

Patent Claims

1. A method for the enzymatic production of an antibody with a modified glycosylation at an N-glycosylation site, comprising
 - a) incubating the antibody affinity ligand-bound monoclonal antibody with a glycosylation at an N-glycosylation site with a first enzyme for a time sufficient and under conditions suitable to modify the glycosylation at the N-glycosylation site,
 - b) optionally recovering the first enzyme and producing thereby a recycled first enzyme,
 - c) recovering the antibody from the antibody affinity ligand,
 - d) incubating the recovered antibody in solution with a second enzyme for a time sufficient and under conditions suitable to modify the glycosylation at the N-glycosylation site of the antibody,
 - e) separating the antibody with the modified glycosylation at the N-glycosylation site from the second enzyme in a cation exchange chromatography, wherein the cation exchange chromatography material has a matrix of cross-linked agarose with sulfopropyl cation exchange groups, and thereby producing the antibody with a modified glycosylation at the N-glycosylation site and optionally a recycled second enzyme, and
 - f) optionally repeating step a) with the first recycled enzyme of step b) at least once and repeating step d) with the second recycled enzyme of step e) at least once.
2. The method according to claim 1, wherein the antibody affinity ligand is an antibody light chain affinity ligand or an antibody Fc-region affinity ligand.
3. The method according to claim 1 or 2, wherein the incubating step a) comprises incubating the antibody affinity ligand-bound monoclonal antibody with the glycosylation at the N-glycosylation site with the first enzyme and a first activated sugar residue for a time sufficient and under

conditions suitable to modify the glycosylation at the N-glycosylation site;
and

5 wherein the incubating step d) comprises incubating the recovered antibody
in solution with the second enzyme and a second activated sugar residue for a
time sufficient and under conditions suitable to modify the glycosylation at
the N-glycosylation site of the antibody to a defined form.

4. The method according to any one of claims 1 to 3, wherein the first enzyme
is a galactosyltransferase and the second enzyme is a sialyltransferase.
- 10 5. The method according to claim 4, wherein the galactosyltransferase is
 β 4GalT1.
6. The method according to any one of claims 4 or 5, wherein the
sialyltransferase is ST6.
7. The method according to any one of claims 1 to 6, wherein the cation
exchange chromatography comprises the following steps
 - 15 i) applying a solution comprising a galactosyltransferase and/or a
sialyltransferase and the antibody with the modified glycosylation at
the N-glycosylation site to a cation exchange chromatography material,
 - ii) optionally washing the cation exchange chromatography material,
 - 20 iii) optionally if a galactosyltransferase is present applying a first solution
to the cation exchange chromatography material and thereby eluting the
galactosyltransferase from the cation exchange chromatography
material,
 - 25 iv) applying a second solution to the cation exchange chromatography
material and thereby eluting the antibody with the modified
glycosylation at the N-glycosylation site from the cation exchange
chromatography material, and
 - v) applying a linear gradient to the cation exchange chromatography
material and thereby eluting the sialyltransferase from the cation
exchange chromatography material.

8. The method according to claim 7, wherein the optional washing step ii) is to remove unbound compounds from the cation exchange chromatography material but without eluting the antibody with the modified glycosylation at the N-glycosylation site; and
- 5 wherein the linear gradient in the applying step v) is a linear salt gradient.
9. The method according to claim 7 or 8, wherein
- the solution of step i) is a 2-(N-morpholino)ethanesulfonic acid buffered solution with a pH value from pH 5.0 to pH 6.5,
 - the solution of step ii) is a 2-(N-morpholino)ethanesulfonic acid
10 buffered solution with a pH value from pH 5.0 to pH 6.5,
 - the solution of step iii) is a tris(hydroxymethyl)aminomethane buffered solution with a pH value from pH 6.6 to pH 8.0,
 - the solution of step iv) is a 2-(N-morpholino)ethanesulfonic acid buffered solution with a pH value from pH 5.0 to pH 6.5 comprising 75
15 mM to 125 mM sodium chloride,
 - the linear gradient is from the solution of step iv) to a 2-(N-morpholino)ethanesulfonic acid buffered solution with a pH value from pH 5.0 to pH 6.5 comprising 750 mM to 1250 mM sodium chloride.
10. The method according to any one of claims 1 to 9, wherein the method
20 comprises the following steps instead of steps a) to c):
- applying a solution comprising the antibody with a glycosylation at the N-glycosylation site to an antibody affinity ligand bound to a solid phase whereby the antibody is bound to the ligand,
 - optionally washing the solid phase with a buffered solution,
 - enzymatically modifying the glycosylation at the N-glycosylation site
25 of the antibody by applying a solution comprising a first glycosylation modifying enzyme for a time sufficient and under conditions suitable for the enzymatic modification to the ligand-bound antibody, optionally washing the modified ligand-bound antibody without disrupting the
30 antibody-ligand interaction,

- optionally recovering the first enzyme and producing thereby a recycled first enzyme, and
 - recovering the antibody from the antibody affinity ligand.
11. The method according to claim 10, wherein the method comprises the following steps:
- applying a buffered solution comprising the antibody with a glycosylation at the N-glycosylation site to an antibody light chain affinity ligand or antibody Fc-region affinity ligand bound to a solid phase antibody light chain affinity ligand chromatography material or antibody Fc-region affinity ligand chromatography material whereby the antibody is bound to the ligand resulting in a ligand-bound antibody,
 - optionally washing the solid phase with a buffered solution without disrupting the antibody-ligand interaction,
 - enzymatically modifying the glycosylation at the N-glycosylation site of the antibody by applying a buffered solution comprising a first glycosylation modifying enzyme and a first activated sugar residue for a time sufficient and under conditions suitable for the enzymatic modification to the ligand-bound antibody, optionally washing the modified ligand-bound antibody without disrupting the antibody-ligand interaction,
 - optionally recovering the first enzyme and producing thereby a recycled first enzyme, and
 - recovering the antibody from the antibody light chain affinity ligand or antibody Fc-region affinity ligand.
12. The method according to any one of claims 1 to 11, wherein the antibody is a bivalent monospecific antibody or a bivalent bispecific antibody or an antibody Fab fragment.
13. The method according to any one of claims 1 to 12, wherein the antibody is a chimeric or humanized or human antibody.

14. The method according to any one of claims 1 to 13, wherein the antibody is a monoclonal antibody.
15. The method according to any one of claims 1 to 14, wherein the antibody is an antibody of the human IgG1 or IgG4 subclass.
- 5 16. The method according to any one of claims 1 to 15, wherein the N-glycosylation site is a Fab region N-glycosylation site or the Fc-region N-glycosylation site at asparagine residue 297, where the numbering is according to Kabat).