivity wash (with Tris buffer only)

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 4: Wash II:	700 mM Tris, pH 7,2
Step 5: Wash III:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 6: Wash IV:	---
Step 7: Elution:	50 mM acetic acid, pH 4,0

e) low conductivity wash (with Tris buffer only; pH 6.0)

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 4: Wash II:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 5: Wash III:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 6: Wash IV:	2 mM Tris, pH 6.0
Step 7: Elution:	50 mM acetic acid, pH 4,0

f) high conductivity wash (with Histidine (His)/Tris buffer only)

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 4: Wash II:	200 mM His/1000 mM Tris, pH 7,0
Step 5: Wash III:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 6: Wash IV:	---
Step 7: Elution:	50 mM acetic acid, pH 4,0

g) low conductivity Tris + high conductivity Histidine (His)/Tris

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 4: Wash II:	200 mM His/1000 mM Tris, pH 7,0
Step 5: Wash III:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 6: Wash IV:	2 mM Tris, pH 8.0
Step 7: Elution:	50 mM acetic acid, pH 4,0

h) low conductivity potassium phosphate (KP) + high conductivity Histidine (His)/Tris

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 4: Wash II:	200 mM His/1000 mM Tris, pH 7,0
Step 5: Wash III:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 6: Wash IV:	0.5 mM potassium phosphate, pH 8.0
Step 7: Elution:	50 mM acetic acid, pH 4,0

i) low conductivity Tris + high conductivity Tris

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 4: Wash II:	700 mM Tris, pH 7,2
Step 5: Wash III:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 6: Wash IV:	2 mM Tris, pH 8.0
Step 7: Elution:	50 mM acetic acid, pH 4,0

j) low conductivity Tris; pH 6.0 + high conductivity Tris

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 4: Wash II:	700 mM Tris, pH 7,2
Step 5: Wash III:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 6: Wash IV:	2 mM Tris, pH 6.0
Step 7: Elution:	50 mM acetic acid, pH 4,0

Results:

Run	HCP total [ng/mg]	PLBL2 [ng/mg]	Clusterin [ng/mg]	Yield [%]
a	218	105.9	27.4	93.44
b	105	0.8	11.2	94.61

Run	HCP total [ng/mg]	PLBL2 [ng/mg]	Clusterin [ng/mg]	Yield [%]
c	114	0.7	11.8	86.67
d	48	14.7	23.2	89.33
e	155	18.7	53.8	107.3
f	106	2.9	21.6	84.9
g	83	0.4	11.8	85
h	91	0.4	9.1	80.34
i	90	0.4	15.7	84.92
j	141	1.5	53	106.9

Example 3

Purification of an anti-amyloid beta antibody (IgG1 isotype) in a protein A chromatography

General conditions were according to the conditions described in Example 2.

Antibody: anti-amyloid beta.

Initial load of PLBL2 determined in solution containing an anti-amyloid beta antibody: 2019.7 ng PLBL2/mg of antibody. Initial load of CHOP determined in solution containing an anti-amyloid beta antibody: 578908 ng CHOP/mg of antibody.

Specific buffer conditions for Protein A chromatography

a) Control (wash with equilibration buffer only)

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 4: Wash II:	25 mM Tris, 25 mM NaCl, pH 7,0

Step 5: Wash III:	---
Step 6: Wash IV:	---
Step 7: Elution:	50 mM acetic acid, pH 4,0

b) low conductivity wash (with Tris buffer only)

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 4: Wash II:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 5: Wash III:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 6: Wash IV:	2 mM Tris, pH 8.0
Step 7: Elution:	50 mM acetic acid, pH 4,0

c) high conductivity wash (with Tris buffer only)

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 4: Wash II:	700 mM Tris, pH 7,2
Step 5: Wash III:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 6: Wash IV:	---
Step 7: Elution:	50 mM acetic acid, pH 4,0

d) low conductivity Tris + high conductivity Histidine (His)/Tris

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 4: Wash II:	200 mM His/1000 mM Tris, pH 7,0
Step 5: Wash III:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 6: Wash IV :	2 mM Tris, pH 8.0
Step 7: Elution:	50 mM acetic acid, pH 4,0

Run	HCP total [ng/mg]	PLBL2 [ng/mg]	Clusterin [ng/mg]	Yield [%]
a	6828	17.3	n.d.	80.3

Run	HCP total [ng/mg]	PLBL2 [ng/mg]	Clusterin [ng/mg]	Yield [%]
b	7794	17.8	n.d.	73.1
c	1595	1.7	n.d.	55.6
d	6132	2.3	n.d.	67.3

Example 4

Purification of an anti-Her2 antibody (IgG1 isotype) in a protein A chromatography

General conditions were according to the conditions described in Example 2.

