We claim:

1. A process for purification of monoclonal antibody from cell culture comprising the following sequential steps

   (a) Affinity chromatography

   (b) Hydrophobic interaction chromatography, optionally followed by other suitable purification steps.

2. The process as claimed in claim 1, wherein affinity chromatography matrix is selected from protein A, protein G and protein L.

3. The process as claimed in claim 1, wherein affinity chromatography matrix is protein A.

4. The process as claimed in claim 1, wherein step (a) comprises loading of a crude mixture containing the desired antibody to the column at suitable pH and/or conductance for binding, followed by column wash prior to elution of the desired antibody in the form of a single peak.

5. The process as claimed in claim 4, wherein column wash comprises:

   (i) First wash with equilibration buffer at suitable pH and/or conductivity

   (ii) Second wash at the same pH as of the first wash buffer and/or a conductivity higher than the first wash buffer

   (iii) Third wash at a pH and/or a conductivity lower than the second wash buffer

   (iv) Elution of an antibody at lower pH and/or higher conductivity than third wash buffer

6. The process as claimed in claim 5, wherein the column wash comprises:

   (i) First wash with equilibration buffer at about pH 7.4 and/or conductivity in the range of 1mS/cm to 30 mS/cm

(ii) Second wash at about pH 7.4 and/or conductivity more than 30 mS / cm

(iii) Third wash at pH in the range of pH 5 to pH 6.5 and/or conductivity in the range of 1 mS/cm to 5 mS / cm.

(iv) Elution of an antibody at about pH 3.5 and/or conductivity higher than 5 mS/cm.

7. The process as claimed in claim 5, wherein buffer component is selected from Tris, acetate and citrate buffer.

8. The process as claimed in claim 5, wherein elution of an antibody is performed at a pH ranging from about pH 3.5 to pH 4, preferably 3.5 to 3.7.

9. The process as claimed in claim 5, wherein elution is performed with additive in the buffer such as sodium chloride, arginine, glycine, preferably sodium chloride.

10. The process as claimed in claim 1, wherein an amount of aggregates in an antibody preparation after step (a) is no greater than 5%, preferably no greater than 2%.

11. The process as claimed in claim 1, wherein step (b) is performed at pH in the range of pH 5 to pH 7 and/or conductivity more than 100 mS/cm.

12. The process as claimed in claim 1, wherein in step (b) an antibody is eluted from the column with down-the-gradient salt concentration.

13. The process as claimed in claim 12, wherein salt is selected from ammonium sulphate, sodium chloride, ammonium chloride and sodium sulphate, preferably, ammonium sulphate.

14. The process as claimed in claim 1, wherein hydrophobic column matrix is selected from phenyl sepharose, butyl sepharose, octyl sepharose, preferably, phenyl sepharose.

15. A process for purification of monoclonal antibody from cell culture comprising the steps of

(a) Protein A affinity chromatography

(b) Hydrophobic interaction chromatography

(c) Ion exchange chromatography

Wherein, step (b) and step (c) can be carried out in any order.

16. The process as claimed in claim 15, wherein step (a) is carried out as claimed in claims 2-10.

17. The process as claimed in claim 15, wherein step (b) is carried out as claimed in claims 11-13.

18. The process as claimed in claim 15, wherein the ion exchange chromatography is selected from cation exchange chromatography and anion exchange chromatography.

19. The process as claimed in claim 18, wherein ion exchange chromatography is anion exchange chromatography.

20. The process as claimed in claim 15, wherein the column matrix for anion exchange chromatography is selected from DEAE sepharose, Mono Q and Q sepharose XL.

21. The process as claimed in claim 20, wherein column matrix is Q sepharose.

22. The process as claimed in claim 15, wherein elution of antibody at step (c) is performed in flow-through-and-wash mode or bind-elute mode.

