CLAIMS

1. A process for the purification of an antibody fragment from periplasmic cell extract comprising:

   a) a first chromatography step to capture the antibody fragment wherein a mixture containing an antibody fragment at a concentration of at least 1.5 g/L is subjected to cation exchange chromatography and subsequently eluted to produce a first eluate containing the antibody fragment; and

   b) a second chromatography step wherein the first eluate is subjected to anion exchange chromatography to capture impurities and produce a flow through containing the antibody fragment,

   wherein the process comprises not more than two chromatography steps.

2. A process for the purification of an antibody fragment from periplasmic cell extract consisting essentially of:

   a) a first chromatography step to capture the antibody fragment wherein a mixture containing an antibody fragment at a concentration of at least 1.5 g/L is subjected to cation exchange chromatography and subsequently eluted to produce a first eluate containing the antibody fragment;

   b) a first ultrafiltration applied to the first eluate;

   c) a second chromatography step wherein the purified first eluate is subjected to anion exchange chromatography to capture impurities and produce a flow through containing the antibody fragment; and

   d) a second ultrafiltration applied to the flow through.

3. The process according to claim 1 or 2, wherein all chromatography steps are performed on a chromatography column.

4. The process according to any one of claims 1 to 3, wherein the cation exchange chromatography is performed in elution mode.

5. The process according to any one of claims 1 to 4, wherein the first cation chromatography step comprises the following steps in sequential order:

   a) loading a mixture, such as a periplasmic cell extract, containing an antibody fragment onto the cation exchange column,

   b) washing the cation exchange column with a wash buffer wherein during the washing the conductivity, pH and salt concentration of the buffer remains essentially unchanged, and

   c) eluting the antibody fragment with an elution buffer.

6. The process according to claim 5, wherein the pH of the washing buffer is identical to the pH of the mixture containing an antibody fragment prior to first chromatography step.

7. The process according to claim 5 or 6, wherein the mixture containing an antibody fragment, prior to the first chromatography step has a pH of between 4.0 to 5.0.

8. The process according to any one of claims 1 to 7, wherein in the first chromatography step the mixture containing an antibody fragment contains bacterial host cell protein in an amount of from about 200 µg/ml to 10,000 µg/ml, before it is subjected to cation exchange chromatography.

9. The process according to any one of claims 1 to 8, wherein the cation exchange chromatography in the first chromatography step is performed at a flow rate of at least 300 cm/h.

10. The process according to any of the preceding claims, wherein the cation exchange chromatography in the first chromatography step is performed at a conductivity of not more 6 mS/cm.

11. The process according to any of the preceding claims, wherein the cation exchange chromatography in the first chromatography step is performed in a chromatography column comprising sulphonyl, sulphopropyl or carboxymethyl coupled to a resin.

12. The process according to any of the preceding claims, wherein the cation exchange chromatography column in the first chromatography step is has a dynamic binding capacity for the antibody fragment of between 50 and 75 g/L resin.

13. The process according to claim 11 or 12, wherein the cation exchange chromatography column resin in the first chromatography step has a mean particular size of at least 50 µm.

14. The process according to any of the preceding claims, wherein in the cation exchange chromatography of the primary capture step between 5 and 100 g antibody fragment per liter resin are loaded.

15. The process according to any of the preceding claims, wherein the anion exchange chromatography in the second chromatography step is performed on a resin comprising quaternary ammonium (Q), diethylaminoethyl (DEAE) or trimethylaminoethyl (TMAE).

16. The process according to any of the preceding claims, wherein the antibody fragment is a Fab, Fab' or scFv.

17. The process according to claim 16, wherein the Fab or Fab' binds specifically to VEGF-A, FcRn, OX40, glycoprotein IIb/IIIa receptor, C5, HER2/neu, TNFα, IL1β or CD40-L.

18. The process according to claim 17, wherein the Fab or Fab' is abciximab, ranibizumab, pexelizumab, CDP870, CDP484 or CDP7657

19. The process according to any of the preceding claims, wherein the antibody fragment recovered from the process contains host cell protein in an amount of not more than 150 parts per million.

