

Claims

1. A method for the production of bispecific antibody construct comprising a first binding domain for FcγRIII and a second binding domain for CD30, the method comprising the following steps
 - (a) chromatographically capturing the antibody construct from a solution;
 - (b) eluting the antibody construct from the capture matrix;
 - (c) reducing the pH in the solution of the eluted antibody construct to low pH in a range of 2.5 pH to 3.9 pH and incubating the antibody construct under these conditions for at least 40h;
 - (d) neutralizing to a pH in the range of pH 4.5 to pH 8.0.
2. The method according to claim 1, wherein the first binding domain of the antibody construct for FcγRIII binds to CD16A.
3. The method according to claim 2, wherein the antibody construct comprises at least four variable domains from the group consisting of
 - (a) heavy chain variable domain specific for CD16A (VH_CD16A) comprising a heavy chain CDR1 having the amino acid sequence set forth in SEQ ID NO :1, a heavy chain CDR2 having the amino acid sequence set forth in SEQ ID NO :2, a heavy chain CDR3 having the amino acid sequence set forth in SEQ ID NO: 3;
 - (b) a light chain variable domain specific for CD16A (VL_CD16A) comprising a light chain CDR1 having an amino acid sequence set forth in SEQ ID NO :4, a light chain CDR2 having an amino acid sequence set forth in SEQ ID NO: 5, and a light chain CDR3 having an amino acid sequence set forth in SEQ ID NO: 6;
 - (c) heavy chain variable domain specific for CD30 (VH_CD30A) comprising a heavy chain CDR1 having the amino acid sequence set forth in SEQ ID NO :7, a heavy chain CDR2 having the amino acid sequence set forth in SEQ ID NO :8, a heavy chain CDR3 having the amino acid sequence set forth in SEQ ID NO: 9;
 - (d) a light chain variable domain specific for CD30A (VL_CD30A) comprising a light chain CDR1 having an amino acid sequence set forth in SEQ ID NO :10, a light chain CDR2 having an amino acid sequence set forth in SEQ ID NO: 11, and a light chain CDR3 having an amino acid sequence set forth in SEQ ID NO: 12.

4. The method according to any of claim 1 to 3, wherein the variable domains of the antibody construct are linked one after another by peptide linkers L1, L2 and L3 consisting of 12 or less amino acid residues and positioned within each of the two polypeptide chains from the N-terminus to the C-terminus in the order: VH_CD30-L1 -VL_CD16A-L2 -VH_CD16A-L3 -VL_CD30.
5. The method according to any of claims 3 or 4, wherein linker L2 of the antibody construct consists of 3 to 9 amino acid residues.
6. The method according to any of claim 1 to 5, wherein the antibody construct comprises an amino acid sequence as set forth in SEQ ID NO:13.
7. The method according to any of claim 1 to 6, wherein the pH in step (c) is in the range of pH 3.0 to pH 3.75.
8. The method according to any of claim 1 to 7, wherein the antibody construct is incubated in step (c) for at least 48h.
9. The method according to any of claim 1 to 8, wherein the chromatographic capturing method in step (a) is selected from the group consisting of a protein L chromatography, an anion exchange chromatography (AEX), a cation exchange chromatography (CEX), a hydrophobic interaction chromatography (HIC) or a mixed mode chromatography (MMC).
10. The method according to any of claim 1 to 9, wherein the method further comprises the additional steps:
 - (e) capturing the antibody construct from the solution by at least one chromatographic method selected from the group consisting of an anion exchange chromatography (AEX), a cation exchange chromatography (CEX), a hydrophobic interaction chromatography (HIC) or a mixed mode chromatography (MMC).
11. The method according to any of claim 1 to 10, wherein the elution of the antibody construct in step (b) is performed using a buffer selected from the group consisting of buffers comprising sodium acetate / acetic acid, sodium formate / formic acid, sodium citrate / citric acid, and sodium succinate / succinic acid.

12. The method according to any of claim 1 to 11, wherein the antibody construct is formulated as a pharmaceutical composition in a step (f).
13. An antibody construct produced by a method according to any of claims 1 to 11.
14. A pharmaceutical composition comprising an antibody construct produced by a method according to any of claims 1 to 11 for the treatment or amelioration of a CD30⁺ proliferative disease or a tumorous disease.
15. A method for the treatment or amelioration of a patient, the method comprising administering to a subject suffers from a CD30⁺ proliferative disease or a tumorous disease an antibody construct produced by a method according to any of claims 1 to 11.