

CLAIMS

What is claimed is:

1. A method of producing a composition comprising vedolizumab, comprising:
providing a composition comprising vedolizumab at a pH at or greater than pH 6.5;
and
incubating the composition comprising vedolizumab for a period of at least 20 minutes-10 hours;
thereby producing a composition comprising vedolizumab.
2. The method of claim 1, wherein the method is a method of producing a composition comprising vedolizumab having a reduced level of basic vedolizumab isoform species, and wherein the method produces a composition comprising vedolizumab having a reduced level of basic vedolizumab isoform species.
3. The method of claim 1 or 2, wherein the method produces a composition comprising vedolizumab having <16%, <15%, <14%, <13%, <12%, <11% or <10% basic vedolizumab isoform species.
4. The method of any one of claims 1-3, wherein the incubation is performed during vedolizumab purification, and wherein the incubation is performed (a) prior to ultrafiltration/diafiltration (UF/DF) of the antibody, or (b) prior to formulation of the antibody in a pharmaceutically acceptable buffer.
5. The method of any one of claims 1-4, wherein the incubation is performed at ambient temperature, or the incubation is performed at 15-30°C, or the incubation is performed at 20-25°C.
- ~~6. The method of any one of claims 1-4, wherein the incubation is performed at 15-30°C, or wherein the incubation is performed at 20-25°C.~~
- ~~76.~~ The method of any one of the previous claims, wherein:
the composition comprising vedolizumab is provided at a pH of about 6.5-8.5.

~~87.~~ The method of any one of the previous claims, wherein the composition comprising vedolizumab is provided

at a pH of about 7.0-8.0,

at a pH of 7.0-7.5, or

at a pH of about pH 6.5, pH 6.6, pH 6.7, pH 6.8, pH 6.9, pH 7.0, pH 7.1, pH 7.2, pH 7.3, pH 7.4, pH 7.5, pH 7.6, pH 7.7, pH 7.8, pH 7.9, pH 8.0, pH 8.1, pH 8.2, pH 8.3, pH 8.4, or pH 8.5.

~~9. The method of any one of the previous claims, wherein the composition comprising vedolizumab is provided at a pH of about 7.0-7.5.~~

~~10. The method of any one of the previous claims, wherein the composition comprising vedolizumab is provided at a pH of about pH 6.5, pH 6.6, pH 6.7, pH 6.8, pH 6.9, pH 7.0, pH 7.1, pH 7.2, pH 7.3, pH 7.4, pH 7.5, pH 7.6, pH 7.7, pH 7.8, pH 7.9, pH 8.0, pH 8.1, pH 8.2, pH 8.3, pH 8.4, or pH 8.5.~~

~~118.~~ The method of any one of the previous claims, wherein:

~~the composition comprising vedolizumab is incubated for a period of about 10-120 hours, a period of about 12-120 hours, a period of about 12-96 hours, a period of about 12-72 hours, a period of about 12-48 hours, a period of about 24-120 hours, a period of about 24-96 hours, a period of about 24-72 hours, or a period of about 24-48 hours;~~

the composition comprising vedolizumab is incubated for a period of at least 12 hours; or

the composition comprising vedolizumab is incubated for a period of about 12 hours, about 24 hours, about 36 hours, about 48 hours, about 72 hours, about 96 hours, or about 120 hours.

~~12. The method of any one of the previous claims, wherein the composition comprising vedolizumab is incubated for a period of about 12-120 hours.~~

~~13. The method of any one of the previous claims, wherein the composition comprising vedolizumab is incubated for a period of about 12-96 hours.~~

~~14. The method of any one of the previous claims, wherein the composition comprising vedolizumab is incubated for a period of about 12-72 hours.~~

~~15. The method of any one of the previous claims, wherein the composition comprising vedolizumab is incubated for a period of about 12-48 hours.~~

~~16. The method of any one of the previous claims, wherein the composition comprising vedolizumab is incubated for a period of at least 12 hours.~~

~~17. The method of any one of the previous claims, wherein the composition comprising vedolizumab is incubated for a period of about 24-120 hours.~~

~~18. The method of any one of the previous claims, wherein the composition comprising vedolizumab is incubated for a period of about 24-96 hours.~~

~~19. The method of any one of the previous claims, wherein the composition comprising vedolizumab is incubated for a period of about 24-72 hours.~~

~~20. The method of any one of the previous claims, wherein the composition comprising vedolizumab is incubated for a period of about 24-48 hours.~~

~~21. The method of any one of the previous claims, wherein the composition comprising vedolizumab is incubated for a period of about 12 hours, about 24 hours, about 36 hours, about 48 hours, about 72 hours, about 96 hours, or about 120 hours.~~

