

CLAIMS

What is claimed:

1. A method for obtaining a composition comprising an anti- $\alpha 4\beta 7$ antibody from a
5 liquid solution comprising the anti- $\alpha 4\beta 7$ antibody and one or more impurities, said method comprising
contacting a matrix comprising Protein A with the liquid solution comprising the anti- $\alpha 4\beta 7$ antibody and one or more impurities, such that the anti- $\alpha 4\beta 7$ antibody binds to the Protein A;
10 washing the matrix comprising Protein A with a wash solution; and
eluting the anti- $\alpha 4\beta 7$ antibody from the matrix comprising Protein A by contacting the matrix with an elution solution having a pH of 3.2 to 4, such that a composition comprising the anti- $\alpha 4\beta 7$ antibody is obtained,
wherein the anti- $\alpha 4\beta 7$ antibody is a humanized antibody, is an IgG1 antibody,
15 comprises a heavy chain variable region comprising a CDR3 domain as set forth in SEQ ID NO: 4, a CDR2 domain as set forth in SEQ ID NO: 3, and a CDR1 domain as set forth in SEQ ID NO: 2; and comprises a light chain variable region comprising a CDR3 domain as set forth in SEQ ID NO: 8, a CDR2 domain as set forth in SEQ ID NO: 7, and a CDR1 domain as set forth in SEQ ID NO: 6.
20
2. The method of claim 1, wherein the composition comprising the anti- $\alpha 4\beta 7$ antibody comprises less than 1% high molecular weight (HMW) aggregate.
3. The method of claim 1 or 2, wherein the Protein A is immobilized on a solid phase.
25
4. The method of claim 3, wherein the solid phase comprises one or more of a bead, a gel, and a resin.
5. The method of any one of claims 1 to 4, wherein the wash solution has a pH of about
30 7.
6. The method of any one of claims 1 to 5, wherein the elution solution comprises citric acid.

7. The method of any one of claims 1 to 6, wherein the elution solution has a pH of 3.2 to 3.7 or 3.3 to 3.8.
- 5 8. A method for obtaining a composition comprising an anti- $\alpha 4\beta 7$ antibody from a liquid solution comprising an anti- $\alpha 4\beta 7$ antibody and one or more impurities, said method comprising
- 10 contacting a solution comprising an anti- $\alpha 4\beta 7$ antibody and at least one impurity with a hydrophobic interaction chromatography (HIC) resin under conditions that allow flow through of the anti- $\alpha 4\beta 7$ antibody through the HIC resin, such that a composition comprising the anti- $\alpha 4\beta 7$ antibody is obtained,
- wherein the HIC resin is characterized as a high hydrophobic HIC resin,
- wherein the anti- $\alpha 4\beta 7$ antibody is a humanized antibody, is an IgG1 antibody, comprises a heavy chain variable region comprising a CDR3 domain as set forth in SEQ ID
- 15 NO: 4, a CDR2 domain as set forth in SEQ ID NO: 3, and a CDR1 domain as set forth in SEQ ID NO: 2; and comprises a light chain variable region comprising a CDR3 domain as set forth in SEQ ID NO: 8, a CDR2 domain as set forth in SEQ ID NO: 7, and a CDR1 domain as set forth in SEQ ID NO: 6.
- 20 9. The method of claim 8, wherein the composition comprising the anti- $\alpha 4\beta 7$ antibody, comprises less than 0.6% HMW aggregate.
10. The method of claim 8 or 9, wherein the HIC resin is equilibrated with a phosphate buffer having a pH of less than about 7.2.
- 25 11. The method of claim 10, wherein the phosphate buffer comprises about 0.35 M to about 0.15 M potassium phosphate.
12. The method of any one of claims 8 to 11, wherein the resin load is about 55 to 75
- 30 mg/ml.
13. The method of any one of claims 8 to 12, wherein the composition comprises less than about 0.22 ppm residual protein A.