Antibody: anti-Her2

Initial load of PLBL2 determined in solution containing an anti-Her2 antibody: 1662.5 ng PLBL2/mg of antibody. Initial load of CHOP determined in solution containing an anti-Her2 antibody: 727070 ng CHOP/mg of antibody.

Specific buffer conditions for Protein A chromatography

a) Control (wash with equilibration buffer only)

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 4: Wash II:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 5: Wash III:	---
Step 6: Wash IV:	---
Step 7: Elution:	50 mM acetic acid, pH 4,0

b) low conductivity wash (with Tris buffer only)

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 4: Wash II:	25 mM Tris, 25 mM NaCl, pH 7,0

Step 5: Wash III:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 6: Wash IV:	2 mM Tris, pH 8.0
Step 7: Elution:	50 mM acetic acid, pH 4,0

c) high conductivity wash (with Tris buffer only)

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 4: Wash II:	700 mM Tris, pH 7,2
Step 5: Wash III:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 6: Wash IV:	---
Step 7: Elution:	50 mM acetic acid, pH 4,0

d) low conductivity Tris + high conductivity Histidine (His)/Tris

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 4: Wash II:	200 mM His/1000 mM Tris, pH 7,0
Step 5: Wash III:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 6: Wash IV:	2 mM Tris, pH 8.0
Step 7: Elution:	50 mM acetic acid, pH 4,0

Run	HCP total [ng/mg]	PLBL2 [ng/mg]	Clusterin [ng/mg]	Yield [%]
a	309	1.2	n.d.	85.5
b	227	1	n.d.	77
c	26	0.2	n.d.	70.9
d	42	0.5	n.d.	83.8

Example 5

Purification of a bispecific anti-Ang2/VEGF-A antibody (IgG1 isotype) in a protein A chromatography

General conditions were according to the conditions described in Example 2.

Antibody: anti-Ang2/VEGF-A

Initial load of PLBL2 determined in solution containing a bispecific anti-Ang2/VEGF-A antibody: 919.7 ng PLBL2/mg of antibody. Initial load of CHOP determined in solution containing an anti-Ang2/VEGF-A: 682304 ng CHOP/mg of antibody.

Specific buffer conditions for Protein A chromatography

a) Control (wash with equilibration buffer only)

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 4: Wash II:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 5: Wash III:	---
Step 6: Wash IV:	---
Step 7: Elution:	50 mM acetic acid, pH 4,0

b) low conductivity wash (with Tris buffer only)

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 4: Wash II:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 5: Wash III:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 6: Wash IV:	2 mM Tris, pH 8.0
Step 7: Elution:	50 mM acetic acid, pH 4,0

c) high conductivity wash (with Tris buffer only)

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0

Step 4: Wash II:	700 mM Tris, pH 7,2
Step 5: Wash III:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 6: Wash IV:	---
Step 7: Elution:	50 mM acetic acid, pH 4,0

d) low conductivity Tris + high conductivity Histidine (His)/Tris

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 4: Wash II:	200 mM His/1000 mM Tris, pH 7,0
Step 5: Wash III:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 6: Wash IV:	2 mM Tris, pH 8.0
Step 7: Elution:	50 mM acetic acid, pH 4,0

Run	HCP total [ng/mg]	PLBL2 [ng/mg]	Clusterin [ng/mg]	Yield [%]
a	3035	1.0	n.d.	85.0
b	1707	0.8	n.d.	79.8
c	655	0.7	n.d.	52
d	1050	0.8	n.d.	92.3

Example 6

Purification of a bispecific anti-FIXa/X antibody (IgG4 isotype) in a protein A chromatography

Purification of anti-FIXa/X antibody was tested in two different chromatograhpy settings:

Setting 1

General conditions were according to the conditions described in Example 2.

Antibody: anti-FIXa/X

Initial load of PLBL2 determined in solution containing an anti-FIXa/X antibody: 557 ng PLBL2/mg of antibody. Initial load of CHOP determined in solution containing an anti-FIXa/X: 387377 ng CHOP/mg of antibody.

