CLAIMS:

1. A perfusion method for manufacturing correctly folded etanercept comprising the following steps: (a) preparing a mixture comprising cells capable of expressing a protein comprising etanercept and a culture medium suitable for conducting such expression; (b) in a suitable reaction vessel containing the mixture, causing the cells to produce the protein comprising etanercept; and (c) periodically or continuously removing spent culture medium from, and adding fresh culture medium to, the reaction vessel, wherein the protein comprising etanercept produced in the method comprises at least 40 wt.%, 50 wt.%, or 60 wt.% of correctly folded etanercept, and wherein the culture medium comprises a Chinese hamster ovary cell medium as a base feed medium and wherein the culture medium comprises dexamethasone, galactose and N-acetylmannosamine (ManNAc).

2. A perfusion method for manufacturing correctly folded etanercept comprising the following steps: (a) preparing a mixture comprising cells capable of expressing a protein comprising etanercept and a culture medium suitable for conducting such expression; (b) in a suitable reaction vessel containing the mixture, causing the cells to produce the protein comprising etanercept; and (c) periodically or continuously removing spent culture medium from, and adding fresh culture medium to, the reaction vessel;

and wherein:

(1) the culture medium comprises a Chinese hamster ovary cell medium, dexamethasone, galactose and N-acetylmannosamine (ManNAc);

(2) prior to step (a), the cells capable of expressing the protein comprising etanercept are grown in a growth phase at a temperature of 28°C to 37°C;

(3) production of the protein comprising etanercept is carried out at a temperature of 33°C to 36°C; and

(4) the protein comprising etanercept comprises at least 40 wt.%, 50 wt.%, or 60 wt.% of correctly folded etanercept, and wherein the

total amount of correctly folded and incorrectly folded protein is produced at a titer of about 0.2 to about 1 g/L.

3. The perfusion method of claim 2 wherein the production of the protein comprising etanercept is conducted at 33°C to 34°C, and the amount of correctly folded etanercept is at least 60 wt.%.

4. The perfusion method of claim 2 in which an alternating tangential flow cell retention device is used to recirculate medium containing waste products and the protein comprising etanercept past a hollow fiber filter whereby the waste products and the protein comprising etanercept are removed from the reaction vessel.

5. The perfusion method of claim 2 wherein the culture medium further comprises at least one feed supplement selected from glutamine and cottonseed hydrolysate.

6. The perfusion method of claim 5 wherein the culture medium comprises cottonseed hydrolysate.

7. A perfusion method for producing correctly folded etanercept said method comprising the steps of: (a) preparing a mixture comprising cells capable of expressing a protein comprising etanercept and a culture medium suitable for conducting such expression; (b) in a suitable reaction vessel containing the mixture, causing the cells to produce the protein comprising etanercept; and (c) periodically or continuously removing spent culture medium from, and adding fresh culture medium to, the reaction vessel; wherein dexamethasone, galactose and N-acetylmannosamine (ManNAc) are present in the culture medium.

8. The perfusion method of claim 7 wherein

(1) prior to step (a), the cells capable of expressing the protein comprising etanercept are grown in a growth phase at a temperature selected from; (i) about 28°C to about 37°C; and (ii) about 35°C to about 36°C; and

(2) production of the protein comprising etanercept is carried out at a temperature selected from (i) greater than about 32°C; (ii) greater than about 33°C; (iii) greater than about 34°C; (iv) greater than about 35°C; (v) the range of about 33°C to about 36°C; (vi) the range of about 35°C to about 36°C; (vii) 32.5°C; (viii) 33.5°C; (ix) 34.5°C; and (x) 35.5°C.

9. The method of claim 8 wherein the protein comprising etanercept produced in the method comprises at least 40 wt.%, 50 wt.%, or 60 wt.% of correctly folded etanercept; the culture medium comprises a Chinese hamster ovary cell base medium, glutamine and cotton hydrolysate; and correctly folded and incorrectly folded protein is produced at a titer of about 0.2 to about 1 g/L.

10. A perfusion method for producing correctly folded etanercept, said method comprising the steps of: (a) preparing a mixture comprising cells capable of expressing a protein comprising etanercept and a culture medium suitable for conducting such expression; (b) in a suitable reaction vessel containing the mixture, causing the cells to produce the protein comprising etanercept; and (c) periodically or continuously removing spent culture medium from, and adding fresh culture medium to, the reaction vessel; wherein:

(i) dexamethasone, galactose and N-acetylmannosamine (ManNAc) are present in the culture medium and

(ii) the culture medium comprises feed media comprising a Chinese hamster ovary cell medium, glutamine and cottonseed hydrolysate, and correctly folded and incorrectly folded protein is produced at titer of about 0.2 to about 1 g/L; and

(iii) production of the protein comprising etanercept is carried out at a temperature selected from (i) greater than about 32°C; (ii) greater than about 33°C; (iii) greater than about 34°C; (iv) greater than about 35°C; (v) the range of about 33°C to about 36°C; (vi) the range of about 35°C to about 36°C; (vii) 32.5°C; (viii) 33.5°C; (ix) 34.5°C; and (x) 35.5°C.

11. The method of claim 10 wherein the production of the protein comprising etanercept is carried out at a temperature of 33°C to 36°C, and the protein comprising etanercept comprises at least 60 wt.% correctly folded etanercept.

12. The method of claim 11 wherein the production of the protein comprising etanercept is carried out at a temperature of 33°C to 34°C.

13. A perfusion method for manufacturing correctly folded etanercept comprising the following steps: (a) preparing a mixture comprising cells capable of expressing a protein comprising etanercept and a culture medium suitable for conducting such expression; (b) in a suitable reaction vessel containing the mixture, causing the cells to produce the protein

comprising etanercept; and (c) periodically or continuously removing spent culture medium from, and adding fresh culture medium to, the reaction vessel; wherein (i) step (b) is carried out at or above 33°C; (ii) the culture medium comprises at least one of dexamethasone, galactose, glutamine, cottonseed hydrolysate, and N-acetylmannosamine (ManNAc); (iii) the protein comprising etanercept comprises at least 60 wt.% correctly folded etanercept; and (iv) the protein comprising etanercept is produced at a titer of about 0.2 to about 1 g/L.

14. The perfusion method of claim 13 wherein the culture medium comprises dexamethasone, galactose and ManNAc.

15. The perfusion method of claim 14 wherein the culture medium comprises a Chinese hamster ovary cell medium.

16. A perfusion method for manufacturing correctly folded etanercept comprising the following steps: (a) preparing a mixture comprising cells capable of expressing a protein comprising etanercept and a culture medium suitable for conducting such expression; (b) in a suitable reaction vessel containing the mixture, causing the cells to produce the protein comprising etanercept; and (c) periodically or continuously removing spent culture medium from, and adding fresh culture medium to, the reaction vessel, wherein the protein comprising etanercept produced in the method comprises at least 40 wt.%, 50 wt.%, or 60 wt.% of correctly folded etanercept, wherein the culture medium comprises a Chinese hamster ovary cell medium as a base feed medium and wherein the culture medium comprises cottonseed hydrolysate.