

CLAIMS

1. A method for obtaining a human VH3 domain-containing antibody in monomeric form comprising:

a) applying a mixture comprising a human VH3 domain-containing antibody in monomeric and multimeric form to a protein A chromatography material wherein said protein A comprises domain D and/or E, under conditions that allow binding of said antibody to protein A, and

b) recovering the human VH3 domain-containing antibody in monomeric form,

wherein the eluate recovered from the protein A chromatography is enriched in monomeric antibody over multimeric antibody with respect to the applied mixture, and

wherein the human VH3 domain-containing antibody does not contain an Fc region.

2. A method for manufacturing a human VH3 domain-containing antibody comprising:

a) expressing the antibody in a host cell,

b) recovering a mixture containing the antibody in monomeric and multimeric form, host cells and other contaminants,

c) purifying the antibody by applying the mixture to at least a protein A chromatography material wherein said protein A comprises domain D and/or E, and

d) recovering the human VH3 containing antibody

wherein the eluate recovered from the protein A chromatography is enriched in monomeric antibody over multimeric antibody with respect to the mixture, and

wherein the human VH3 domain containing antibody does not contain an Fc region.

3. A method of separating a human VH3 domain-containing antibody in monomeric form from the antibody in multimeric form comprising:

a) applying a mixture comprising a human VH3 domain-containing antibody in monomeric and multimeric form to a protein A chromatography material wherein said protein A comprises domain D and/or E,

b) allowing binding of said antibody to protein A,

c) applying an elution buffer that selectively disrupts binding of the antibody in monomeric form,

d) recovering the resulting eluate, and optionally

e) applying a second elution buffer that disrupts binding of the antibody in multimeric form and recovering this second eluate,

wherein the human VH3 domain-containing antibody does not contain an Fc region.

4. A method of separating a human VH3 domain-containing antibody in monomeric form from the antibody in multimeric form comprising:

- a) applying a mixture comprising a human VH3 domain-containing antibody in monomeric and multimeric form to a protein A chromatography material wherein said protein A comprises domain D and/or E,
- b) allowing binding of the antibody in multimeric form,
- c) recovering the antibody in monomeric form in the flow-through, and optionally
- d) applying an elution buffer that selectively disrupts binding of the antibody in multimeric form, and
- e) recovering the eluate resulting from d);

wherein the human VH3 domain-containing antibody does not contain an Fc region.

5. A method according to any preceding claim wherein a first solution is added to the protein A chromatography material after applying the mixture comprising the human VH3 domain-containing antibody in monomeric and multimeric form such that unbound material is removed in the solution.

6. A method according to claims 1, 2 or 5, wherein an elution buffer is applied to the protein A chromatography material such that the bound antibody is released.

7. A method according to any preceding claim wherein said protein A is native recombinant protein A.

8. A method according to any preceding claim wherein the VH3 domain containing antibody is selected from Fab', F(ab')₂, scFv, Fab-Fv, Fab-scFv, Fab-(scFv)₂, Fab-(Fv)₂, diabodies, triabodies, and tetrabodies.

9. A method according to any preceding claim wherein the VH3 domain-containing antibody comprises at least 2 human VH3 domains

10. A method according to any preceding claim wherein the VH3 domain-containing antibody specifically binds OX40.

11. A method according to claim 10 wherein said VH3 domain-containing antibody comprises:

- heavy chain CDR1, CDR2 and CDR3 as defined in SEQ ID NO: 1, SEQ ID NO:2 and SEQ ID NO:3, respectively; and
- light chain CDR1, CDR2, and CDR3 as defined in SEQ ID NO: 4, SEQ ID NO:5 and SEQ ID NO: 6, respectively.