Specific buffer conditions for Protein A chromatography

a) high conductivity wash (with Tris buffer only)

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 4: Wash II:	700 mM Tris, pH 7,2
Step 5: Wash III:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 6: Wash IV:	---
Step 7: Elution:	50 mM acetic acid, pH 4,0

b) low conductivity Tris + high conductivity Histidine (His)/Tris

Step 1: Equilibration:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 2: Load	
Step 3: Wash I:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 4: Wash II:	200 mM His/1000 mM Tris, pH 7,0
Step 5: Wash III:	25 mM Tris, 25 mM NaCl, pH 7,0
Step 6: Wash IV:	2 mM Tris, pH 8.0
Step 7: Elution:	50 mM acetic acid, pH 4,0

Run	HCP total [ng/mg]	PLBL2 [ng/mg]	Clusterin [ng/mg]	Yield [%]
a	1632	19.1	n.d.	79
b	2148	1.1	n.d.	77

Setting 2

General chromatography conditions

Column resin: Protein A material “Mab Select SuRe” (GE-Healthcare) Ø 1cm, Height: 20,1 cm, CV: 15,79 ml

Equipment: Äkta Avant 150

Flow rate: 300 cm/h during all steps

A solution containing an anti-FIXa/X antibody, was applied to a Protein A affinity column after equilibration (step 1) of the column.

Initial load of PLBL2 determined in solution containing an anti-FIXa/X antibody: 557 ng PLBL2/mg of antibody.

The chromatographic steps were performed according to the following general scheme:

Step 1: Equilibration:
Step 2: Load of antibody containing solution
Step 3: Wash I
Step 4: Wash II
Step 5: Wash III (additional wash)
Step 6: Elution

Specific buffer conditions for Protein A chromatography

a) high conductivity wash (with NaSO4 buffer only)

Step 1: Equilibration:	20 mM NaPO4, pH 7,5
Step 2: Load	
Step 3: Wash I:	450 mM NaSO4, 20 mM NaAc, pH 4,8
Step 4: Wash II:	20 mM NaPO4, pH 7,5
Step 5: Wash III:	---
Step 6: Elution:	35 mM acetic acid, pH 4,0

b) low conductivity wash (Tris 1 mM) + high conductivity wash (with NaSO4)

Step 1: Equilibration:	20 mM NaPO4, pH 7,5
Step 2: Load	
Step 3: Wash I:	450 mM NaSO4, 20 mM NaAc, pH 4,8
Step 4: Wash II:	20 mM NaPO4, pH 7,5
Step 5: Wash III:	1 mM Tris, pH 8.0
Step 6: Elution:	50 mM acetic acid, pH 4,0

c) low conductivity wash (Tris 2 mM) + high conductivity wash (with NaSO4)

Step 1: Equilibration:	20 mM NaPO4, pH 7,5
Step 2: Load	
Step 3: Wash I:	450 mM NaSO4, 20 mM NaAc, pH 4,8
Step 4: Wash II:	20 mM NaPO4, pH 7,5
Step 5: Wash III:	2 mM Tris, pH 8.0
Step 6: Elution:	35 mM acetic acid, pH 4,0

d) low conductivity wash (Tris 4 mM) + high conductivity wash (with NaSO4)

Step 1: Equilibration:	20 mM NaPO4, pH 7,5
Step 2: Load	
Step 3: Wash I:	450 mM NaSO4, 20 mM NaAc, pH 4,8
Step 4: Wash II:	20 mM NaPO4, pH 7,5
Step 5: Wash III:	4 mM Tris, pH 8.0
Step 6: Elution:	50 mM acetic acid, pH 4,0

e) low conductivity wash (Tris 6 mM) + high conductivity wash (with NaSO4)

Step 1: Equilibration:	20 mM NaPO4, pH 7,5
Step 2: Load	
Step 3: Wash I:	450 mM NaSO4, 20 mM NaAc, pH 4,8
Step 4: Wash II:	20 mM NaPO4, pH 7,5
Step 5: Wash III:	6 mM Tris, pH 8.0
Step 6: Elution:	50 mM acetic acid, pH 4,0

f) low conductivity wash (Tris 4 mM, pH 7.8) + high conductivity wash (with NaSO4)

Step 1: Equilibration:	20 mM NaPO4, pH 7,5
Step 2: Load	
Step 3: Wash I:	450 mM NaSO4, 20 mM NaAc, pH 4,8
Step 4: Wash II:	20 mM NaPO4, pH 7,5
Step 5: Wash III:	4 mM Tris, pH 7.8
Step 6: Elution:	50 mM acetic acid, pH 4,0

g) low conductivity wash (Tris 4 mM, pH 8.2) + high conductivity wash (with NaSO4)

