What is claimed is:

1. A perfusion method for manufacturing 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 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.

2. The perfusion method of claim 1, wherein the culture medium comprises a base feed medium selected from SFM4CHO® medium and BalanCD™/Hycell® medium.

3. The perfusion method of claim 1 wherein the culture medium comprises at least one supplement selected from the group consisting of dexamethasone, ManNAc and galactose.

4. The perfusion method of claim 3 wherein the base feed medium is BalanCD™/Hycell® medium, and the culture medium comprises dexamethasone, galactose and ManNAc.

5. The perfusion method of claim 4 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.

6. The perfusion method of claim 5 wherein the production temperature is in the range of 33°C to about 36°C.

7. The perfusion method of claim 6 wherein 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 produced during the production phase is produced in titers of about 0.2 to about 1 g/L.

8. The composition of claim 7 wherein the production temperature is 33.5°C.

9. The composition of claim 7 wherein the culture medium further comprises at least one feed supplement selected from the group consisting of CHOZN and cottonseed hydrolysate.

10. The perfusion method of claim 3 where in the base feed medium is SFM4CHO® medium, and the culture medium comprises dexamethasone, galactose and ManNAc.

11. The perfusion method of claim 10 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.

12. The composition of claim 11 wherein the production temperature is in the range of 33°C to 36°C.

13. The perfusion method of claim 12 wherein 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 produced during the production phase is produced in titers of about 0.2 to about 1 g/L.

14. The composition of claim 13 wherein the production temperature is 33.5°C.

15. The composition of claim 13 wherein the culture medium further comprises at least one supplement selected from the group consisting of CHOZN and cottonseed hydrolysate.

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 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 BalanCD™/Hycell® medium, dexamethasone, galactose and 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;

(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 produced during the production phase is produced in titers of about 0.2 to about 1 g/L.

17. The perfusion method of claim 16 wherein the production phase is conducted at 33-34°C, and the amount of correctly folded etanercept is at least 60 wt.%.

18. The perfusion method of claim 16 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.

19. The perfusion method of claim 16 wherein the culture medium further comprises at least one feed supplement selected from CHOZN, Feed 1, Feed 2, Efficient Feed A, glutamine and cottonseed hydrolysate.

20. The perfusion method of claim 19 wherein the culture medium comprises CHOZN and cottonseed hydrolysate.

21. Correctly folded etanercept produced by a perfusion method 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 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 BalanCD/Hycell® medium, dexamethasone, galactose and 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;

(4) 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 total amount of correctly folded and incorrectly folded protein produced during the production phase is produced in titers of about 0.2 to about 1 g/L.

22. The correctly folded etanercept of claim 21, wherein the production temperature is 33-34°C and wherein the protein comprising etanercept comprises at least 60 wt.% correctly folded etanercept.

23. Correctly folded etanercept of claim 21, wherein the culture medium further comprises at least one of CHOZN and cottonseed hydrolysate.

24. Correctly folded etanercept of claim 22, wherein the culture medium further comprises CHOZN and cottonseed hydrolysate.

25. Correctly folded etanercept of claim 24, wherein the culture medium further comprises a feed supplement selected from (a) at least one of feed 1, Feed 2 and Efficient Feed A; and (b) all three of the foregoing supplements in combination.

26. A perfusion method for producing correctly folded etanercept having a glycosylation profile substantially similar to that of commercially available etanercept sold under the tradename Enbrel®, 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 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 ManNAc are present in the culture medium to achieve said substantially matching glycosylation profile.

27. The perfusion method of claim 26 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.

28. The method of claim 27 wherein the 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 BalanCD™/Hycell® base medium, EX-Cell CHOZN Platform Feed, glutamine and cotton hydrolysate to achieve a

production titer of correctly folded and incorrectly folded protein produced during the production phase of about 0.2 to about 1 g/L.

29. The method of claim 28 where the culture medium comprises one or more feed supplements selected from (a) at least one of feed 1, Feed 2 and Efficient Feed A; and (b) all three of the foregoing supplements in combination.

30. A perfusion method for producing correctly folded etanercept having a glycosylation profile substantially similar to that of commercially available etanercept sold under the tradename Enbrel®, 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 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 ManNAc are present in the culture medium in amounts sufficient to achieve said substantially matching glycosylation profile and

(ii) the culture medium comprises feed media comprising BalanCD™/Hycell®, CHOZN, glutamine and cottonseed hydrolysate in amounts sufficient to achieve a production titer of correctly folded and incorrectly folded protein produced during the production phase 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.

