

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, and
- adding a supplement comprising uridine, manganese, and galactose to the
- production medium, thereby producing a composition comprising the humanized anti-
- 10 $\alpha 4\beta 7$ antibody,
- wherein the mammalian host cell is genetically engineered to express a humanized anti- $\alpha 4\beta 7$ antibody which 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
- 15 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 comprises a decreased amount of basic isoform (as determined by Cation Exchange Chromatography (CEX)) of the
- 20 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 but in the absence of the supplement; or
- the composition comprises about 16% or less, about 14% or less, or about 13% or less basic isoform (as determined by CEX) of the humanized anti- $\alpha 4\beta 7$ antibody.
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- ~~3. The method of claim 1, wherein the composition comprises about 16% or less basic isoform (as determined by CEX) of the humanized anti- $\alpha 4\beta 7$ antibody.~~
- ~~4. The method of claim 3, wherein the composition comprises about 14% or less basic isoform of the humanized anti- $\alpha 4\beta 7$ antibody.~~
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- ~~5. The method of claim 3, wherein the composition comprises about 13% or less basic isoform of the humanized anti- $\alpha 4\beta 7$ antibody.~~

~~63.~~ The method of ~~any one of claims 1 to 52~~, wherein the supplement is added to the production medium or is added to a feed medium which is subsequently added to the production medium.

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~~74.~~ The method of any one of claims 1 to ~~63~~, wherein the cumulative concentration of uridine added in the production medium between supplementation and harvest is about 1 to about 7 mM; wherein the cumulative concentration of manganese in the production medium between supplementation and harvest is about 0.002 to about 0.015 mM; and/or wherein the cumulative concentration of galactose in the production medium between supplementation and harvest is about 3 to about 20 mM.

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~~85.~~ The method of any one of claims 1 to ~~74~~, wherein manganese is added as a supplement multiple times to the production medium by about 0.1 to 10 μ M, about 0.2 to 1.5 μ M, about 0.2 to 5 μ M, about 0.25 to 2 μ M, about 0.3 to 1.2 μ M, or about 0.3 to 0.8 μ M with each addition;

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

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wherein galactose is added as a supplement multiple times to the production medium by about 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 about 1.2 mM or 1.4 mM with each addition.

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~~9. — The method of claim 8, wherein manganese is added as a supplement multiple times to the production medium by about 0.2 to 1.5 μ M with each addition.~~

~~10. — The method of any one of claims 1 to 9, wherein uridine is added as a supplement multiple times to the production medium by about 25 to 1000 μ M, about 75 to 750 μ M, about 55 to 620 μ M, about 100 to 600 μ M, about 150 to 450 μ M, about 100 to 700 μ M, about 100 to 600 μ M, or about 170 to 630 μ M with each addition.~~

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11. — The method of claim 10, wherein uridine is added as a supplement multiple times to the production medium by about 100 to 700 μ M.

12. — The method of any one of claims 1 to 11, wherein galactose is added as a supplement multiple times to the production medium by about 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 about 1.2 mM or 1.4 mM with each addition.

13. — The method of claim 12, wherein the galactose is added as a supplement multiple times to the production medium by about 0.5 to 3.5 mM.

146. The method of ~~any one of~~ claims 8 to 135, 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.

15. — The method of any one of claims 8 to 14, wherein the supplement is added beginning on day 4 of the production phase culture.

147. The method of any one of claims 1-156, wherein the feed medium further comprises zinc.

148. The method of claim 147, wherein the cumulative concentration of zinc added to the production medium between supplementation and harvest is about 0.005 mM to about 0.045 mM.

149. The method of any one of claims 1-178, 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.

~~1910.~~ The method of any one of claims 1-~~189~~, wherein the method is a fed batch method.

~~20.—The method of claim 19, wherein the method is a production phase and wherein the supplement is added to the production medium beginning on about day four of the production phase.~~

~~211.~~ 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 comprising zinc, thereby producing a composition comprising the humanized anti- $\alpha 4\beta 7$ antibody,

wherein the mammalian host cell is genetically engineered to express a humanized anti- $\alpha 4\beta 7$ antibody which 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.

~~2212.~~ The method of claim ~~211~~, wherein the composition comprises a decreased amount of basic isoform 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 the same conditions in the absence of zinc; or

the composition comprises about 16% or less, about 14% or less, about 13% or less basic isoform of the humanized anti- $\alpha 4\beta 7$ antibody.