14. The method of any one of claims 8 to 13, wherein the composition contains less than about 0.3 ppm host cell protein (HCP).
- 5 15. The method of any one of claims 8 to 14, wherein the high hydrophobic HIC resin has a mean pore size of about 50 to 150 μm .
16. The method of any one of claims 8 to 14, wherein the high hydrophobic HIC resin has a mean pore size of about 100 nm and/or a pore size of about 100 μm .
- 10 17. A method for producing a composition comprising an anti- $\alpha 4\beta 7$ antibody from a liquid solution comprising the anti- $\alpha 4\beta 7$ antibody and one or more impurities, said method comprising
- 15 contacting the liquid solution comprising the anti- $\alpha 4\beta 7$ antibody and one or more impurities with a mixed mode chromatography resin, such that the anti- $\alpha 4\beta 7$ antibody binds to the resin;
- washing the mixed mode chromatography resin with a wash solution; and
- eluting the anti- $\alpha 4\beta 7$ antibody from the mixed mode chromatography resin by contacting the resin with an elution solution having a pH at or above pH 3.9, such that a composition
- 20 comprising the anti- $\alpha 4\beta 7$ antibody is obtained,
- wherein the anti- $\alpha 4\beta 7$ antibody comprises a heavy chain variable region set forth in SEQ ID NO:1, and a light chain variable region set forth in SEQ ID NO:2.
18. The method of claim 17, wherein the composition comprising the anti- $\alpha 4\beta 7$ antibody,
- 25 comprises less than 1% HMW aggregate.
19. The method of claim 17 or 18, wherein the elution solution has a pH at or above pH 4.1.
- 30 20. The method of any one of claims 17 to 19, wherein the elution solution has a pH of about pH 3.9 to about pH 4.4.

21. The method of any one of claims 17 to 20, wherein the elution solution has a conductivity of 30 mS/cm or less.
22. The method of claim 21, wherein the elution solution has a conductivity of about 20 mS/cm to about 30 mS/cm.
23. The method of any one of claims 17 to 22, wherein the elution solution comprises NaCl at a concentration of about 160 mM to about 240 mM.
24. The method of any one of claims 17 to 23, wherein the mixed mode chromatography resin is Capto Adhere ImpRes.
25. The method of any one of claims 17 to 24, wherein the method further comprises purifying the anti- $\alpha 4\beta 7$ antibody using a cation exchange (CEX) resin.
26. The method of claim 26, wherein the CEX resin is operated in bind/elute mode.
27. A method for producing a composition comprising an anti- $\alpha 4\beta 7$ antibody from a liquid solution comprising an anti- $\alpha 4\beta 7$ antibody and one or more impurities, said method comprising
- contacting the liquid solution comprising the anti- $\alpha 4\beta 7$ antibody and one or more impurities with a mixed mode chromatography resin, such that the anti- $\alpha 4\beta 7$ antibody binds to the resin;
- washing the mixed mode chromatography resin with a wash solution; and
- eluting the anti- $\alpha 4\beta 7$ antibody from the mixed mode chromatography resin by contacting the resin with an elution solution having a pH at or below pH 4.2 and a conductivity at or below 28 mS/cm, such that a composition comprising the anti- $\alpha 4\beta 7$ antibody is obtained,
- wherein the anti- $\alpha 4\beta 7$ antibody comprises a heavy chain variable region set forth in SEQ ID NO:1, and a light chain variable region set forth in SEQ ID NO:2.
28. The method of claim 27, wherein the composition comprises an increased yield of an anti- $\alpha 4\beta 7$ antibody relative to a control composition comprising the anti- $\alpha 4\beta 7$ antibody

obtained in like manner using a control elution solution having a pH above pH 4.2 and/or a control conductivity above 28 mS/cm.

