CLAIMS

What is claimed is :

1. A method for isolating a protein of interest from nucleic acids and other host cell proteins present in cell culture supernatant comprising said protein, the method comprising:
   (a) combining said cell culture supernatant with a first precipitating agent to remove precipitated proteins and/or other components to produce a secondary cell culture supernatant, the first precipitating agent being calcium chloride and/or a fatty acid;
   (b) introducing a precipitating polymer into said secondary cell culture supernatant to precipitate the protein of interest from the secondary cell culture supernatant.

2. The method of claim 1, wherein the precipitating polymer is polyethylene glycol (PEG).

3. The method of claim 2, wherein the PEG is selected from PEG2000, PEG4000, PEG6000 and PEG20000.

4. The method of claim 2, wherein the PEG is introduced into the secondary cell culture supernatant in an amount of from about 5 to about 30%.

5. The method of claim 1, wherein the pH in step (b) is about from 4 to 9.

6. The method of claim 5, wherein the pH is from 5.0 to 8.5.

7. The method of claim 1, wherein the first precipitating agent is a fatty acid used with a salt.
8. The method of claim 7, wherein the fatty acid is caprylic acid.

9. The method of claim 8 wherein the fatty acid is present in step (a) in a concentration of from about 0.1% to about 10% (v/v).

10. The method of claim 9 wherein the fatty acid is present in step (a) in a concentration of about 1%.

11. The method of claim 1, wherein the pH in step (a) is from about 4 to about 6.

12. The method of claim 11, wherein the pH in step (a) is from about 4.5 to about 5.5.

13. The method of claim 7, wherein the salt is sodium acetate or sodium citrate.

14. The method of claim 1, wherein the first precipitating agent is calcium chloride.

15. The method of claim 1 further comprising subsequent to steps (a) and (b), subsequently precipitating impurities with calcium chloride as step (c).

16. The method of claim 15, wherein the concentration of calcium chloride is from about 10 mM to about 400 mM.

17. The method of claim 15, wherein the pH in step (c) is from about 6 to about 9.

18. The method of claim 1, further comprising a subsequent precipitation using cold ethanol.

19. The method of claim 15, further comprising a subsequent precipitation using cold ethanol.

20. The method of claim 1, wherein the protein of interest is an antibody.

21. The method of claim 1, wherein the antibody is immunoglobulin G.

22. The method of claim 1, wherein said method is carried out using a two-step

precipitation reactor, optionally wherein the two steps are continuous.

23. The method of claim 1, performed without using chromatography.

24. The method of claim 15, performed without using chromatography.

25. The method of claim 19, performed without using chromatography.

26. The method of claim 4, wherein the PEG is introduced into the secondary cell culture supernatant in an amount of from about 10 to about 21%.