229. A method of purifying a humanized anti- $\alpha 4\beta 7$ antibody or an antigen binding portion thereof from a clarified cell culture harvest comprising

(i) providing a clarified cell culture harvest obtained from a culture of recombinant host cells expressing the anti- $\alpha 4\beta 7$ antibody or an antigen binding portion thereof, and

(ii) purifying the anti- $\alpha 4\beta 7$ antibody or an antigen binding portion thereof from the cell culture harvest, wherein the antibody is exposed to a pH at or below 4.0 for no more than 24 hours,

wherein the anti- $\alpha 4\beta 7$ antibody or antigen binding portion thereof comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:1, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:5.

[2310](#). The method of claim [229](#), wherein the anti- $\alpha 4\beta 7$ antibody, or an antigen binding portion thereof, has a reduced level of basic isoform species (determined by CEX) as compared to a control, wherein the control is a composition comprising the anti- $\alpha 4\beta 7$ antibody, or an antigen binding portion thereof, produced by the same method, wherein the antibody is exposed to a pH at or below 4.0 (e.g., pH 3.6-4.0) for a longer duration of time, i.e., greater than 24 hours.

[2411](#). The method of claim [229](#) or [2310](#), wherein the anti- $\alpha 4\beta 7$ antibody, or an antigen binding portion thereof is vedolizumab, or an antigen binding portion thereof.

[2512](#). The method of any one of the previous claims, wherein the composition comprising vedolizumab, or an antigen binding portion thereof, comprises a first basic isoform peak (BP1) and a second basic isoform peak (BP2), and wherein the method produces a composition comprising vedolizumab, or an antigen binding portion thereof, having a reduced level of BP2.

[2613](#). The method of claim [2512](#), wherein the method produces a composition comprising vedolizumab, or an antigen binding portion thereof, having less than 2%, less than 1.5%, less than 1%, or less than 0.7% BP2.

[2714](#). The method of any one of the previous claims, wherein the composition comprising vedolizumab is derived from a mammalian cell culture expressing vedolizumab.

[2815](#). The method of claim [2714](#), wherein the mammalian cell culture is a Chinese Hamster Ovary (CHO) cell culture.

[2916](#). The method of claim [2815](#), wherein the CHO cell culture comprises CHO cells that lack dihydrofolate reductase (DHFR) expression [or CHO cells that lack glutamine synthetase \(GS\) expression](#).

~~30.—The method of claim 28, wherein the CHO cell culture comprises CHO cells that lack glutamine synthetase (GS) expression.~~

~~31~~17. The method of any one of the previous claims, wherein the method further comprises purifying the composition comprising vedolizumab from mammalian host cell protein (HCP) using one or more chromatographic separation steps selected from the group consisting of affinity chromatography, cation exchange chromatography, anion exchange chromatography, and ceramic hydroxyapatite (CHT) chromatography.

~~32.—The method of claim 31, wherein the composition comprising vedolizumab is purified using an affinity chromatography resin comprising Protein A.~~

~~33~~18. The method of claim ~~32~~17, wherein the composition comprising vedolizumab is purified using affinity chromatography prior to the incubation or following the incubation;
the composition comprising vedolizumab is purified using cation exchange chromatography prior to the incubation or following the incubation;
the composition comprising vedolizumab is purified using anion exchange chromatography prior to the incubation or following the incubation; or
the composition comprising vedolizumab is purified using CHT chromatography prior to the incubation or following the incubation.

~~34.—The method of claim 32, wherein the composition comprising vedolizumab is purified using affinity chromatography following the incubation.~~

~~35.—The method of claim 31, wherein the composition comprising vedolizumab is purified using cation exchange chromatography.~~

~~36.—The method of claim 35, wherein the composition comprising vedolizumab is purified using cation exchange chromatography prior to the incubation.~~

~~37.—The method of claim 35, wherein the composition comprising vedolizumab is purified using cation exchange chromatography following the incubation.~~

~~38. — The method of claim 31, wherein the composition comprising vedolizumab is purified using anion exchange chromatography.~~

~~39. — The method of claim 38, wherein the composition comprising vedolizumab is purified using anion exchange chromatography prior to the incubation.~~

~~40. — The method of claim 38, wherein the composition comprising vedolizumab is purified using anion exchange chromatography following the incubation.~~

~~41. — The method of claim 31, wherein the composition comprising vedolizumab is purified using CHT chromatography.~~

~~42. — The method of claim 41, wherein the composition comprising vedolizumab is purified using CHT chromatography prior to the incubation.~~

~~43. — The method of claim 41, wherein the composition comprising vedolizumab is purified using CHT chromatography following the incubation.~~

~~44~~19. The method of any one of claims 1-~~43~~18, wherein the method comprises incorporating the composition into a pharmaceutical formulation.

