

Patent Claims

1. A method for purifying a protein from a sample comprising the protein and at least one impurity with hydrolytic activity, comprising the following steps:
 - i)
 - 5 a) applying the sample comprising the protein and at least one impurity with hydrolytic activity to a chromatography material,
 - b) applying a solution which comprises a polysorbate to the chromatography material, and
 - c) recovering the protein from the chromatography material, or
 - 10 ii)
 - a) adding a polysorbate to the sample comprising the protein and at least one impurity with hydrolytic activity,
 - b) applying the mixture of step a) to a chromatography material, and
 - c) recovering the protein from the chromatography material, or
 - 15 iii)
 - a) adding a polysorbate to the sample comprising the protein and at least one impurity with hydrolytic activity,
 - b) applying the mixture of step a) to a chromatography material,
 - c) applying a solution which comprises a polysorbate to the chromatography material, and
 - 20 d) recovering the protein from the chromatography material.
2. A method for reducing the hydrolytic activity in a sample comprising a protein and at least one impurity with hydrolytic activity, comprising the following steps:
 - 25 i)

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- 5 a) applying the sample comprising the protein and at least one impurity with hydrolytic activity to a chromatography material,
- b) applying a solution which comprises a polysorbate to the chromatography material, and
- c) recovering the protein from the chromatography material, or
- ii)
- a) adding a polysorbate to the sample comprising the protein and at least one impurity with hydrolytic activity,
- b) applying the mixture of step a) to a chromatography material, and
- 10 c) recovering the protein from the chromatography material, or
- iii)
- a) adding a polysorbate to the sample comprising the protein and at least one impurity with hydrolytic activity,
- b) applying the mixture of step a) to a chromatography material,
- 15 c) applying a solution which comprises a polysorbate to the chromatography material, and
- d) recovering the protein from the chromatography material.
3. The method according to any one of claims 1 to 2, wherein the impurity is a hydrolase.
- 20 4. The method according to any one of claims 1 to 3, wherein the impurity is an esterase.
5. The method according to any one of claims 1 to 4, wherein the polysorbate is selected from the group comprising polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 65 or polysorbate 80.
- 25 6. The method according to any one of claims 1 to 5, wherein the polysorbate is polysorbate 20 or polysorbate 80.

7. The method according to any one of claims 1 to 6, wherein the polysorbate is added to a final concentration of at least about 0.001% (w/v).
8. The method according to any one of claims 1 to 7, wherein the polysorbate is added to a final concentration of from about 0.001% (w/v) to about 10% (w/v).
- 5 9. The method according to any one of claims 1 to 8, wherein the chromatography material is an affinity chromatography material, a cation exchange chromatography material, an anion exchange chromatography material, a hydrophobic interaction chromatography material or a mixed mode chromatography material.
- 10 10. The method according to any one of claims 1 to 9, wherein the chromatography material is an affinity chromatography material or a cation exchange chromatography material.
- 15 11. The method according to any one of claims 1 to 10, wherein the chromatography material is an affinity chromatography material selected from Protein A chromatography or a chromatography material with a camelid-derived single domain antibody fragment as ligand.
12. The method according to any one of claims 1 to 11, wherein the affinity chromatography material is CaptureSelect FcXL or MabSelect SuRe.
- 20 13. The method according to any one of claims 1 to 10, wherein the cation exchange chromatography material is SP Sepharose Fast Flow or Poros 50HS or Poros XS.
14. The method according to any one of claims 1 to 13, wherein the protein is a monoclonal antibody.
- 25 15. The method according to any one of claims 1 to 14, wherein the protein is a humanized Type II anti-CD20 antibody, or an anti-VEGF/Ang2 antibody or an anti-HER2 antibody.
16. A liquid antibody preparation with reduced hydrolytic activity obtained by a method according to any one of the preceding claims.
17. A method for producing a protein comprising the following steps:

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- a) cultivating a mammalian cell comprising a nucleic acid encoding the protein,
 - b) recovering the protein from the cell or the cultivation medium, and
 - c) purifying the protein with a method according to any one of claims 1 to 15,

and thereby producing the protein.

18. A liquid composition comprising an antibody and a polysorbate, wherein the polysorbate is degraded during storage of the liquid composition by 20% or less per year.
- 10 19. A liquid composition according to claim 18, wherein the antibody is a humanized Type II anti-CD20 antibody, or an anti-VEGF/Ang2 antibody or an anti-HER2 antibody.
20. A liquid composition according to any one of claims 18 or 19, wherein the antibody is obinutuzumab, faricimab, trastuzumab or pertuzumab.

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