29. The method of claim 27 or 28, wherein the elution solution has a pH at or below 4.0.
30. The method of claim 27 or 28, wherein the elution solution has a pH of about pH 4.2 to about pH 3.8.
31. The method of any one of claims 27 to 30, wherein the elution solution has a conductivity of about 18 mS/cm to about 28 mS/cm.
32. The method of any one of claims 27 to 31, wherein the elution solution comprises NaCl at a concentration of about 160 mM to about 240 mM.
33. The method of any one of claims 27 to 32, wherein the mixed mode chromatography resin is contacted with at least 55 g of the anti- $\alpha 4\beta 7$ antibody per liter of resin.
34. The method of claim 33, wherein the mixed mode chromatography resin is contacted with about 55 g to about 80 g of the anti- $\alpha 4\beta 7$ antibody per liter of resin.
35. The method of any one of claims 27 to 34, wherein the mixed mode chromatography resin has strong anion exchange, hydrogen bonding, and hydrophobic interaction functionality on a smaller bead size, optionally wherein the mixed mode chromatography resin is Capto Adhere ImpRes.
36. The method of any one of claims 27 to 35, wherein the method further comprises purifying the anti- $\alpha 4\beta 7$ antibody using a cation exchange (CEX) resin.
37. The method of claim 36, wherein the CEX resin is operated in bind/elute mode.
38. A method for producing a composition comprising an anti- $\alpha 4\beta 7$ antibody from a liquid solution comprising an anti- $\alpha 4\beta 7$ antibody and one or more impurities, said method comprising

contacting the liquid solution comprising the anti- $\alpha 4\beta 7$ antibody and one or more impurities with a cation exchange (CEX) resin, such that the anti- $\alpha 4\beta 7$ antibody binds to the resin;

washing the CEX resin with a wash solution; and

- 5 eluting the anti- $\alpha 4\beta 7$ antibody from the CEX resin by contacting the resin with an elution solution having a conductivity at or below 16 mS/cm, such that a composition comprising the anti- $\alpha 4\beta 7$ antibody is obtained,

wherein the anti- $\alpha 4\beta 7$ antibody comprises a heavy chain variable region set forth in SEQ ID NO:1, and a light chain variable region set forth in SEQ ID NO:2.

10

39. The method of claim 38, wherein the composition comprising the anti- $\alpha 4\beta 7$ antibody, comprises about 1% or less HMW aggregate.

40. The method of claim 38 or 39, wherein the elution solution has a conductivity at or
15 below 14 mS/cm.

41. The method of claim 38 or 39, wherein the elution solution has a conductivity of about 11-16 mS/cm.

20 42. The method of claim 38 or 39, wherein the elution solution has a conductivity of about 12-14 mS/cm.

43. The method of any one of claims 38 to 42, wherein the elution solution comprises NaCl at a concentration of about 70 mM to about 110 mM.

25

44. The method of any one of claims 38 to 43, wherein the elution solution has a pH from about pH 5 to about pH 6.

45. The method of claim 44, wherein the elution solution has a pH from about pH 5.1 to
30 about pH 5.8.

46. The method of any one of claims 38 to 45, wherein the anti- $\alpha 4\beta 7$ antibody is loaded on the CEX resin at a concentration of about 25-70 g antibody per liter of resin.

