

CLAIMS:

1.- A method for manufacturing a protein of interest wherein said protein is expressed in a host cell, comprising:

- a) culturing said host cells under conditions such that they express said protein,
- b) collecting the host cells from the cell culture fluid,
- c) adding buffer to said host cells,
- d) measuring and/or adjusting the pH of the buffer, and
- e) subjecting the host cells to heat treatment at a temperature of between 30°C to 70°C and wherein the redox potential of said buffer is maintained below -0 mV during said heat treatment.

2.- A method according to claim 1 wherein the heat treatment is performed in a container and said redox potential is maintained by reducing the amount of oxygen (O₂) present in the gaseous phase in the container during protein extraction.

3.- A method according to claim 1 or 2, wherein nitrogen (N₂) is added to the gaseous phase of the container.

4.- A method according to claim 3 wherein said gaseous phase contains at least 50% N₂.

5.- A method according to claim 3 or 4 wherein said N₂ is added to the container as an overlay in the gaseous phase of the container or by sparging the N₂ through the buffer containing the host cells obtained in c).

6.- A method according to any preceding claim wherein said heat treatment step is performed at 55°C to 65°C.

7.- A method according to any preceding claim wherein said temperature is maintained for 1 to 18 hours.

8.- A method according to any preceding claim wherein the pH of the buffer is or is adjusted to a pH of 6 to 9.

9.- A method according to any preceding claim wherein the protein is a recombinant protein encoded in an expression vector.

10.- A method according to claim 9 wherein the protein is a recombinant antibody.

11.- A method according to claim 10, wherein said recombinant antibody is a recombinant antibody fragment.

12.- A method according to claim 10 or 11, wherein said recombinant antibody or recombinant antibody fragment specifically binds to TNF-alpha or CD154.