31. The method of claim 30 wherein 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% correctly folded etanercept.

32. The method of claim 31 wherein production of the protein comprising etanercept is carried out at a temperature of 33-34°C.

33. The method of claim 31 wherein the culture medium further comprises one or more feed supplements selected from (a) at least one of feed 1, Feed 2 and Efficient Feed A; and (b) all three of the foregoing supplements in combination.

34. A perfusion method for manufacturing 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 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 and ManNAc; (iii) the protein comprising etanercept comprises at least 60 wt% correctly folded etanercept; (iv) the protein comprising etanercept is produced in titers of 0.2 to about 1 g/L; and (v) the etanercept has a glycosylation profile substantially similar to that of commercially available etanercept sold under the tradename Enbrel®.

35. The perfusion method of claim 34 wherein the culture medium comprises dexamethasone, galactose and ManNAc.

36. The perfusion method of Claim 35 where the glycosylation profile is characterized by a degree of sialylation substantially similar to that of etanercept commercially available under the tradename Enbrel®.

37. The perfusion method of claim 35 wherein the culture medium comprises a 1:1 mixture of BalanCD™ CHO Growth A and HyClone™ Hycell CHO.

What is claimed is:

1. A perfusion method for manufacturing 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 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.

2. The perfusion method of claim 1, wherein the culture medium comprises a base feed medium selected from SFM4CHO® medium and BalanCD™/Hycell® medium.

3. The perfusion method of claim 1 wherein the culture medium comprises at least one supplement selected from the group consisting of dexamethasone, N-acetylmannosamine (ManNAc) and galactose.

4. The perfusion method of claim 1, wherein the culture medium comprises at least one feed supplement selected from the group consisting of CHOZN, Feed 1, Feed 2, Efficient Feed A, glutamine and cottonseed hydrolysate.

5. The perfusion method of claim 3 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.

6. The perfusion method of claim 5 wherein the production temperature is in the range of 33°C to about 36°C.

7. The perfusion method of claim 6 wherein 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 produced during the production phase is produced in titers of about 0.2 to about 1 g/L.

8. The composition of claim 7 wherein the production temperature is 33.5°C.

9. The composition of claim 7 wherein the culture medium further comprises at least one feed supplement selected from the group consisting of CHOZN, Feed 1, Feed 2, Efficient Feed A, glutamine and cottonseed hydrolysate.

10. The perfusion method of claim 1 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.

11. Correctly folded etanercept produced by a perfusion method 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 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 glutamine;

(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;

(4) 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 total amount of correctly folded and incorrectly folded protein produced during the production phase is produced in titers of about 0.2 to about 1 g/L.

12. The correctly folded etanercept of claim 11, wherein the production temperature is 33-34°C and wherein the protein comprising etanercept comprises at least 60 wt.% correctly folded etanercept.

13. Correctly folded etanercept of claim 11, wherein the culture medium further comprises at least one of dexamethasone, galactose, ManNAc, CHOZN, and cottonseed hydrolysate.

14. Correctly folded etanercept of claim 12, wherein the culture medium further comprises galactose and cottonseed hydrolysate.

15. Correctly folded etanercept of claim 14, wherein the culture medium further comprises a feed supplement selected from (a) at least one of feed 1, Feed 2 and Efficient Feed A; and (b) all three of the foregoing supplements in combination.

16. A perfusion method for producing correctly folded etanercept having a glycosylation profile substantially similar to that of commercially available etanercept sold under the tradename Enbrel®, 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 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 is present in the culture medium to achieve said substantially matching glycosylation profile.

17. The perfusion method of claim 16 wherein

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.

18. The method of claim 17 wherein the 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 and cottonseed hydrolysate to achieve a production titer of correctly folded and incorrectly folded protein produced during the production phase of about 0.2 to about 1 g/L.

19. The method of claim 18 where the culture medium further comprises at least one of: feed 1, Feed 2, Efficient Feed A, dexamethasone, galactose, and ManNAc;.

20. A perfusion method for manufacturing 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 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 glutamine, galactose and cottonseed; (iii) the protein comprising etanercept comprises at least 60 wt% correctly folded etanercept; (iv) the protein comprising etanercept is produced in titers of 0.2 to about 1 g/L; and (v) the etanercept has a glycosylation profile substantially similar to that of commercially available etanercept sold under the tradename Enbrel®.

21. The perfusion method of claim 20 wherein the culture medium comprises glutamine and cottonseed hydrolysate.

22. The perfusion method of Claim 21 where the glycosylation profile is characterized by a degree of sialylation substantially similar to that of etanercept commercially available under the tradename Enbrel®.

23. The perfusion method of claim 16 wherein

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.