

Patent Claims

1. A method for producing an antibody comprising the following steps:
 - forming an antibody-antibody light chain affinity ligand complex, wherein the antibody light chain affinity ligand is immobilized on a solid phase, by applying a solution comprising the antibody to the immobilized antibody light chain affinity ligand,
 - incubating the complex formed in the previous step with one or more enzymes to modify the glycosylation of the antibody,and thereby producing the antibody.
2. The method according to claim 1, wherein the incubation step comprises
 - incubating the complex formed in the forming step with one or more enzymes and one or more activated sugar residues to modify the glycosylation of the antibody.
3. The method according to claim 1 or 2, comprising after the incubation step the step of
 - recovering the modified antibody from the antibody light chain affinity ligand.
4. The method according to any one of claims 1 to 3, comprising the following steps in the following order
 - applying the solution comprising the antibody to an antibody light chain affinity ligand bound to a solid phase, whereby the antibody is bound by the ligand,
 - optionally washing the solid phase with a buffered solution,
 - enzymatically modifying the glycosylation at the N-glycosylation site of the antibody by either
 - applying a first solution comprising a first glycosylation modifying enzyme for a time sufficient and under conditions suitable for the enzymatic modification of the ligand-bound antibody, optionally washing the modified ligand-bound

antibody, applying a second solution comprising a second glycosylation modifying enzyme for a time sufficient and under conditions suitable for the enzymatic modification of the modified ligand-bound antibody, optionally washing the two-times modified ligand-bound antibody,

or

- applying a first solution comprising a first glycosylation modifying enzyme for a time sufficient and under conditions suitable for at least a partial enzymatic modification of the ligand-bound antibody, applying after a defined period of time a second solution comprising a second glycosylation modifying enzyme for a time sufficient and under conditions suitable for the enzymatic modification of the modified ligand-bound antibody, optionally washing the two-times modified ligand-bound antibody,

or

- applying a solution comprising a first and a second glycosylation modifying enzyme for a time sufficient and under conditions suitable for the enzymatic modification of the ligand-bound antibody, optionally washing the modified ligand-bound antibody,

- releasing the antibody from the antibody light chain affinity ligand,

and thereby producing the antibody.

5. The method according to claim 4, wherein the solution comprising the antibody is a buffered solution.

6. The method according to claim 4 or 5, wherein the antibody has an N-glycosylation site.

7. The method according to any one of claims 4 to 6, wherein the binding by the ligand results in a ligand-bound antibody.

8. The method according to any one of claims 4 to 7, wherein the optional washing step does not desorb the antibody/disrupting the ligand antibody complex.
- 5 9. The method according to any one of claims 4 to 8, wherein in the enzymatically modifying step,
the first solution is a first buffered solution;
the first solution further comprises a first activated sugar residue;
the second solution is a second buffered solution;
the second solution further comprises a second activated sugar;
10 the solution comprising the first and the second glycosylation modifying enzyme is a buffered solution; and
the solution comprising the first and the second glycosylation modifying enzyme further comprises a first and second activated sugar.
- 15 10. The method according to any one of claims 1 to 9, wherein the antibody is a glycosylation modified antibody.
11. The method according to any one of claims 1 to 10, wherein the antibody is selected from the group of antibodies consisting of a full length antibody, a bivalent monospecific antibody, a bispecific antibody, a bivalent bispecific antibody, a trivalent bispecific antibody, a tetravalent bispecific antibody, a trivalent trispecific antibody, an antibody Fab fragment, and a tetravalent tetraspecific antibody.
20
12. The method according to any one of claims 1 to 11, wherein the antibody is a bivalent or trivalent or tetravalent bispecific antibody or a Fab fragment.
13. The method according to any one of claims 1 to 12, wherein the antibody is a
25 chimeric or humanized or human antibody.
14. The method according to any one of claims 4 to 13, wherein the first glycosylation modifying enzyme is a galactosyltransferase and the second glycosylation modifying enzyme is a sialyltransferase.
15. The method according to any one of claims 4 to 14, wherein the solution
30 comprises a chromatographically purified antibody, the first glycosylation

modifying enzyme is GalT1, and the incubation with the first glycosylation modifying enzyme is for 24 hours at 37°C or room temperature.

- 5 16. The method according to any one of claims 4 to 15, wherein the solution comprises a chromatographically purified antibody, the second glycosylation modifying enzyme is ST6, and the incubation with the second glycosylation modifying enzyme is for 24 hours at 37 °C or room temperature.
- 10 17. The method according to any one of claims 4 to 16, wherein the solution is a buffered, cell-free cultivation supernatant comprising the antibody, the first glycosylation modifying enzyme is GalT1, the second glycosylation modifying enzyme is ST6, which is added 6 to 24 hours, preferably 24 hours, after the first glycosylation modifying enzyme, the total incubation time is 24 hours to 48 hours, preferably 30 hours, at 37 °C or room temperature.
- 15 18. The method according to any one of claims 1 to 11, 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.