Step 1: Equilibration:	20 mM NaPO4, pH 7,5
Step 2: Load	
Step 3: Wash I:	450 mM NaSO4, 20 mM NaAc, pH 4,8
Step 4: Wash II:	20 mM NaPO4, pH 7,5
Step 5: Wash III:	4 mM Tris, pH 8.2
Step 6: Elution:	50 mM acetic acid, pH 4,0

h) low conductivity wash (Tris 2 mM) + high conductivity wash (with Histidine (His)/Tris 1 M)

Step 1: Equilibration:	20 mM NaPO4, pH 7,5
Step 2: Load	
Step 3: Wash I:	200 mM His/1000 mM Tris, pH 7,0
Step 4: Wash II:	20 mM NaPO4, pH 7,5
Step 5: Wash III:	2 mM Tris, pH 8.0
Step 6: Elution:	35 mM acetic acid, pH 4,0

i) low conductivity wash (Tris 2 mM) + high conductivity wash (Histidine (His)/Tris 0.85 M)

Step 1: Equilibration:	20 mM NaPO4, pH 7,5
Step 2: Load	
Step 3: Wash I:	200 mM His/850 mM Tris, pH 7,0
Step 4: Wash II:	20 mM NaPO4, pH 7,5
Step 5: Wash III:	2 mM Tris, pH 8.0
Step 6: Elution:	50 mM acetic acid, pH 4,0

j) low conductivity wash (Tris 2 mM) + high conductivity wash (Histidine (His)/Tris 0.7 M)

Step 1: Equilibration:	20 mM NaPO4, pH 7,5
Step 2: Load	
Step 3: Wash I:	200 mM His/700 mM Tris, pH 7,0
Step 4: Wash II:	20 mM NaPO4, pH 7,5
Step 5: Wash III:	2 mM Tris, pH 8.0
Step 6: Elution:	50 mM acetic acid, pH 4,0

k) low conductivity wash (Tris 2 mM) + high conductivity wash (Histidine (His)/Tris 0.55 M)

Step 1: Equilibration:	20 mM NaPO4, pH 7,5
Step 2: Load	
Step 3: Wash I:	200 mM His/550 mM Tris, pH 7,0
Step 4: Wash II:	20 mM NaPO4, pH 7,5
Step 5: Wash III:	2 mM Tris, pH 8.0
Step 6: Elution:	50 mM acetic acid, pH 4,0

Run	HCP total [ng/mg]	PLBL2 [ng/mg]	Clusterin [ng/mg]	Yield [%]
a	1518	204.2	n.d.	82
b	646	1	n.d.	73.8
c	737	1.2	n.d.	79
d	595	1.4	n.d.	78.5
e	685	1.8	n.d.	79.5
f	692	1.4	n.d.	78.2
g	707	1.1	n.d.	76.4
h	299	0.5	n.d.	79
i	140	0.4	n.d.	70
j	100	0.5	n.d.	71.9
k	112	0.7	n.d.	73

Example 7

General procedure/conditions:

Mock Cell Culture Fluid

Null harvested cell culture fluid was produced using non-transfected CHO-DP12 cells cultured in serum-free media. Fermentation was performed at the 2 L-scale using a representative cell culture process. At the end of 14 days of fermentation, cell culture fluid was harvested via centrifugation and sterile filtration. This harvested cell culture fluid (HCCF) was then stored at −70 °C until experimentation.

Purified PLBL2

Recombinant CHO PLBL2 with a C-terminal hexahistidine-tag was expressed in 35 L-scale transient transfections and purified from harvested cell culture fluid as previously described (Vanderlaan et al., 2015). Purified PLBL2 was then formulated in a PBS solution and stored at −70°C until experimentation.

Purified Antibody

Recombinant humanized antibodies were expressed in CHO cells and purified using column chromatography to ensure PLBL2 concentration was below 20 ng/mg. Prior to beginning each study, each antibody was buffer-exchanged into PBS using PD-10 desalting columns (GE Healthcare).

Preparation of Load Material for Protein A Chromatography

To normalize the population and abundance of host cell proteins in the Protein A load across antibodies, purified antibodies were diluted to the same concentration with PBS and spiked into HCCF from a non-producing cell line to give a final antibody titer of 5 g/L. A control was also prepared wherein PBS was added instead of the purified antibody to evaluate non-specific host cell protein binding to the Protein A resin in the absence of antibody.