~~45~~20. The method of claim ~~44~~19, wherein the pharmaceutical formulation is a dry, lyophilized pharmaceutical formulation or a liquid pharmaceutical formulation.

~~46. — The method of claim 45, wherein the lyophilized pharmaceutical formulation is a dry, lyophilized pharmaceutical formulation.~~

~~47~~21. The method of claim ~~46~~20, further comprising a step of reconstituting the dry, lyophilized pharmaceutical formulation with a liquid so that it is suitable for administration.

~~48. — The method of claim 44, wherein the pharmaceutical formulation is a liquid pharmaceutical formulation.~~

~~49~~22. A composition comprising vedolizumab, wherein the composition is produced by or is obtainable by the method of any one of claims 1-~~48~~21.

~~50. — A composition comprising vedolizumab, wherein the composition is obtainable by the method of any one of claims 1-48.~~

~~51~~23. The composition of claim ~~49 or 50~~22, wherein the basic vedolizumab isoform species comprises less than 16%, less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, or less than 10% of the vedolizumab species present in the composition.

~~52~~24. A low basic species composition comprising an anti- $\alpha 4\beta 7$ antibody, wherein the composition comprises less than 16%, less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, or less than 10% total basic isoform species of the anti- $\alpha 4\beta 7$ antibody, wherein the basic isoform species have a net positive charge relative to a main isoform of the anti- $\alpha 4\beta 7$ antibody and can be quantified by determining the relative area of peaks that elute more slowly from a cation exchange (CEX) resin than a peak corresponding to the main isoform, and wherein the anti- $\alpha 4\beta 7$ antibody comprises a heavy chain variable region comprising SEQ ID NO:1, and a light chain variable region comprising SEQ ID NO:~~25~~.

~~53~~25. The composition of claim ~~52~~24, wherein the composition comprises a first basic isoform peak (BP1) and a second basic isoform peak (BP2).

~~54~~26. The composition of claim ~~53~~25, wherein the composition comprises less than 2% BP2, less than 1.5% BP2, less than 1% BP2, or less than 0.7% BP2.

~~55. — The composition of claim 53, wherein the composition comprises less than 1.5% BP2.~~

~~56. — The composition of claim 53, wherein the composition comprises less than 1% BP2.~~

~~57. — The composition of claim 53, wherein the composition comprises less than 0.7% BP2.~~

~~58~~27. The composition of claim ~~53~~25, wherein the ratio of BP1 to BP2 is at least 3, at least 5, at least 7, or at least 10.

~~59. The composition of claim 53, wherein the ratio of BP1 to BP2 is at least 5.~~

~~60. The composition of claim 53, wherein the ratio of BP1 to BP2 is at least 7.~~

~~61. The composition of claim 53, wherein the ratio of BP1 to BP2 is at least 10.~~

~~6228.~~ A pharmaceutical composition comprising the composition of any one of claims ~~5224-6127~~ and a pharmaceutically acceptable carrier or excipient.

~~6329.~~ A method of producing a composition comprising vedolizumab, comprising:
(a) contacting a sample containing vedolizumab and host cell protein (HCP) with an anion exchange resin in the presence of a loading buffer, wherein the loading buffer has a conductivity of 11 mS/cm or less, such that HCP binds to the anion exchange resin; and
(b) collecting the flow through material from the anion exchange resin, wherein the eluate comprises vedolizumab.

~~6430.~~ The method of claim ~~6329~~, wherein the method is a method of producing a composition comprising vedolizumab having a reduced amount of HCP, and the flow through material comprises vedolizumab and a reduced amount of HCP.

~~6531.~~ The method of claim ~~63 or 6429~~, wherein the loading buffer has a conductivity of 9 mS/cm to 11 mS/cm or wherein the loading buffer has a conductivity of 10 mS/cm or less; 9 mS/cm or less; or wherein the loading buffer has a conductivity of about 9 mS/cm, 9.5 mS/cm, 10 mS/cm, 10.5 mS/cm, or 11 mS/cm.