23. A process for purification of monoclonal antibody from cell culture consisting:

a) Cell separation

b) Protein A chromatography

c) Low-pH incubation

d) Neutralization and reconditioning

e) Hydrophobic interaction chromatography

f) Ultrafiltration-diafiltration

g) Anion exchange chromatography

h) Nano-filtration

i) Ultrafiltration-diafiltration

j) Microfiltration

wherein step (e) to (j) can be carried out in any order.

24. The process as claimed in claim 23, wherein step (a) is carried out as claimed in claims 2-9.

25. The process as claimed in claim 23, wherein step (b) is carried out as claimed in claims 10-12.

26. The process as claimed in claim 23, wherein diafiltration medium is selected from phosphate, acetate, citrate, succinate and combination thereof.

27. The process as claimed in claims 1-26, wherein antibody is recovered from the column with the buffer components selected from citrate, acetate, phosphate, preferably, citrate.

28. The process as claimed in claims 1-26, wherein antibody is recovered from the column with additive selected from sodium chloride, arginine, glycine, preferably sodium chloride.

29. The process as claimed in claims 1-28, wherein elution of an antibody is performed at pH in the range of pH 5 to pH 7, preferably at pH 6.5.

30. The process as claimed in claim 1-29, wherein the overall recovery of an antibody is in the range of not less than 50%, preferably, not less than 70%, more preferably, in the range of 85% to 90% of the initial amount.

31. The process as claimed in claim 1-30, wherein purified antibody preparation contains no more than 1% aggregate.

32. The process as claimed in claim 1-31, wherein antibody is selected from anti-HER antibody, anti-TNF antibody, anti-VEGF antibody, anti-CD20 antibody, anti-CD52 antibody, anti-RANKL, anti-IgE antibody.

33. The process as claimed in claim 1-32, wherein an antibody is selected from trastuzumab, pertuzumab, infliximab, adalimumab, bevacizumab, ranibizumab, rituximab, bectumomab, epratuzumab.

34. The process as claimed in claim 1-33, wherein an antibody is adalimumab, trastuzumab and rituximab.

We claim:

1. A process for purification of monoclonal antibody from cell culture comprising the following sequential steps

   (a) Affinity chromatography

   (b) Hydrophobic interaction chromatography, optionally followed by other suitable purification steps, wherein hydrophobic interaction chromatography is performed in bind-elute mode.

2. The process as claimed in claim 1, wherein affinity chromatography matrix is selected from protein A, protein G and protein L.

3. The process as claimed in claim 1, wherein affinity chromatography matrix is protein A.

4. The process as claimed in claim 1, wherein step (a) comprises loading of a crude mixture containing the desired antibody to the column at suitable pH and/or conductance for binding, followed by column wash prior to elution of the desired antibody in the form of a single peak.

5. The process as claimed in claim 4, wherein column wash comprises:

   (i) First wash with equilibration buffer at suitable pH and/or conductivity

   (ii) Second wash at the same pH as of the first wash buffer and/or a conductivity higher than the first wash buffer

   (iii) Third wash at a pH and/or a conductivity lower than the second wash buffer

   (iv) Elution of an antibody at lower pH and/or higher conductivity than third wash buffer

6. The process as claimed in claim 5, wherein the column wash comprises:

(i) First wash with equilibration buffer at about pH 7.4 and/or conductivity in the range of 1mS/cm to 30 mS/cm

(ii) Second wash at about pH 7.4 and/or conductivity more than 30 mS / cm

(iii) Third wash at pH in the range of pH 5 to pH 6.5 and/or conductivity in the range of 1 mS/cm to 5 mS / cm.

(iv) Elution of an antibody at about pH 3.5 and/or conductivity higher than 5 mS/cm.

7. The process as claimed in claim 5, wherein buffer component is selected from Tris, acetate and citrate buffer.

8. The process as claimed in claim 5, wherein elution of an antibody is performed at a pH ranging from about pH 3.5 to pH 4, preferably 3.5 to 3.7.