CLAIMS

1. A process for the purification of an antibody fragment from periplasmic cell extract from gram-negative bacteria selected from *E. coli strains* MC4100, TG1, TG2, DHB4, DH5α, DH1, BL21, K12, XL1Blue, JM109 and W3110 comprising:

   a) a first chromatography step to capture the antibody fragment wherein a mixture containing an antibody fragment at a concentration of at least 1.5 g/L is subjected to cation exchange chromatography and subsequently eluted to produce a first eluate containing the antibody fragment; and

   b) a second chromatography step wherein the first eluate is subjected to anion exchange chromatography to capture impurities and produce a flow through containing the antibody fragment,

   wherein the process comprises not more than two chromatography steps.

2. A process for the purification of an antibody fragment from periplasmic cell extract from gram-negative bacteria selected from *E. coli strains* MC4100, TG1, TG2, DHB4, DH5α, DH1, BL21, K12, XL1Blue, JM109 and W3110 consisting essentially of:

   a) a first chromatography step to capture the antibody fragment wherein a mixture containing an antibody fragment at a concentration of at least 1.5 g/L is subjected to cation exchange chromatography and subsequently eluted to produce a first eluate containing the antibody fragment;

   b) a first ultrafiltration applied to the first eluate;

   c) a second chromatography step wherein the purified first eluate is subjected to anion exchange chromatography to capture impurities and produce a flow through containing the antibody fragment; and

   d) a second ultrafiltration applied to the flow through.

3. The process according to claim 1 or 2, wherein all chromatography steps are performed on a chromatography column.

4. The process according to any one of claims 1 to 3, wherein the cation exchange chromatography is performed in elution mode.

5. The process according to any one of claims 1 to 4, wherein the first cation chromatography step comprises the following steps in sequential order:

   a) loading a mixture, such as a periplasmic cell extract, containing an antibody fragment onto the cation exchange column,

   b) washing the cation exchange column with a wash buffer wherein during the washing the conductivity, pH and salt concentration of the buffer remains essentially unchanged, and

   c) eluting the antibody fragment with an elution buffer.

6. The process according to claim 5, wherein the pH of the washing buffer is identical to the pH of the mixture containing an antibody fragment prior to first chromatography step.

7. The process according to claim 5 or 6, wherein the mixture containing an antibody fragment, prior to the first chromatography step has a pH of between 4.0 to 5.0.

8. The process according to any one of claims 1 to 7, wherein in the first chromatography step the mixture containing an antibody fragment contains bacterial host cell protein in an amount of from about 200 µg/ml to 10,000 µg/ml, before it is subjected to cation exchange chromatography.

9. The process according to any one of claims 1 to 8, wherein the cation exchange chromatography in the first chromatography step is performed at a flow rate of at least 300 cm/h.

10. The process according to any of the preceding claims, wherein the cation exchange chromatography in the first chromatography step is performed at a conductivity of not more 6 mS/cm.

11. The process according to any of the preceding claims, wherein the cation exchange chromatography in the first chromatography step is performed in a chromatography column comprising sulphonyl, sulphopropyl or carboxymethyl coupled to a resin.

12. The process according to any of the preceding claims, wherein the cation exchange chromatography column in the first chromatography step is has a dynamic binding capacity for the antibody fragment of between 50 and 75 g/L resin.

13. The process according to claim 11 or 12, wherein the cation exchange chromatography column resin in the first chromatography step has a mean particular size of at least 50 µm.

14. The process according to any of the preceding claims, wherein in the cation exchange chromatography of the primary capture step between 5 and 100 g antibody fragment per liter resin are loaded.

15. The process according to any of the preceding claims, wherein the anion exchange chromatography in the second chromatography step is performed on a resin comprising quaternary ammonium (Q), diethylaminoethyl (DEAE) or trimethylaminoethyl (TMAE).

16. The process according to any of the preceding claims, wherein the antibody fragment is a Fab, Fab' or scFv.

17. The process according to claim 16, wherein the Fab or Fab' binds specifically to VEGF-A, FcRn, OX40, glycoprotein IIb/IIIa receptor, C5, HER2/neu, TNFα, IL1β or CD40-L.

18. The process according to claim 17, wherein the Fab or Fab' is abciximab, ranibizumab, pexelizumab, CDP870, CDP484 or CDP7657

19. The process according to any of the preceding claims, wherein the antibody fragment recovered from the process contains host cell protein in an amount of not more than 150 parts per million.