CLAIMS

What is claimed:

1. A method for obtaining a composition comprising an anti-α4β7 antibody from a liquid solution comprising the anti-α4β7 antibody and one or more impurities, said method comprising

contacting a matrix comprising Protein A with the liquid solution comprising the anti-α4β7 antibody and one or more impurities, such that the anti-α4β7 antibody binds to the Protein A;

washing the matrix comprising Protein A with a wash solution; and

eluting the anti-α4β7 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-α4β7 antibody is obtained,

wherein the anti-α4β7 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 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.

2. The method of claim 1, wherein the composition comprising the anti-α4β7 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.~~

~~4. The method of claim 3, wherein the solid phase comprises one or more of a bead, a gel, and a resin.~~

~~5~~3. The method of ~~any one of~~ claim~~s~~ 1 ~~to~~or ~~4~~2, wherein the wash solution has a pH of about 7.

~~6~~4. The method of any one of claims 1 to ~~5~~3, wherein the elution solution comprises citric acid and/or the elution solution has a pH of 3.2 to 3.7 or 3.3 to 3.8.

~~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.~~

~~8~~5. A method for obtaining a composition comprising an anti-α4β7 antibody from a liquid solution comprising an anti-α4β7 antibody and one or more impurities, said method comprising

contacting a solution comprising an anti-α4β7 antibody and at least one impurity with a hydrophobic interaction chromatography (HIC) resin under conditions that allow flow through of the anti-α4β7 antibody through the HIC resin, such that a composition comprising the anti-α4β7 antibody is obtained,

wherein the HIC resin is characterized as a high hydrophobic HIC resin,

wherein the anti–α4β7 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 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.

~~9~~6. The method of claim ~~8~~5, wherein the composition comprising the anti-α4β7 antibody, comprises less than 0.6% HMW aggregate.

~~10~~7. The method of claim ~~8~~5 or ~~9~~6, wherein the HIC resin is equilibrated with a phosphate buffer having a pH of less than about 7.2.

~~11~~8. The method of claim ~~10~~7, wherein the phosphate buffer comprises about 0.35 M to about 0.15 M potassium phosphate.

~~12~~9. The method of any one of claims ~~8~~5 to ~~11~~8, wherein the resin load is about 55 to 75 mg/ml.

~~13~~10. The method of any one of claims ~~8~~5 to ~~12~~9, wherein the composition comprises less than about 0.22 ppm residual protein A and/or the composition contains less than about 0.3 ppm host cell protein (HCP).

~~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).~~

~~15~~11. The method of any one of claims ~~8~~5 to ~~14~~10, 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.~~

~~17~~12. A method for producing a composition comprising an anti-α4β7 antibody from a liquid solution comprising the anti-α4β7 antibody and one or more impurities, said method comprising

contacting the liquid solution comprising the anti-α4β7 antibody and one or more impurities with a mixed mode chromatography resin, such that the anti-α4β7 antibody binds to the resin;

washing the mixed mode chromatography resin with a wash solution; and

eluting the anti-α4β7 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 comprising the anti-α4β7 antibody is obtained,

wherein the anti-α4β7 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~~13. The method of claim ~~17~~12, wherein the composition comprising the anti-α4β7 antibody, 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.~~

2014. The method of ~~any one of~~ claim~~s~~ ~~17~~12 ~~to~~or ~~19~~13, wherein the elution solution has a pH of about pH 3.9 to about pH 4.4.

~~21~~15. The method of any one of claims ~~17~~12 to ~~20~~14, wherein the elution solution has a conductivity of 30 mS/cm or less, or wherein the elution solution has a conductivity of about 20 mS/cm to about 30 mS/cm.

~~22. The method of claim 21, wherein the elution solution has a conductivity of about 20 mS/cm to about 30 mS/cm.~~

~~23~~16. The method of any one of claims ~~17~~12 to ~~22~~15, 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~~17. The method of any one of claims ~~17~~12 to ~~24~~16, wherein the method further comprises purifying the anti-α4β7 antibody using a cation exchange (CEX) resin.

~~26~~18. The method of claim ~~26~~17, wherein the CEX resin is operated in bind/elute mode.

~~27~~19. A method for producing a composition comprising an anti-α4β7 antibody from a liquid solution comprising an anti-α4β7 antibody and one or more impurities, said method comprising

contacting the liquid solution comprising the anti-α4β7 antibody and one or more impurities with a mixed mode chromatography resin, such that the anti-α4β7 antibody binds to the resin;

washing the mixed mode chromatography resin with a wash solution; and

eluting the anti-α4β7 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-α4β7 antibody is obtained,

wherein the anti-α4β7 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~~20. The method of claim ~~27~~19, wherein the composition comprises an increased yield of an anti-α4β7 antibody relative to a control composition comprising the anti-α4β7 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~~21. The method of claim ~~27~~19 or ~~28~~20, wherein the elution solution has a pH at or below 4.0 or wherein the elution solution has a pH of about pH 4.2 to about pH 3.8.

~~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~~22. The method of any one of claims ~~27~~19 to ~~30~~21, wherein the elution solution has a conductivity of about 18 mS/cm to about 28 mS/cm and/or the elution solution comprises NaCl at a concentration of about 160 mM to about 240 mM.

~~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~~23. The method of any one of claims ~~27~~19 to ~~32~~22, wherein the mixed mode chromatography resin is contacted with at least 55 g or with about 55 g to about 80 g of the anti-α4β7 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-α4β7 antibody per liter of resin.~~

~~35~~24. The method of any one of claims ~~27~~19 to ~~34~~23, 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~~25. The method of any one of claims ~~27~~19 to ~~35~~24, wherein the method further comprises purifying the anti-α4β7 antibody using a cation exchange (CEX) resin.