9. The process as claimed in claim 5, wherein elution is performed with additive in the buffer such as sodium chloride, arginine, glycine, preferably sodium chloride.

10. The process as claimed in claim 1, wherein an amount of aggregates in an antibody preparation after step (a) is no greater than 5%, preferably no greater than 2%.

11. The process as claimed in claim 1, wherein step (b) is performed at pH in the range of pH 5 to pH 7 and/or conductivity more than 100 mS/cm.

12. The process as claimed in claim 1, wherein in step (b) an antibody is eluted from the column with down-the-gradient salt concentration.

13. The process as claimed in claim 12, wherein salt is selected from ammonium sulphate, sodium chloride, ammonium chloride and sodium sulphate, preferably, ammonium sulphate.

14. The process as claimed in claim 1, wherein hydrophobic column matrix is selected from phenyl sepharose, butyl sepharose, octyl sepharose, preferably phenyl sepharose.

15. A process as claimed in claim 1 comprising the steps of

(a) Protein A affinity chromatography

(b) Hydrophobic interaction chromatography

(c) Ion exchange chromatography

16. The process as claimed in claim 15, wherein step (a) is carried out as claimed in claims 2-10.

17. The process as claimed in claim 15, wherein step (b) is carried out as claimed in claims 11-13.

18. The process as claimed in claim 15, wherein the ion exchange chromatography is selected from cation exchange chromatography and anion exchange chromatography.

19. The process as claimed in claim 18, wherein ion exchange chromatography is anion exchange chromatography.

20. The process as claimed in claim 15, wherein the column matrix for anion exchange chromatography is selected from DEAE sepharose, Mono Q and Q sepharose XL.

21. The process as claimed in claim 20, wherein column matrix is Q sepharose.

22. The process as claimed in claim 15, wherein elution of antibody at step (c) is performed in flow-through-and-wash mode or bind-elute mode.

23. A process as claimed in claim 1 consisting:

a) Cell separation

b) Protein A chromatography

c) Low-pH incubation

d) Neutralization and reconditioning

e) Hydrophobic interaction chromatography

f) Ultrafiltration-diafiltration

g) Anion exchange chromatography

h) Nano-filtration

i) Ultrafiltration-diafiltration

j) Microfiltration

24. The process as claimed in claim 23, wherein step (a) is carried out as claimed in claims 2-9.

25. The process as claimed in claim 23, wherein step (b) is carried out as claimed in claims 10-12.

26. The process as claimed in claim 23, wherein diafiltration medium is selected from phosphate, acetate, citrate, succinate and combination thereof.

27. The process as claimed in claims 1-26, wherein antibody is recovered from the column with the buffer components selected from citrate, acetate, phosphate, preferably citrate.

28. The process as claimed in claims 1-26, wherein antibody is recovered from the column with additive selected from sodium chloride, arginine, glycine, preferably sodium chloride.

29. The process as claimed in claims 1-28, wherein elution of an antibody is performed at pH in the range of pH 5 to pH 7, preferably at pH 6.5.

30. The process as claimed in claim 1-29, wherein the overall recovery of an antibody is in the range of not less than 50%, preferably not less than 70%, more preferably in the range of 85% to 90% of the initial amount.

31. The process as claimed in claim 1-30, wherein purified antibody preparation contains no more than 1% aggregate.

32. The process as claimed in claim 1-31, wherein antibody is selected from anti-HER antibody, anti-TNF antibody, anti-VEGF antibody, anti-CD20 antibody, anti-CD52 antibody, anti-RANKL, anti-IgE antibody.

33. The process as claimed in claim 1-32, wherein an antibody is selected from trastuzumab, pertuzumab, infliximab, adalimumab, bevacizumab, ranibizumab, rituximab, bectumomab, epratuzumab.

34. The process as claimed in claim 1-33, wherein an antibody is adalimumab, trastuzumab and rituximab.