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, comprising:
   (a) combining said cell culture supernatant with calcium chloride to remove precipitated proteins and/or other components to produce a secondary cell culture supernatant;
   (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 or 3, wherein the PEG is introduced into the secondary cell culture supernatant in an amount of from about 5 to about 30%, preferably in an amount of from about 10 to about 21%.

5. The method of any one of claims 1-4, wherein the pH in step (b) is about from 4 to 9, preferably about from 5.0 to 8.5.

6. The method of any one of claims 1-5, wherein the calcium chloride is at a concentration of from about 10 mM to about 400 mM.

7. The method of any one of claims 1-6, wherein the pH in step (a) is from about 6 to about 9.

8. The method of any one of claims 1-7 further comprising precipitating host cell proteins using a fatty acid, preferably between step (a) and (b).

9. The method of claim 8, wherein the fatty acid is caprylic acid.

10. The method of claim 8 or 9, wherein the fatty acid is present in a concentration of from about 0.1% to about 10% (v/v), for example about 1%.

11. The method of any one of claims 8-10, wherein the pH in the step of precipitating host cell proteins using a fatty acid is from about 4 to about 6, preferably from about 4.5 to about 5.5.

12. The method of any one of claims 8-11, wherein the fatty acid is used with a salt, preferably selected among sodium acetate and sodium citrate.

13. The method of any one of claims 1-12, comprising a subsequent precipitation using cold ethanol.

14. The method of any one of claims 1-13, wherein the protein of interest is an antibody, preferably an immunoglobulin G.

15. The method of any one of claims 1-14, implemented in a continuous manner.