47. The method of any one of claims 38 to 46, wherein the anti- $\alpha 4\beta 7$ antibody is loaded on the CEX resin at a concentration of about 30-60 g antibody per liter of resin.
- 5 48. The method of any one of claims 38 to 47, wherein the CEX resin is a strong CEX resin, optionally wherein the CEX resin is Nuvia HR-S.
49. The method of any one of claims 38 to 48, wherein the method further comprises purifying the anti- $\alpha 4\beta 7$ antibody using a mixed mode chromatography resin.
- 10 50. The method of claim 49, wherein the mixed mode chromatography resin is operated in bind/elute mode.
51. A method for producing a composition comprising an anti- $\alpha 4\beta 7$ antibody from a liquid solution comprising a major isoform of an anti- $\alpha 4\beta 7$ antibody and one or more basic isoform species, the method comprising
- 15 contacting the liquid solution comprising the anti- $\alpha 4\beta 7$ antibody and one or more basic isoform species with a cation exchange (CEX) resin, such that the anti- $\alpha 4\beta 7$ antibody binds to the resin;
- 20 washing the CEX resin with a wash solution; and
- eluting the anti- $\alpha 4\beta 7$ antibody from the CEX resin by contacting the resin with an elution solution having a conductivity at or above 11 mS/cm, such that a composition comprising the anti- $\alpha 4\beta 7$ antibody is obtained,
- wherein the anti- $\alpha 4\beta 7$ antibody comprises a heavy chain variable region set forth in SEQ ID NO:1, and a light chain variable region set forth in SEQ ID NO:2.
- 25 52. The method of claim 51, wherein the composition comprising the anti- $\alpha 4\beta 7$ antibody, comprises about 4% to about 20% basic isoform.
- 30 53. The method of claim 51 or 52, wherein the elution solution has a conductivity at or above 12 mS/cm.

54. The method of claim 51 or 52, wherein the elution solution has a conductivity of about 11-16 mS/cm.
55. The method of claim 51 or 52, wherein the elution solution has a conductivity of
5 about 12-14 mS/cm.
56. The method of any one claims 51 to 55, wherein the elution solution has a pH from about pH 5 to about pH 6.
- 10 57. The method of claim 56, wherein the elution solution has a pH from about pH 5.1 to about pH 5.8.
58. The method of any one of claims 51 to 57, wherein the anti- $\alpha 4\beta 7$ antibody is loaded on the CEX resin at a concentration of about 25-70 g antibody per liter of resin.
15
59. The method of claim 58, wherein the anti- $\alpha 4\beta 7$ antibody is loaded on the CEX resin at a concentration of about 30-60 g antibody per liter of resin.
60. The method of any one of claims 51 to 59, wherein the CEX resin is Nuvia HR-S.
20
61. The method of any one of claims 51 to 60, wherein the method further comprises purifying the anti- $\alpha 4\beta 7$ antibody using a mixed mode chromatography resin.
62. The method of claim 61, wherein the mixed mode chromatography resin is operated
25 in bind/elute mode.
63. The method of any one of claims 1 to 62, wherein the antibody was produced in a Chinese Hamster Ovary (CHO) cell.
- 30 64. The method of claim 63, wherein the host cell is a GS-CHO cell.
65. The method of any one of claims 1 to 64, wherein the composition obtained comprises purified anti- $\alpha 4\beta 7$ antibody, and wherein the method further comprises a

subsequent step of formulating the anti- $\alpha 4\beta 7$ antibody into a formulation suitable for human use.

66. The method of any one of claims 1 to 65, wherein the method comprises formulating
5 the purified anti- $\alpha 4\beta 7$ antibody into a dry, lyophilized formulation.

67. The method of claim 66, wherein the method further comprises reconstituting the dry, lyophilized formulation with a liquid so that it is suitable for administration.

10 68. The method of any one of claims 1 to 65, wherein the method comprises formulating purified anti- $\alpha 4\beta 7$ antibody into a liquid formulation so that the anti- $\alpha 4\beta 7$ antibody is suitable for administration by subcutaneous injection.

69. The method of any one of claims 1 to 68, wherein the anti- $\alpha 4\beta 7$ antibody comprises a
15 heavy chain variable region sequence as set forth in SEQ ID NO: 1, and a light chain variable region sequence as set forth in SEQ ID NO: 5.

70. The method of any one of claims 1 to 68, wherein the anti- $\alpha 4\beta 7$ antibody is
vedolizumab.

20

71. A composition comprising an anti- $\alpha 4\beta 7$ antibody, wherein said composition is obtainable by the method of any one of claims 1-70.