~~23.—The method of claim 22, wherein the composition comprises about 16% or less basic isoform of the humanized anti- $\alpha 4\beta 7$ antibody.~~

~~24.—The method of claim 23, wherein the composition comprises about 14% or less basic isoform of the humanized anti- $\alpha 4\beta 7$ antibody.~~

~~25.—The method of claim 23, wherein the composition comprises about 13% or less basic isoform of the humanized anti- $\alpha 4\beta 7$ antibody.~~

~~2613.~~ The method of ~~any one of~~ claims ~~21-2511 or 12~~, wherein the concentration of zinc in the production medium is 2 μ M to 60 μ M.

5 ~~2714.~~ The method of any one of claims ~~2411-2613~~, wherein the method comprises supplementing the production medium with zinc by adding a feed medium comprising zinc to the production medium, and/or wherein the feed medium is added to the production medium beginning on about day four of a production phase.

10 ~~28.~~ ~~The method of claim 27, wherein the feed medium is added to the production medium beginning on about day four of a production phase.~~

~~2915.~~ The method of any one of claims 1-~~2814~~, wherein the production medium comprises 5.0 to 8.8 g/L lysine and 3.0 to 12.0 g/L arginine.

15 ~~3016.~~ The method of claim ~~2915~~, wherein the production medium comprises 4.5 to 5.5 g/L lysine or 5.5 to 8.8 g/L lysine.

20 ~~31.~~ ~~The method of claim 29, wherein the production medium comprises 5.5 to 8.8 g/L lysine.~~

~~3217.~~ The method of ~~any one of~~ claims ~~29-3115 or 16~~, wherein the production medium comprises 5.4 to 7.4 g/L arginine or 7.4 to 12 g/L arginine.

25 ~~33.~~ ~~The method of any one of claims 29-31, wherein the production medium comprises 7.4 to 12 g/L arginine.~~

30 ~~3418.~~ 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 in a production phase, such that a composition comprising the humanized anti- $\alpha 4\beta 7$ antibody is produced, wherein the production medium has an average temperature of about 37 degrees Celsius, wherein the host cell is genetically engineered to express a humanized IgG1 anti- $\alpha 4\beta 7$ antibody,

wherein the humanized anti- $\alpha 4\beta 7$ 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.

~~3519~~. The method of claim ~~3418~~, wherein the method is a method producing a composition comprising 2.5% or less HMW species (as determined by SEC) of the humanized anti- $\alpha 4\beta 7$ antibody.

~~3620~~. 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 growth medium in an expansion phase, wherein the mammalian host cell is genetically engineered to express a humanized anti- $\alpha 4\beta 7$ antibody, and

culturing the mammalian host cell in a production medium in a production phase, such that a composition comprising the humanized anti- $\alpha 4\beta 7$ antibody is produced,

wherein the mammalian host cell is cultured at a temperature that is approximately the same in both the expansion phase and the production phase, and

wherein the humanized anti- $\alpha 4\beta 7$ 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, wherein the temperature is from 36 to 38 degrees Celsius, and wherein the method is a method of producing a composition comprising high levels of monomer (as determined by SEC) of the humanized anti- $\alpha 4\beta 7$ antibody, relative to a control culture cultured under substantially similar conditions but with a different temperature between the expansion and the production phases.

~~37. — The method of claim 36, wherein the method is a method of producing a composition comprising high levels of monomer (as determined by SEC) of the humanized anti- $\alpha 4\beta 7$ antibody, relative to a control culture cultured under substantially~~

~~similar conditions but with a different temperature between the expansion and the production phases.~~

5 ~~38. The method of claim 36 or 37, wherein the temperature is from 36 to 38 degrees Celsius.~~

10 ~~39~~21. The method of ~~any one of claims 36–38~~20, wherein the average temperature is from 36.5 to 37.5 degrees Celsius or the temperature is an average temperature of about 37 degrees Celsius.

~~40. The method of claim 36 or 37, wherein the temperature is an average temperature of about 37 degrees Celsius.~~

15 ~~41~~22. The method of any one of claims 1-~~40~~21, wherein the production medium has a temperature ranging from 36 to 38 degrees Celsius.

~~42. The method of claim 41, wherein the temperature ranges from 36.5 to 37.5 degrees Celsius.~~

20 ~~43. The method of claim 42, wherein the temperature is an average temperature of about 37 degrees Celsius.~~

~~44~~23. The method of any one of claims 1-~~43~~22, wherein the production medium has a pH ranging from 6.5 to 7;

25 has a pH ranging from 6.8 to 7.0; and/or
has a glucose level that is maintained at about 7 g/L or less during the production phase.

