CLAIMS

1. A method of selectively reducing one or more unpaired cysteines of a recombinant monoclonal antibody during production of the antibody, wherein the method comprises:

(a) providing a cell capable of recombinant expression of the antibody;

(b) culturing the cell in a cell culture medium, wherein the cells are cultured at a first temperature and then shifted to a second temperature, wherein the second temperature is lower than the first temperature,

wherein the cells are maintained in culture until at least 90% or more of the unpaired cysteines are de-cysteinylated; and

(c) harvesting the antibodies from the cell culture to obtain a preparation of the antibody.

2. The method of claim 1, wherein the cysteine is in the complementary determining region (CDR) of the antibody and/or the antibody is an anti-IL-17 antibody.

3. The method of any preceding claim, wherein the antibody comprises:

(a) a VH sequence having at least about 90% or more sequence identity to the amino acid sequence of SEQ ID NO: 3; and

(b) a VL sequence having at least about 90% or more sequence identity to the amino acid sequence of SEQ ID NO: 4.

4. The antibody of claim 1, wherein the antibody is secukinumab, biosimilar or variant thereof.

5. The method of any preceding claim, wherein the method comprises selectively reducing light chain (LC) Cys97.

6. The method of any preceding claim, wherein (i) the pH of the cell culture is maintained at a constant level; or (ii) the pH of the cell culture is maintained at a constant level and is maintained at a pH of between about 6.7 to about 7.1

7. The method of claim 6, wherein the pH of the cell culture is maintained until at least 90% or more of the unpaired cysteines are de-cysteinylated.

8. The method of any preceding claim, wherein:

(i) the second temperature is between about 3°C to about 5°C lower than the first temperature;
(ii) the second temperature is about 4°C lower than the first temperature;
(iii) the first temperature is about 37ºC; and/or
(iv) the first temperature is about 37ºC and the second temperature is about 33ºC.

9. The method of any preceding claim, wherein: (i) the cells are cultured at the first temperature for between about 8 days to about 13 days before culturing the cells at the second temperature; or (ii) the cells are cultured at the first temperature for about 10 days before culturing the cells at the second temperature.

10. The method of any preceding claim, wherein the cells are maintained in culture for between about 14 days to about 17 days.

11. The method of any preceding claim, wherein step (b) further comprises:
(i) stirring or agitating the cell culture at a rate of about 160 to about 180 rpm (e.g., 170 rpm);
(ii) maintaining a dissolved oxygen (DO) concentration of between about 20% to about 50%, or between about 30% to about 40%;
(iii) inoculating the cell culture medium with the cells at a seeding cell density of between about 0.2 x 10^6 cells/ml to about 0.6 x 10^6 cells/ml, or at a seeding cell density of about 0.4 x 10^6 cells/ml;
(iv) supplementing the cell culture medium with cell feed, or supplementing the cell culture medium with cell feed each day from about day 2, 3 or 4 of the culture to the penultimate day of the culture;
(v) addition of supplemental glucose to the cell culture medium to a concentration between about 2g/L to about 7g/L; and/or
(vi) addition of an antifoam emulsion, or addition of an antifoam emulsion wherein the antifoam emulsion is a silicone antifoam emulsion.

12. The method of any preceding claim, wherein: (i) the cell is a eukaryotic cell; or (ii) the cell is a eukaryotic cell and is a CHO cell.

13. The method of any preceding claim, wherein the cell culture medium is a chemically defined medium and/or animal-component free; or the cell culture medium is a chemically defined medium and/or animal-component free and is supplemented with a mannosidase I inhibitor.

14. The method of claim 13, wherein: (i) the mannosidase I inhibitor is Kifunensine; or (ii) the mannosidase I inhibitor is Kifunensine and is present in the cell culture medium at a concentration of less than about 5 μg/kg.

15. The method of any preceding claim, wherein the antibodies are harvested from the cell culture by centrifugation, flocculation, depth filtration and/or tangential flow filtration.

16. The method of any one of the preceding claims, wherein the method further comprises:

(d) passing the harvested antibodies through one or more chromatography material(s),

thereby obtaining a purified preparation of antibodies.

17. The method of any one of the preceding claims, wherein the method further comprises:

(d) passing a sample of the harvested antibodies through one or more chromatography material(s), thereby binding the antibodies to the chromatography material(s);

(e) contacting the bound antibodies with a wash buffer, wherein the wash buffer is at a pH above about 7.0; and

(f) eluting the antibodies from the chromatography material(s).

18. The method of claim 16 or 17, wherein the chromatography material is cation-exchange (CEX) material.

19. The method of any one of claims 16 to 18, wherein:

(i) about 10 g/L of antibodies or less are loaded onto the chromatography material(s), and the wash buffer is at a pH of about 8.8 to about 9.0; or

(ii) more than about 10g/L of antibodies are loaded onto the chromatography material(s), and the wash buffer is at a pH of about 7.0 to about 8.4, or at a pH of about 8.0.

20. The method of any one of claims 17 to 19, wherein the wash buffer is a Tris buffer.

21. The method of any one of claims 16 to 20, wherein:

(i) the chromatography material(s) are washed with one or more equilibration buffer(s) and/or loading buffer(s) prior to step (e);
(ii) the bound antibodies are contacted with one or more pre-wash buffers prior to step (e);
(iii) the bound antibodies are contacted with one or more post-wash buffers after step (e); and/or
(iv) the bound antibodies are contacted with one or more re-equilibration buffers after step (e).

22. The method of claim 21, wherein:(a) the equilibrium buffer(s), loading buffer(s) and/or re-equilibrium buffer(s) are at a pH of about 5.5; and/or (b) the pre- and/or post-wash buffers are at pH of about 7.4.

23. The method of any one of claims 17 to 22, wherein step (f) comprises passing an elution buffer through the chromatography material, wherein the elution buffer is at a pH of about 5.5.

24. A purified preparation of antibodies obtainable by the method of any one of the preceding claims.

25. A purified preparation of secukinumab, biosimilar or variant thereof, wherein:
(i) LC Cys97 is un-cysteinylated in at least 90%, 95%, 96%, 97%, 98%, 99% or more of the antibodies;
(ii) the % level of intact antibodies is at least about 98.5%, 99.0%, 99.9% or more; and/or
(iii) the antibodies retain biological activity and/or structure as compared to a reference approved antibody.