

CLAIMS

What is claimed:

- 5 1. A method of producing a composition comprising a humanized anti- $\alpha 4\beta 7$ antibody, said method comprising
 - culturing a mammalian host cell in a production medium having a temperature ranging from 36 to 38 degrees Celsius and a pH of 6.5 to 7, and
 - adding a supplement comprising uridine, manganese, and galactose to the
 - 10 production medium, thereby producing a composition comprising the humanized anti- $\alpha 4\beta 7$ antibody,
 - wherein the composition comprises 16% or less basic isoform of the humanized anti- $\alpha 4\beta 7$ antibody, as determined by cation exchange chromatography (CEX),
 - wherein the mammalian host cell is genetically engineered to express a humanized
 - 15 anti- $\alpha 4\beta 7$ antibody which is vedolizumab,
 - wherein the mammalian host cell is a Chinese hamster ovary (CHO) cell.
2. The method of claim 1, wherein the composition comprises a 14% or less, or 13% or less basic isoform of the humanized anti- $\alpha 4\beta 7$ antibody.
- 20 3. The method of claim 1 or 2, wherein the supplement is added to the production medium or is added to a feed medium which is subsequently added to the production medium.
- 25 4. The method of any one of claims 1 to 3, wherein the cumulative concentration of uridine added in the production medium between supplementation and harvest is 1 to 7 mM; wherein the cumulative concentration of manganese in the production medium between supplementation and harvest is 0.002 to 0.015 mM; and/or wherein the cumulative concentration of galactose in the production medium between supplementation
- 30 and harvest is 3 to 20 mM.

5. The method of any one of claims 1 to 4, wherein manganese is added as a supplement multiple times to the production medium by 0.1 to 10 μM , 0.2 to 1.5 μM , 0.2 to 5 μM , 0.25 to 2 μM , 0.3 to 1.2 μM , or 0.3 to 0.8 μM with each addition;

uridine is added as a supplement multiple times to the production medium by 25 to 1000 μM , 75 to 750 μM , 55 to 620 μM , 100 to 600 μM , 150 to 450 μM , 100 to 700 μM , 100 to 600 μM , or 170 to 630 μM with each addition; and/or

wherein galactose is added as a supplement multiple times to the production medium by 0.1 to 10 mM, 0.2 to 7.5 mM, 0.5 to 5 mM, 0.4 to 2.8 mM, 0.5 to 3.5 mM, 0.7 to 2.9 mM, 0.75 to 2.5 mM or by 1.2 mM or 1.4 mM with each addition.

6. The method of claim 5, wherein the supplement is added daily or every two days, and/or the supplement is added beginning on day 4 of the production phase culture.

7. The method of any one of claims 1-6, wherein the feed medium further comprises zinc.

8. The method of claim 7, wherein the cumulative concentration of zinc added to the production medium between supplementation and harvest is 0.005 mM to 0.045 mM.

9. The method of any one of claims 1-8, wherein the method further decreases the percentage of acidic species of the humanized anti- $\alpha 4\beta 7$ antibody relative to the percentage of acidic species produced in a control mammalian host cell expressing the humanized anti- $\alpha 4\beta 7$ antibody that is cultured under substantially similar conditions but in the absence of the supplement.

10. The method of any one of claims 1-9, wherein the method is a fed batch method.

11. The method of any one of claims 1-10, wherein the production medium comprises 4.5 to 5.5 g/L lysine or 5.0 to 8.8 g/L lysine and 3.0 to 12.0 g/L arginine.