~~37~~26. The method of claim ~~36~~25, wherein the CEX resin is operated in bind/elute mode.

~~38~~27. A method for producing a composition comprising an anti-α4β7 antibody from a liquid solution comprising an anti-α4β7 antibody and one or more impurities, said method comprising

contacting the liquid solution comprising the anti-α4β7 antibody and one or more impurities with a cation exchange (CEX) resin, such that the anti-α4β7 antibody binds to the resin;

washing the CEX resin with a wash solution; and

eluting the anti-α4β7 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-α4β7 antibody is obtained,

wherein the anti-α4β7 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.

~~39~~28. The method of claim ~~38~~27, wherein the composition comprising the anti-α4β7 antibody, comprises about 1% or less HMW aggregate.

~~40~~29. The method of claim ~~38~~27 or ~~39~~28, wherein the elution solution has a conductivity at or below 14 mS/cm, about 11-16 mS/cm or about 12-14 mS/cm.

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

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

~~43~~30. The method of any one of claims ~~38~~27 to ~~42~~29, wherein the elution solution comprises NaCl at a concentration of about 70 mM to about 110 mM.

~~44~~31. The method of any one of claims ~~38~~27 to ~~43~~30, wherein the elution solution has a pH from about pH 5 to about pH 6 or about pH 5.1 to about pH 5.8.

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

~~46~~32. The method of any one of claims ~~38~~27 to ~~45~~31, wherein the anti-α4β7 antibody is loaded on the CEX resin at a concentration of about 25-70 g antibody per liter of resin; or the anti-α4β7 antibody is loaded on the CEX resin at a concentration of about 30-60 g antibody per liter of resin.

~~47. The method of any one of claims 38 to 46, wherein the anti-α4β7 antibody is loaded on the CEX resin at a concentration of about 30-60 g antibody per liter of resin.~~

~~48~~33. The method of any one of claims ~~38~~27 to ~~47~~32, wherein the CEX resin is a strong CEX resin, optionally wherein the CEX resin is Nuvia HR-S.

~~49~~34. The method of any one of claims ~~38~~27 to ~~48~~33, wherein the method further comprises purifying the anti-α4β7 antibody using a mixed mode chromatography resin.

~~50~~35. The method of claim ~~49~~34, wherein the mixed mode chromatography resin is operated in bind/elute mode.

~~51~~36. A method for producing a composition comprising an anti-α4β7 antibody from a liquid solution comprising a major isoform of an anti-α4β7 antibody and one or more basic isoform species, the method comprising

contacting the liquid solution comprising the anti-α4β7 antibody and one or more basic isoform species with a cation exchange (CEX) resin, such that the anti-α4β7 antibody binds to the resin;

washing the CEX resin with a wash solution; and

eluting the anti-α4β7 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-α4β7 antibody is obtained,

wherein the anti-α4β7 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.

~~52~~37. The method of claim ~~51~~36, wherein the composition comprising the anti-α4β7 antibody, comprises about 4% to about 20% basic isoform.

~~53~~38. The method of claim ~~51~~36 or ~~52~~37, wherein the elution solution has a conductivity at or above 12 mS/cm, about 11-16 mS/cm or about 12-14 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 about 12-14 mS/cm.~~

~~56~~39. The method of any one claims ~~51~~36 to ~~55~~38, wherein the elution solution has a pH from about pH 5 to about pH 6 or from about pH 5.1 to about pH 5.8.

~~57. The method of claim 56, wherein the elution solution has a pH from about pH 5.1 to about pH 5.8.~~

~~58~~40. The method of any one of claims ~~51~~36 to ~~57~~39, wherein the anti-α4β7 antibody is loaded on the CEX resin at a concentration of about 25-70 g or about 30-60 g antibody per liter of resin.

~~59. The method of claim 58, wherein the anti-α4β7 antibody is loaded on the CEX resin at a concentration of about 30-60 g antibody per liter of resin.~~

~~60~~41. The method of any one of claims ~~51~~36 to ~~59~~40, wherein the CEX resin is Nuvia HR-S.

~~61~~42. The method of any one of claims ~~51~~36 to ~~60~~41, wherein the method further comprises purifying the anti-α4β7 antibody using a mixed mode chromatography resin.

~~62~~43. The method of claim ~~61~~42, wherein the mixed mode chromatography resin is operated in bind/elute mode.

~~63~~44. The method of any one of claims 1 to ~~62~~43, wherein the antibody was produced in a Chinese Hamster Ovary (CHO) cell.

~~64. The method of claim 63, wherein the host cell is a GS-CHO cell.~~

~~65~~45. The method of any one of claims 1 to ~~64~~44, wherein the composition obtained comprises purified anti-α4β7 antibody, and wherein the method further comprises a subsequent step of formulating the anti-α4β7 antibody into a formulation suitable for human use.

~~66~~46. The method of any one of claims 1 to ~~65~~45, wherein the method comprises formulating the purified anti-α4β7 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.~~

~~68~~47. The method of any one of claims 1 to ~~65~~45, wherein the method comprises formulating purified anti-α4β7 antibody into a liquid formulation so that the anti-α4β7 antibody is suitable for administration by subcutaneous injection.

~~69~~48. The method of any one of claims 1 to ~~68~~47, wherein the anti-α4β7 antibody comprises a 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~~49. The method of any one of claims 1 to ~~68~~47, wherein the anti-α4β7 antibody is vedolizumab.

~~71~~50. A composition comprising an anti-α4β7 antibody, wherein said composition is obtainable by the method of any one of claims 1-~~70~~49.