~~66. The method of claim 63 or 64, wherein the loading buffer has a conductivity of 10 mS/cm or less.~~

~~67. The method of claim 63 or 64, wherein the loading buffer has a conductivity of 9 mS/cm or less.~~

~~68. The method of claim 63 or 64, wherein the loading buffer has a conductivity of about 9 mS/cm, 9.5 mS/cm, 10 mS/cm, 10.5 mS/cm, or 11 mS/cm.~~

~~6932. The method of any one of claims 6329-6831, wherein the HCP is a Chinese Hamster Ovary (CHO) cell protein.~~

~~7033. The method of claim 6932, wherein the HCP is derived from a CHO cell that lacks dihydrofolate reductase (DHFR) expression or a CHO cell that lacks glutamine synthetase (GS) expression.~~

~~71. The method of claim 69, wherein the HCP is derived from a CHO cell that lacks glutamine synthetase (GS) expression.~~

~~7234. The method of any one of claims 6329-7133, further comprising contacting the anion exchange resin with a wash buffer.~~

~~7335. The method of claim 7234, wherein:~~
~~_____the wash buffer has a conductivity of less than 11 mS/cm;~~
~~the wash buffer has a conductivity of 9 mS/cm to 11 mS/cm; or~~
~~the wash buffer has the same conductivity as the loading buffer.~~

~~74. The method of claim 72, wherein the wash buffer has a conductivity of 9 mS/cm to 11 mS/cm.~~

~~75. The method of claim 72, wherein the wash buffer has the same conductivity as the loading buffer.~~

~~7636. The method of any one of claims 6329-7535, wherein the loading buffer and/or the wash buffer comprises sodium chloride and/or sodium phosphate.~~

~~77. The method of any one of claims 63-75, wherein the wash buffer comprises sodium chloride and/or sodium phosphate.~~

~~7837. The method of any one of claims 6329-7736, wherein:~~

the anion exchange resin is formatted as an anion exchange column or an anion exchange membrane; and/or

the anion exchange resin comprises a quaternary amine functional group.

~~79. The method of any one of claims 63-78, wherein the anion exchange resin comprises a quaternary amine functional group.~~

~~8038.~~ The method of any one of claims ~~6329-7937~~, wherein the sample containing vedolizumab and HCP is derived from a mammalian cell culture following one or more chromatographic separation steps.

~~8139.~~ The method of claim ~~8038~~, wherein the one or more chromatographic separation steps comprise one or more steps selected from the group consisting of affinity chromatography, cation exchange chromatography, and ceramic hydroxyapatite (CHT) chromatography.

~~8240.~~ The method of any one of claims ~~6329-8139~~, wherein:

~~the amount of HCP in the flow through material is 8 ppm or less, 7.5 ppm or less, 7 ppm or less, 6.5 ppm or less, 6 ppm or less, 5.5 ppm or less, 5 ppm or less, 4.5 ppm or less, 4 ppm or less, 3.5 ppm or less, 3 ppm or less, 2.5 ppm or less, or 2 ppm or less; or~~

~~the amount of HCP in the flow through material is reduced by at least 50% relative to the amount of HCP in the flow through material produced when the method is performed using the same sample with a loading buffer having a conductivity greater than 12 mS/cm.~~

~~83. The method of any one of claims 63-82, wherein the amount of HCP in the flow through material is reduced by at least 50% relative to the amount of HCP in the flow through material produced when the method is performed using the same sample with a loading buffer having a conductivity greater than 12 mS/cm.~~

~~8441.~~ The method of any one of claims ~~6329-8340~~, wherein the method further comprises processing the flow through material to exchange the elution buffer by a process comprising ultrafiltration and/or diafiltration to a buffer comprising one or more pharmaceutically acceptable carriers or excipients.

~~8542.~~ The method of any one of claims ~~6329-8441~~, wherein the method comprises incorporating the composition into a pharmaceutical formulation.

~~8643.~~ The method of claim ~~8542~~, wherein the pharmaceutical formulation is a ~~dry, lyophilized pharmaceutical formulation or a liquid pharmaceutical formulation.~~

~~87.—The method of claim 86, wherein the lyophilized pharmaceutical formulation is a dry, lyophilized pharmaceutical formulation.~~

~~8844.~~ The method of claim ~~8743~~, further comprising a step of reconstituting the dry, lyophilized pharmaceutical formulation with a liquid so that it is suitable for administration.

~~89.—The method of claim 85, wherein the pharmaceutical formulation is a liquid pharmaceutical formulation.~~

~~9045.~~ The method of claim ~~8943~~ or ~~4820~~, wherein the liquid pharmaceutical formulation is suitable for subcutaneous administration to a human.

~~9146.~~ A composition comprising vedolizumab produced by ~~or is obtainable by~~ the method of any one of claims ~~6329-9045~~.