12. The method of claim 11, wherein the production medium comprises 5.5 to 8.8 g/L lysine.

13. The method of claim 11 or 12, wherein the production medium comprises 5.4 to 7.4 g/L arginine or 7.4 to 12 g/L arginine.
- 5 14. The method of any one of claims 1-13, wherein the production medium has a pH ranging from 6.8 to 7.0; and/or
has a glucose level that is maintained at 7 g/L or less during the production phase.
15. The method of any one of claims 1-14, wherein the production phase is 14 days or
10 less or the production phase ranges from 10 days to 17 days.
16. The method of any one of claims 1-15, which is performed in a large scale bioreactor.
- 15 17. The method of any one of claims 1-16, wherein the production phase results in a titer of the humanized anti- $\alpha 4\beta 7$ antibody of greater than 3 g/L; 3 to 8 g/L, or 5 to 7 g/L.
18. The method of any one of claims 1-17, wherein the method comprises harvesting and purification of the antibody.
- 20 19. The method of claim 18, wherein the method further comprises preparing a pharmaceutical formulation of the purified antibody which is suitable for human therapeutic use.
20. A composition comprising humanized anti- $\alpha 4\beta 7$ antibodies, wherein the
25 composition is produced using or is obtainable by any one of the methods of claims 1-19,
wherein the composition comprises 16% or less basic isoform of the humanized anti- $\alpha 4\beta 7$ antibody, as determined by cation exchange chromatography (CEX).
21. The composition of claim 20, comprising a population of humanized anti- $\alpha 4\beta 7$
30 antibodies having (i) 90% or more, or (ii) 92-95%, total asialo-, agalacto, core fucosylated biantennary glycan (G0F), asialo-, monogalacto, core fucosylated biantennary glycan (G1F), and/or asialo-, digalacto, core fucosylated biantennary glycan (G2F) glycosylation variants.

22. The method of any one of claims 1-10, wherein the method is a method of producing a composition having a decreased amount of a G0F glycoform (as determined by Hydrophilic Interaction Chromatography (HILIC) of the humanized anti- α 4 β 7 antibody, in comparison to a control mammalian host cell expressing the humanized anti- α 4 β 7 antibody that is cultured under substantially similar conditions but in the absence of the supplement.

23. The method of claim 22, wherein the composition comprises at least about a 15% decreased or a 20% decreased level of the G0F glycoform of the humanized anti- α 4 β 7 antibody in comparison to a control mammalian host cell expressing the humanized anti- α 4 β 7 antibody that is cultured under substantially similar conditions in the absence of the supplement.

24. The method of any one of claims 1-10, wherein the method is a method of producing a composition having 65% or less, 60% or less, or 55% or less G0F glycoform (as determined by HILIC) of the humanized anti- α 4 β 7 antibody.

25. The method of any one of claims 1-10, wherein the method is a method of producing a composition having an increased amount of a G1F glycoform (as determined by HILIC) of the humanized anti- α 4 β 7 antibody in comparison to a control mammalian host cell expressing the humanized anti- α 4 β 7 antibody that is cultured under substantially similar conditions in the absence of the supplement.

26. The method of claim 25, wherein the composition comprises at least a 2-fold or at least a 3-fold increase in the G1F glycoform of the humanized anti- α 4 β 7 antibody in comparison to the control.

27. The method of any one of claims 1-10, wherein the method is a method of producing a composition having 25% or more or 30% or more G1F glycoform (as determined by HILIC) of the humanized anti- α 4 β 7 antibody.

28. The method of any one of claims 1-10, wherein the method is a method of producing a composition having an increased amount of a G2F glycoform (as determined by HILIC) of the humanized anti- $\alpha 4\beta 7$ antibody in comparison to a control mammalian host cell expressing the humanized anti- $\alpha 4\beta 7$ antibody that is cultured under substantially similar conditions in the absence of the supplement.

29. The method of claim 28, wherein the composition comprises at least a 3-fold increase or a 4-fold increase in the G2F glycoform of the humanized anti- $\alpha 4\beta 7$ antibody in comparison to the control.

30. The method of any one of claims 1-10, wherein the method is a method of producing a composition having 3% or more or 4% or more G2F glycoform (as determined by HILIC) of the humanized anti- $\alpha 4\beta 7$ antibody.

