

One aspect as reported herein is the use of 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 0.5 mS/cm or less, wherein the host cell protein is phospholipase B-like 2 (PLBL2) or Clusterin.

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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,
- 5 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,
- 10 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.

15 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,
- 20 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 0.5 mS/cm or less, wherein the amount of a host cell protein is reduced and wherein said host cell protein is phospholipase B-like 2 (PLBL2) or Clusterin,
- 25 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.

As reported herein is 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,
- 5 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.

One aspect as reported herein is 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,
- 10 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 0.5 mS/cm or less, wherein the amount of a host cell protein is reduced and wherein said
15 host cell protein is phospholipase B-like 2 (PLBL2) or Clusterin.

In one embodiment 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 the human IgG1 isotype antibody is an antibody against Influenza B or an antibody against
20 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 the low conductivity aqueous solution has a conductivity value
25 of about 0.5 mS/cm or less.

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 0.5 mS/cm or less, wherein the host cell protein is phospholipase B-like 2 (PLBL2) or Clusterin.
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2. Use according to claim 1, wherein the low conductivity aqueous solution comprises 0.1 mM to 8 mM Tris.
- 10 3. Use according to claim 1, wherein the low conductivity aqueous solution comprises 0.05 mM to 2 mM potassium phosphate.
4. Use according any one of claims 1 to 3, wherein the low conductivity aqueous solution has a pH of 7 or higher.
- 15 5. Use according to any one of claims 1 to 4, wherein the low conductivity aqueous solution wash step is preceded or succeeded by a high conductivity aqueous solution wash step.
6. Use according to claim 5, wherein the high conductivity aqueous solution has a conductivity value of 20 mS/cm or higher.
- 20 7. Use according to any one of claims 5 to 6, wherein the high conductivity aqueous solution comprises Histidine.
8. Use according to any one of claims 1 to 7, 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.
- 25 9. Use according to any one of claims 1 to 7, 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.

10. Method for producing a human IgG4 or IgG1 isotype antibody comprising the steps of
 - 5 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,
 - 10 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 0.5 mS/cm or less, wherein the amount of a host cell protein is reduced and wherein said host cell protein is phospholipase B-like 2 (PLBL2) or Clusterin,
 - 15 e) recovering the human IgG4 or IgG1 isotype antibody from the protein A chromatography materialand thereby producing the human IgG4 or IgG1 isotype antibody.
- 20 11. 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
 - 25 the low conductivity aqueous solution has a conductivity value of 0.5 mS/cm or less, wherein the amount of a host cell protein is reduced and wherein said host cell protein is phospholipase B-like 2 (PLBL2) or Clusterin.
- 30 12. Method according to any one of claims 10 to 11, wherein the low conductivity aqueous solution comprises 0.1 mM to 8 mM Tris.

13. Method according to any one of claims 10 to 12, wherein the low conductivity aqueous solution comprises 0.05 mM to 2 mM potassium phosphate.
- 5 14. Method according to any one of claims 10 to 13, wherein the low conductivity aqueous solution has a pH of 7 or higher.
- 10 15. Method according to any one of claims 10 to 14, 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.
- 15 16. Method according to claim 15, wherein the high conductivity aqueous solution has a conductivity value of 20 mS/cm or higher.
17. Method according to claim 15, wherein the medium conductivity aqueous solution has a conductivity value of from more than 0.5 mS/cm to less than 20 mS/cm.
18. Method according to any one of claims 15 to 17, wherein the high or medium conductivity aqueous solution comprises Histidine.
- 20 19. Method according to any one of claims 10 to 18, 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.
- 25 20. Method according to any one of claims 10 to 18, 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.