~~92.—A composition comprising vedolizumab, wherein the composition is obtainable by the method of any one of claims 63-90.~~

~~9347.~~ The composition of claim ~~91 or 9246~~, wherein the amount of HCP in the composition is 8 ppm or less, 7.5 ppm or less, 7 ppm or less, 6.5 ppm or less, 6 ppm or less, 5.5 ppm or less, 5 ppm or less, 4.5 ppm or less, 4 ppm or less, 3.5 ppm or less, 3 ppm or less, 2.5 ppm or less, or 2 ppm or less.

~~9448.~~ A method of increasing the yield of vedolizumab recovered following elution from a mixed mode chromatography resin, comprising equilibrating the mixed mode chromatography resin with an equilibration buffer, loading a solution comprising vedolizumab and a loading buffer onto the mixed mode chromatography resin such that vedolizumab binds the mixed mode chromatography resin, washing the mixed mode

chromatography resin with a wash buffer, and eluting vedolizumab from the mixed mode chromatography resin with an elution buffer, wherein the equilibration buffer, the loading buffer, and/or the wash buffer have a pH at or below 7.0.

~~9549.~~ The method of claim ~~9448~~, wherein:

~~_____ the equilibration buffer, the loading buffer, and/or the wash buffer have a pH of 6.0-7.0, or wherein the equilibration buffer, the loading buffer, and/or the wash buffer have a pH of 6.5-7.0 or a pH of 6.6-6.8.~~

~~96.—The method of claim 94, wherein the equilibration buffer, the loading buffer, and/or the wash buffer have a pH of 6.5-7.0.~~

~~97.—The method of claim 94, wherein the equilibration buffer, the loading buffer, and/or the wash buffer have a pH of 6.6-6.8.~~

~~9850.~~ The method of any one of claims ~~9448-9749~~, wherein the equilibration buffer, the loading buffer, and/or the wash buffer have a salt concentration of 30 mM to 70 mM, ~~40 mM to 70 mM, 50 mM to 65 mM, or 55 mM-65 mM.~~

~~99.—The method of claim 98, wherein the equilibration buffer, the loading buffer, and/or the wash buffer have a salt concentration of 40 mM to 70 mM.~~

~~100.—The method of claim 98, wherein the equilibration buffer, the loading buffer, and/or the wash buffer have a salt concentration of 50 mM to 65 mM.~~

~~101.—The method of claim 98, wherein the equilibration buffer, the loading buffer, and/or the wash buffer have a salt concentration of 55 mM-65 mM.~~

~~10251.~~ The method ~~of any one~~ of claims ~~98-101~~ ~~50~~, wherein the salt comprises sodium chloride and/or sodium phosphate.

~~10352.~~ The method of any one of claims ~~9448-9749~~, wherein the equilibration buffer, the loading buffer, and/or the wash buffer have a sodium chloride concentration of 30 mM to 70 mM, ~~40 mM to 70 mM, 40 mM to 60 mM, 45 mM to 55 mM, or about 50 mM.~~

~~104.—The method of claim 103, wherein the equilibration buffer, the loading buffer, and/or the wash buffer have a sodium chloride concentration of 40 mM to 70 mM.~~

~~105.—The method of claim 103, wherein the equilibration buffer, the loading buffer, and/or the wash buffer have a sodium chloride concentration of 40 mM to 60 mM.~~

~~106.—The method of claim 103, wherein the equilibration buffer, the loading buffer, and/or the wash buffer have a sodium chloride concentration of 45 mM 55 mM.~~

~~107.—The method of claim 103, wherein the equilibration buffer, the loading buffer, and/or the wash buffer have a sodium chloride concentration of about 50 mM.~~

~~108~~53. The method ~~of any one~~ of claims ~~103–107~~ 52, wherein the equilibration buffer, the loading buffer, and/or the wash buffer further comprise sodium phosphate.

~~109~~54. The method of any one of claims ~~94–108~~53, wherein:

the equilibration buffer, the loading buffer, and/or the wash buffer have the same pH;
the equilibration buffer, the loading buffer, and/or the wash buffer have the same salt concentration; or
the equilibration buffer, the loading buffer, and/or the wash buffer are the same buffer.

~~110.—The method of any one of claims 94–109, wherein the equilibration buffer, the loading buffer, and/or the wash buffer have the same salt concentration.~~

~~111.—The method of any one of claims 94–108, wherein the equilibration buffer, the loading buffer, and/or the wash buffer are the same buffer.~~

~~112~~55. The method of any one of claims ~~94–111~~54, wherein the mixed mode resin is a ceramic hydroxyapatite resin.