31. The method of any one of claims 22-30, wherein the method further decreases the percentage of acidic species of the humanized anti- $\alpha 4\beta 7$ antibody relative to the percentage of acidic species produced in a control mammalian host cell expressing the humanized anti- $\alpha 4\beta 7$ antibody that is cultured under substantially similar conditions in the absence of the supplement; or

wherein the method further increases the percentage of main isoform species of the humanized anti- $\alpha 4\beta 7$ antibody relative to the percentage of main isoform species produced in a control mammalian host cell expressing the humanized anti- $\alpha 4\beta 7$ antibody that is cultured under substantially similar conditions in the absence of a feed medium comprising uridine, manganese, and galactose added to the production medium.

32. The method of any one of claims 22-31, wherein the feed medium is added to the production medium beginning on about day four of the production phase; or wherein the production medium has a pH of 6.8 to 7.

33. The method of any one of claims 22-32, wherein the method comprises harvesting and purification of the antibody.

34. The method of claim 33, wherein the method further comprises preparing a pharmaceutical formulation of the purified antibody which is suitable for human therapeutic use.
- 5 35. A composition comprising a humanized anti- $\alpha 4\beta 7$ antibody, wherein the composition is obtainable by any one of the methods of claims 22-32, wherein the composition comprises 16% or less basic isoform of the humanized anti- $\alpha 4\beta 7$ antibody, as determined by cation exchange chromatography (CEX).
- 10 36. A cell culture comprising a host cell genetically engineered to express a humanized anti- $\alpha 4\beta 7$ antibody, and a production medium supplemented with uridine, manganese, and galactose (UMG), wherein the humanized anti- $\alpha 4\beta 7$ antibody vedolizumab, wherein the host cell is a Chinese hamster ovary (CHO) cell, wherein the cell culture has a temperature ranging from 36 to 38 degrees Celsius and a pH of 6.5 to 7, and
- 15 wherein the cell culture comprises about 16% or less basic isoform of the humanized anti- $\alpha 4\beta 7$ antibody, as determined by cation exchange chromatography (CEX).
37. The cell culture of claim 36, wherein the production medium comprises
- 20 supplemented uridine at a concentration of 1 to 7 mM, supplemented manganese at a concentration of 0.002 to 0.015 mM, and supplemented galactose at a concentration of 3 to 20 mM on the day of harvest.
38. The cell culture of claim 36 or 37, wherein the production medium further
- 25 comprises zinc.
39. The cell culture of claim 38, wherein the production medium comprises supplemented zinc at a concentration of 0.005 mM to 0.045 mM on the day of harvest.
- 30 40. The cell culture of any one of claims 36-39, wherein the expressed humanized anti- $\alpha 4\beta 7$ antibody has an isoform distribution comprising:
- a. 15% or less, 14% or less, 13% or less, or 12% or less basic isoform; and/or

- b. at least 65%, at least 68%, at least 70%, at least 72%, or at least 75% major isoform; and/or
wherein the expressed humanized anti- $\alpha 4\beta 7$ antibody has a fucosylated N-glycan content comprising:
- 5 c. 65% or less, 60% or less, or 55% or less G0F;
d. 25% or more, 27% or more, or 30% or more G1F; and/or
e. 2.5% or more, 3% or more, 3.5% or more, 4% or more, or 4.5% or more G2F.
- 10 41. The cell culture of any one of claims 36-40, wherein the expressed humanized anti- $\alpha 4\beta 7$ antibody has a total fucosylated N-glycan content (G0F + G1F + G2F) of at least 88%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, or at least 95%.
- 15 42. The cell culture of any one of claims 36-40, wherein the expressed humanized anti- $\alpha 4\beta 7$ antibody has a total fucosylated N-glycan content (G0F + G1F + G2F) of 92-95%; and/or wherein the expressed humanized anti- $\alpha 4\beta 7$ antibody has a total fucosylated N-glycan content (G0F + G1F + G2F) of 91-92%, 91-92.5%, or 91-93%.