Packed-Bed Column Chromatography

All packed-bed column chromatography experiments were performed using a 0.66 cm inner diameter by 20 cm bed height MabSelect SuRe (GE Healthcare) Protein A resin column. For each purification, the column was first equilibrated for 3 column volumes (CVs) with 25 mM tris, 25 mM NaCl, pH 7.7 (equilibration buffer). Then Protein A load was applied to a target load density of 30 g antibody/L resin, after which the column was washed for 3 CVs with equilibration buffer, 3 CVs of different types of washing buffers, and again with 3 CVs of equilibration buffer. Subsequently, antibody was eluted at low pH with 0.1 to 0.15 M acetic acid, and eluate pools were collected starting at 0.5 OD at the beginning of the elution peak; pooling was terminated after 2.8 CVs. For the control run with PBS-spiked null HCCF, a 2.8 CV mock elution pool was generated starting from 1 CV to 3.8 CVs after the start of the elution phase. At the end of every run, each Protein A eluate was then titrated to pH 5.0 using 1.5 M tris base. The column was then cleaned with a 0.1 M sodium hydroxide solution. All phases had a volumetric flow rate of 20 CV/h except for the load, first equilibration wash, and elution phases, which had a flow rate of 15 CV/h.

A) Purification of an exemplary antibody (IgG4 isotype), Antibody A, in a protein A chromatography

Specific washing buffer conditions for purification of Antibody A (IgG4 isotype) using the general procedure of Example 7 (as outlined above):

a) 0.4 M Potassium Phosphate, pH 7.0
b) 25 mM Tris, 25 mM NaCl, pH 7.7
c) 0.75 M Arg-HCl, pH 7.0
d) 0.6 M NaCl, pH 7.0
e) Deionized water

Results:

	HCP total [ng/mg]	PLBL2 [ng/mg]
	Load: 1067817	Load: 7668
a	1434	167
b	1440	107
c	331	40
d	1864	66
e	1204	31

B) Purification of an exemplary antibody (IgG1 isotype), Antibody B, in a protein A chromatography

Specific washing buffer conditions for purification of Antibody B (IgG1 isotype) using the general procedure of Example 7:

a) Deionized water

Results:

	HCP total [ng/mg]	PLBL2 [ng/mg]
	Load: 1067817	Load: 7668
a	2630	71

C) Purification of an exemplary antibody (IgG4 isotype), Antibody C, in a protein A chromatography

Specific washing buffer conditions for purification of Antibody C (IgG4 isotype) using the general procedure of Example 7:

a) 0.4 M Potassium Phosphate, pH 7.0
b) 25 mM Tris, 25 mM NaCl, pH 7.7
c) 0.75 M Arg-HCl, pH 7.0
d) 0.6 M NaCl, pH 7.0
e) Deionized water

Results:

	HCP total [ng/mg]	PLBL2 [ng/mg]
	Load: 1067817	Load: 7668
a	314	434
b	361	369
c	214	82
d	780	214
e	410	68

D) Purification of an exemplary antibody (IgG1 isotype), Antibody D, in a protein A chromatography

Specific washing buffer conditions for purification of Antibody D (IgG1 isotype) using the general procedure of Example 7:

a) Deionized water

	HCP total [ng/mg]	PLBL2 [ng/mg]
	Load: 1067817	Load: 7668
a	6427	28

E) Purification of an exemplary antibody (IgG1 isotype), Antibody E, in a protein A chromatography

Specific washing buffer conditions for purification of Antibody E (IgG1 isotype) using the general procedure of Example 7:

a) 0.4 M Potassium Phosphate, pH 7.0
b) 31 mM Tris, pH 8.5
c) 55 mM Tris, pH 9.0
d) Deionized water

Results:

	HCP total [ng/mg]	PLBL2 [ng/mg]
	Load: 169706	Load: 333
a	1307	36
b	884	7
c	601	0.2
d	929	8

F) Purification of an exemplary antibody (IgG1 isotype), Antibody F, in a protein A chromatography

Specific washing buffer conditions for purification of Antibody F using the general procedure of Example 7:

a) 25 mM Tris, pH 9.0

Results:

	HCP total [ng/mg]	PLBL2 [ng/mg]
	Load: 994582	Load: 1363
a	806	0.5

Patent Claims

1. Use of a low conductivity aqueous solution in a wash step of a protein A chromatography for reducing the content of a host cell protein wherein the protein A chromatography is used to purify a human IgG4 or IgG1 isotype antibody, wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less.