30 ~~45. The method of claim 44, wherein the production medium has a pH ranging from 6.8 to 7.0.~~

~~46. The method of any one of claims 1-45, wherein the production medium has a glucose level that is maintained at about 7 g/L or less a during the production phase.~~

~~47~~24. The method of any one of claims 1-~~46~~23, wherein the production phase is 14 days or less or the production phase ranges from 10 days to 17 days.

~~48~~—. ~~The method of any one of claims 1-46, wherein the production phase ranges from 10 days to 17 days.~~

~~49~~25. The method of any one of claims 1-~~48~~24, which is performed in a large scale bioreactor.

~~50~~—. ~~The method of claim 49, wherein the large scale bioreactor is selected from the group consisting of a 200 liter (L) bioreactor, a 2000 L bioreactor, a 3000L, and a 6000 L bioreactor.~~

~~51~~26. The method of any one of claims 1-~~50~~25, wherein the production phase results in a titer of the humanized anti- $\alpha 4\beta 7$ antibody of greater than 3 g/L; about 3 to about 8 g/L, or about 5 to about 7 g/L.

~~52~~—. ~~The method of claim 51, wherein the titer of the humanized anti- $\alpha 4\beta 7$ antibody is about 3 to about 8 g/L.~~

~~53~~—. ~~The method of claim 52, wherein the titer of the humanized anti- $\alpha 4\beta 7$ antibody is about 5 to about 7 g/L.~~

~~54~~27. The method of any one of claims 1-~~53~~26, wherein the mammalian host cell is a Chinese Hamster Ovary (CHO) cell.

~~55~~—. ~~The method of claim 54, wherein the CHO cell is a GS-CHO cell.~~

~~56~~28. The method of any one of claims 1-~~55~~27, wherein the humanized anti- $\alpha 4\beta 7$ antibody comprises a heavy chain variable domain comprising an amino acid sequence as set forth in SEQ ID NO: 1, and comprises a light chain variable domain comprising an amino acid sequence as set forth in SEQ ID NO: 5; or is vedolizumab.

~~57~~—. ~~The method of any one of claims 1-55, wherein the humanized anti- $\alpha 4\beta 7$ antibody is vedolizumab.~~

5829. The method of any one of claims 1-5728, wherein the method comprises harvesting and purification of the antibody.

~~59.— The method of claim 58, wherein the purification comprises (i) purification steps that remove any cellular debris, unwanted proteins, salts, minerals or other undesirable elements, and (ii) purification of the antibody from contaminant soluble proteins and polypeptides.~~

6030. The method of claim ~~58 or 5929~~, wherein the method further comprises preparing a pharmaceutical formulation of the purified antibody which is suitable for human therapeutic use.

~~61.— The method of claim 60, wherein the pharmaceutical formulation is a liquid pharmaceutical formulation.~~

~~62.— The method of claim 61, wherein the liquid pharmaceutical formulation is prepared by ultrafiltration/diafiltration.~~

~~63.— The method of claim 60, wherein the pharmaceutical formulation is a lyophilized dry antibody formulation.~~

~~64.— The method of claim 63, wherein the pharmaceutical formulation of the antibody is a dry antibody formulation lyophilized from a liquid pharmaceutical antibody formulation prepared by ultrafiltration/diafiltration following the purification.~~

6531. A composition comprising humanized anti- $\alpha 4\beta 7$ antibodies, wherein the composition is produced using or is obtainable by any one of the methods of claims 1-6430.

~~66. — A composition comprising humanized anti- α 4 β 7 antibodies, wherein the composition is obtainable by any one of the methods of claims 1-64.~~

~~67~~32. The composition of claim ~~65 or 66~~31, comprising a population of humanized anti- α 4 β 7 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.

~~68~~33. The method of any one of claims 1-~~20~~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.

~~69~~34. The method of claim ~~68~~33, 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.

~~70. — The method of claim 69, wherein the composition comprises at least about a 20% decrease in 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.~~

~~71~~35. The method of any one of claims 1-~~20~~10, wherein the method is a method of producing a composition having about 65% or less, about 60% or less, or about 55% or less G0F glycoform (as determined by HILIC) of the humanized anti- α 4 β 7 antibody.

~~72. — The method of claim 71, wherein the composition comprises about 60% or less G0F glycoform of the humanized anti- α 4 β 7 antibody.~~

~~73. The method of claim 71, wherein the composition comprises about 55% or less G1F glycoform of the humanized anti- $\alpha 4\beta 7$ antibody.~~

5 ~~7436.~~ The method of any one of claims 1-~~2010~~, 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- $\alpha 4\beta 7$ 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.

10 ~~7537.~~ The method of claim ~~7436~~, wherein the composition comprises at least about a 2-fold ~~or at least about a 3-fold~~ increase in the G1F glycoform of the humanized anti- $\alpha 4\beta 7$ antibody in comparison to the control.

15 ~~76. The method of claim 74, wherein the composition comprises at least about a 3-fold increase in the G1F glycoform of the humanized anti- $\alpha 4\beta 7$ antibody in comparison to the control.~~

20 ~~7738.~~ The method of any one of claims 1-~~2010~~, wherein the method is a method of producing a composition having about 25% or more ~~or about 30% or more~~ G1F glycoform (as determined by HILIC) of the humanized anti- $\alpha 4\beta 7$ antibody.

~~78. The method of claim 77, wherein the composition comprises about 30% or more G1F glycoform of the humanized anti- $\alpha 4\beta 7$ antibody.~~

25 ~~7939.~~ The method of any one of claims 1-~~2010~~, 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.

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~~8040.~~ The method of claim ~~7939~~, wherein the composition comprises at least about a 3-fold increase or about a 4-fold increase in the G2F glycoform of the humanized anti- $\alpha 4\beta 7$ antibody in comparison to the control.

5 ~~81.—The method of claim 79, wherein the composition comprises at least about a 4-fold increase in the G2F glycoform of the humanized anti- $\alpha 4\beta 7$ antibody in comparison to the control.~~

10 ~~8241.~~ The method of any one of claims 1-~~2010~~, wherein the method is a method of producing a composition having about 3% or more or about 4% or more G2F glycoform (as determined by HILIC) of the humanized anti- $\alpha 4\beta 7$ antibody.

~~83.—The method of claim 82, wherein the composition comprises about 4% or more G2F glycoform of the humanized anti- $\alpha 4\beta 7$ antibody.~~

15 ~~8442.~~ The method of any one of claims ~~6833-8341~~, 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

20 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
25 comprising uridine, manganese, and galactose added to the production medium.

30 ~~85.—The method of any one of claims 68-83, 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.~~

~~86. — The method of any one of claims 68-85, wherein the method is a fed batch method.~~

~~8743. The method of any one of claims 6833-8642, 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 about 6.8 to about 7.1.~~

~~88. — The method of any one of claims 68-87, wherein the production medium has a pH of about 6.8 to about 7.1.~~

~~8944. The method of any one of claims 6833-8843, wherein the method comprises harvesting and purification of the antibody.~~

~~90. — The method of claim 89, wherein the purification comprises (i) purification steps that remove any cellular debris, unwanted proteins, salts, minerals or other undesirable elements, and (ii) purification of the antibody from contaminant soluble proteins and polypeptides.~~

~~9145. The method of claim 89 or 9044, wherein the method further comprises preparing a pharmaceutical formulation of the purified antibody which is suitable for human therapeutic use.~~

~~92. — The method of claim 91, wherein the pharmaceutical formulation is a liquid pharmaceutical formulation.~~

~~93. — The method of claim 92 wherein the liquid pharmaceutical formulation is prepared by ultrafiltration/diafiltration.~~

~~94. — The method of claim 91, wherein the pharmaceutical formulation is a lyophilized dry antibody formulation.~~

~~95. — The method of claim 94, wherein the pharmaceutical formulation of the antibody is a dry antibody formulation lyophilized from a liquid pharmaceutical antibody formulation prepared by ultrafiltration/diafiltration following the purification.~~

[9646](#). A composition comprising a humanized anti- α 4 β 7 antibody, wherein the composition is obtainable by any one of the methods of claims [6833-8843](#).

[9747](#). A cell culture comprising a host cell genetically engineered to express a humanized anti- α 4 β 7 antibody, and a production medium supplemented with uridine, manganese, and galactose (UMG), wherein the humanized anti- α 4 β 7 antibody is an IgG1 antibody and 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.

[9848](#). The cell culture of claim [9747](#), wherein the production medium comprises supplemented uridine at a concentration of about 1 to about 7 mM, supplemented manganese at a concentration of about 0.002 to about 0.015 mM, and supplemented galactose at a concentration of ~~about~~ about 3 to about 20 mM on the day of harvest.

[9949](#). The cell culture of claim [9747](#) or [9848](#), wherein the production medium further comprises zinc.

~~10050~~. The cell culture of claim [9949](#), wherein the production medium comprises supplemented zinc at a concentration of about 0.005 mM to 0.045 mM on the day of harvest.