2. Use according to claim 1, wherein the host cell protein is phospholipase B-like 2 (PLBL2) or Clusterin.

3. Use according to any one of claims 1 to 2, wherein the low conductivity aqueous solution comprises about 0.1 mM to about 8 mM Tris.

4. Use according to any one of claims 1 to 2, wherein the low conductivity aqueous solution comprises about 0.05 mM to about 2 mM potassium phosphate.

5. Use according any one of claims 1 to 4, wherein the low conductivity aqueous solution has a pH of about 7 or higher.

6. Use according to any one of claims 1 to 5, wherein the low conductivity aqueous solution wash step is preceded or succeeded by a high conductivity aqueous solution wash step.

7. Use according to claim 6, wherein the high conductivity aqueous solution has a conductivity value of about 20 mS/cm or higher.

8. Use according to any one of claims 6 to 7, wherein the high conductivity aqueous solution comprises Histidine.

9. Use according to any one of claims 1 to 8, wherein the human IgG4 isotype antibody is an antibody against P-selectin or an antibody against factor IXa and factor X or an antibody against IL-13 or an antibody against amyloid beta.

10. Use according to any one of claims 1 to 8, wherein the human IgG1 isotype antibody is an antibody against Influenza B or an antibody against VEGF-A or an antibody against CD22 or a bispecific antibody against HER3 and EGFR or an antibody against amyloid beta or an antibody against Her2 or a

bispecific antibody against Ang2 and VEGF-A or a bispecific antibody against carcinoembryonic antigen (CEA) and CD3.

11. Method for producing a human IgG4 or IgG1 isotype antibody comprising the steps of

a) cultivating a cell comprising a nucleic acid encoding a human IgG4 or IgG1 isotype antibody,

b) recovering the human IgG4 or IgG1 isotype antibody from the cell or the cultivation medium,

c) contacting the human IgG4 or IgG1 isotype antibody with a protein A chromatography material,

d) washing the protein A chromatography material with a low conductivity aqueous solution, wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less,

e) recovering the human IgG4 or IgG1 isotype antibody from the protein A chromatography material

and thereby producing the human IgG4 or IgG1 isotype antibody.

12. Method for purifying a human IgG4 or IgG1 isotype antibody from a sample comprising the steps of

a) providing a sample comprising a human IgG4 or IgG1 isotype antibody,

b) purifying the human IgG4 or IgG1 isotype antibody with a protein A chromatography method/step, comprising washing the protein A chromatography material with a low conductivity aqueous solution, wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less.

13. Method according to any one of claims 11 to 12, wherein the amount of a host cell protein is reduced and wherein said host cell protein is phospholipase B-like 2 (PLBL2) or Clusterin.

14. Method according to any one of claims 11 to 13, wherein the low conductivity aqueous solution comprises about 0.1 mM to about 8 mM Tris.

15. Method according to any one of claims 11 to 14, wherein the low conductivity aqueous solution comprises about 0.05 mM to about 2 mM potassium phosphate.

16. Method according to any one of claims 11 to 15, wherein the low conductivity aqueous solution has a pH of about 7 or higher.

17. Method according to any one of claims 11 to 16, wherein the method additionally comprises washing the affinity chromatography material with a high conductivity aqueous solution and/or with a medium conductivity aqueous solution before or after washing the protein A chromatography material with low conductivity aqueous solution.

18. Method according to claim 17, wherein the high conductivity aqueous solution has a conductivity value of about 20 mS/cm or higher.

19. Method according to claim 17, wherein the medium conductivity aqueous solution has a conductivity value of from more than 0.5 mS/cm to less than 20 mS/cm.

20. Method according to any one of claims 17 to 19, wherein the high or medium conductivity aqueous solution comprises Histidine.

21. Method according to any one of claims 11 to 20, wherein the human IgG4 isotype antibody is an antibody against P-selectin or an antibody against factor IXa and factor X or an antibody against IL-13 or an antibody against amyloid beta.

22. Method according to any one of claims 11 to 20, wherein the human IgG1 isotype antibody is an antibody against Influenza B or an antibody against VEGF-A or an antibody against CD22 or a bispecific antibody against HER3 and EGFR or an antibody against amyloid beta or an antibody against Her2 or a bispecific antibody against Ang2 and VEGF-A or a bispecific antibody against carcinoembryonic antigen (CEA) and CD3.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/069604

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K1/22 C07K16/10 C07K16/22 C07K16/24 C07K16/28
C07K16/46
ADD.
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)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, EMBASE

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 October 2016	26/10/2016

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Form PCT/ISA/210 (second sheet) (April 2005)

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