~~10151~~. The cell culture of any one of claims [9747-10050](#), wherein the antibody 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.

~~10252~~. A cell culture comprising a host cell genetically engineered to express a humanized anti- α 4 β 7 antibody, and a production medium supplemented with zinc, wherein the humanized anti- α 4 β 7 antibody is an IgG1 antibody and 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.

~~103~~53. The cell culture of claim ~~102~~52, wherein the production medium comprises supplemented zinc at a concentration of about 0.005 mM to 0.045 mM on the day of harvest and/or wherein the production medium further comprises uridine, manganese, and galactose (UMG).

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~~104.~~ The cell culture of claim ~~102~~ or ~~103~~, wherein the production medium further comprises uridine, manganese, and galactose (UMG).

~~105.~~ The cell culture of claim ~~100~~, wherein the production medium comprises supplemented uridine at a concentration of about 1 to about 7 mM, supplemented manganese at a concentration of about 0.002 to about 0.015 mM, and supplemented galactose at a concentration of about about 3 to about 20 mM on the day of harvest.

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~~106~~54. The cell culture of claim ~~100~~50, wherein the production medium comprises supplemented uridine at a concentration of about 2 to about 5 mM, supplemented manganese at a concentration of about 0.001 to about 0.01 mM, and supplemented galactose at a concentration of about ~~about~~ 10 to about 15 mM on the day of harvest.

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~~107~~55. The cell culture of any one of claims ~~97~~47-~~106~~54, wherein the expressed humanized anti- $\alpha 4\beta 7$ antibody has an isoform distribution comprising:

a. 16% or less, 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:

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.

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~~108.~~ The cell culture of any one of claims ~~97-107~~, wherein the expressed humanized anti- $\alpha 4\beta 7$ antibody has a fucosylated N-glycan content comprising:

~~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.~~

~~109~~56. The cell culture of any one of claims ~~97~~47-~~108~~55, 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%.

~~110~~57. The cell culture of any one of claims ~~97~~47-~~108~~55, 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%.

~~111.~~ The cell culture of any one of claims ~~97-108~~, 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%.~~

~~112.~~ The cell culture of any one of claims ~~97-111~~, wherein the cell culture further comprises arginine and/or lysine.

~~113~~58. The cell culture of any one of claims ~~97~~47-~~112~~57, wherein the host cell is a CHO cell.

~~114.~~ The cell culture of claim ~~113~~, wherein the CHO cell is deficient in the gene encoding glutamine synthetase (GS).

~~115~~59. A humanized anti- $\alpha 4\beta 7$ antibody produced by the cell culture of any one of claims ~~97~~47-~~114~~58.

[11660](#). A method of producing a composition comprising a humanized anti- $\alpha 4\beta 7$ antibody, said method comprising

culturing a mammalian host cell genetically engineered to express the humanized anti- $\alpha 4\beta 7$ antibody in a first production medium having a first pH; and culturing the mammalian host cell in a second production medium having a second pH;

wherein the second pH is lower than the first pH, and

wherein the humanized anti- $\alpha 4\beta 7$ 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.

[11761](#). The method of claim [11660](#), wherein the second pH is 0.1 to 0.5 pH units lower than the first pH and/or the first pH is in the range of pH 6.8-7.2, and wherein the second pH is in the range of pH 6.7-6.95.

~~118. The method of claim 116 or 117, wherein the first pH is in the range of pH 6.8-7.2, and wherein the second pH is in the range of pH 6.7-6.95.~~

[11962](#). The method of ~~any one of~~ claims ~~116-118~~[60 or 61](#), wherein the mammalian host cell is cultured at the first pH for 120 hours or less, 85-110 hours; or 90-100 hours.

~~120. The method of any one of claims 116-118, wherein the mammalian host cell is cultured at the first pH for 85-110 hours.~~

~~121. The method of any one of claims 116-118, wherein the mammalian host cell is cultured at the first pH for 90-100 hours.~~

~~122~~63. The method of any one of claims ~~116~~60-~~121~~62, further comprising harvesting the anti- $\alpha 4\beta 7$ antibody from the second production medium.

5 ~~123. The method of claim 122, wherein the anti- $\alpha 4\beta 7$ antibody is harvested following culture of the mammalian host cell in the first production medium and the second production medium for a period of 13-15 days.~~

10 ~~124~~64. The method of any one of claims ~~116~~60-~~123~~63, wherein the composition has an increased level of the anti- $\alpha 4\beta 7$ antibody major isoform, relative to a control composition in which the mammalian host cell is cultured at the first pH without a pH shift.