

WHAT IS CLAIMED IS:

1. A method of producing an antibody composition, wherein the level of total afucosylated (TAF) glycans in the antibody composition is less than about 10%, the method comprising maintaining glycosylation-competent cells in a cell culture medium comprising fucose, wherein fucose is present in the culture medium at a concentration of about 0.17 g/L to about 1.0 g/L.
2. A method of producing an antibody composition, wherein the level of total afucosylated (TAF) glycans in the antibody composition is less than about 10%, the method comprising maintaining glycosylation-competent cells in a cell culture medium comprising fucose, wherein fucose is present in the culture medium at a concentration of about 0.1 g/L to about 1.0 g/L, and wherein the glycosylation-competent cells are not genetically modified to alter activity of an enzyme of the de novo pathway or the salvage pathway.
3. A method of producing an antibody composition, comprising maintaining glycosylation-competent cells in a cell culture medium comprising fucose and glucose, wherein fucose is present in the culture medium at a concentration of about 0.1 g/L to about 1.0 g/L and glucose is added to the cell culture medium according to a glucose feeding schedule that achieves an average glucose concentration of about 10 g/L or less.
4. The method of any one of claims 1 to 3, wherein fucose is present in the culture medium at a concentration less than about 0.75 g/L.
5. The method of claim 4, wherein fucose is present in the culture medium at a concentration less than about 0.6 g/L.
6. The method of claim 5, wherein fucose is present in the culture medium at a concentration of about 0.2 g/L to about 0.5 g/L.
7. The method of any one of claims 1 to 6, wherein the fucose is present in the culture medium the entire duration the glycosylation-competent cells are maintained in cell culture.
8. The method of any one of claims 1 to 6, comprising maintaining the glycosylation-competent cells in a first cell culture medium for an initial time period and subsequently maintaining the glycosylation-competent cells in a second cell culture medium, wherein the first cell culture medium does not comprise fucose at a concentration of about 0.1 g/L to about 1.0 g/L and the second cell culture medium comprises fucose at a concentration of about 0.1 g/L to about 1.0 g/L.
9. The method of claim 8, wherein the initial time period is about 24 to about 72 hours.

10. The method of claim 8, wherein the initial time period is about or greater than about 72 hours but less than or about 156 hours.
11. The method of any one of claims 8 to 10, wherein fucose is added to the first culture medium on the 6th day post-cell culture inoculation to obtain the second cell culture medium.
12. The method of any one of claims 1 to 11, wherein the concentration of fucose fluctuates by about 0.2 g/L or less during the time the glycosylation-competent cells are maintained in the cell culture medium comprising fucose.
13. The method of claim 12, wherein the concentration of fucose fluctuates by about 0.1 g/L or less during the time the glycosylation-competent cells are maintained in the cell culture medium comprising fucose.
14. The method of any one of claims 1 to 13, wherein the cell culture medium comprises an initial glucose concentration for an initial time period.
15. The method of claim 14, wherein the initial glucose concentration is about 1 g/L to about 15 g/L.
16. The method of claim 15, wherein the initial glucose concentration is about 12 g/L $\pm$ 1 g/L.
17. The method of any one of claims 1, 2, and 4 to 16, further comprising adding glucose to the cell culture medium according to a glucose feeding schedule.
18. The method of claim 17, wherein the glucose feeding schedule is initiated at about 4 to about 6 days post-cell culture inoculation.
19. The method of claim 18, wherein the glucose feeding schedule is initiated at about 6 days post-cell culture inoculation.
20. The method of any one of claims 17 to 19, wherein the glucose feeding schedule achieves an average glucose concentration of about 10 g/L or less in the cell culture medium.
21. The method of any one of claims 3 and 17 to 20, wherein the glucose feeding schedule achieves an average glucose concentration of about 9 g/L or less in the cell culture medium.
22. The method of claim 21, wherein the glucose feeding schedule achieves an average glucose concentration of about 6 g/L or less in the cell culture medium.
23. The method of claim 22, wherein the glucose feeding schedule achieves an average glucose concentration of about 0.5 g/L to about 4 g/L in the cell culture medium.
24. The method of any one of claims 17 to 23, wherein the glucose feeding schedule achieves an average glucose concentration based on the concentration of fucose in the cell culture medium.

25. The method of claim 24, wherein the average glucose concentration is calculated based on Formula I:

$$T = 3.354 - 1.388F + 0.111G + [F - 0.4375] \times [1.9527(F - 0.4375)]$$

(Formula I)

wherein T is the targeted % total afucosylated (TAF) glycans in the antibody composition and is about 2.5% to about 6%, about 2.75% to about 5.5%, or about 3% to about 5%, F is the concentration (g/L) of fucose in the medium, and G is the average glucose concentration (g/L) in the medium.

26. The method of claim 24 or 25, wherein (i) the concentration of fucose is about 0.2 ± 0.1 g/L and the average glucose concentration is about 2 to about 4 g/L; (ii) the concentration of fucose is about 0.5 ± 0.1 g/L and the average glucose concentration is about 3 to about 6 g/L; or (iii) the concentration of fucose is about 0.75 ± 0.1 g/L and the average glucose concentration is about 4.5 to about 9 g/L.
27. The method of any one of claims 1 to 26, wherein the pH of the cell culture medium is about 6.85 to about 7.05.
28. The method of claim 27, wherein the pH of the cell culture medium is about 6.90 to about 7.00.
29. The method of any one of claims 1 and 3 to 34, wherein the glycosylation-competent cells are not genetically modified to alter activity of an enzyme of the de novo pathway or the salvage pathway.
30. The method of claims 2 and 29, wherein the glycosylation-competent cells are not genetically modified to knock-out a gene encoding GDP-keto-6-deoxymannose-3,5-epimerase, 4-reductase.
31. The method of any one of claims 3 to 30, wherein the level of total afucosylated (TAF) glycans in the antibody composition is less than about 10%.
32. The method of any one of claims 1 to 31, wherein the level of TAF glycans is about 2% to about 6%.
33. The method of claim 32, wherein the level of TAF glycans is about 2% to about 5%.
34. The method of claim 33, wherein the level of TAF glycans in the antibody composition is about 2% to about 4%.
35. The method of any one claims 1 to 34, wherein the level of high mannose glycans in the antibody composition is less than about 3.5%.
36. The method of claim 35, wherein the level of high mannose glycans in the antibody composition is about 0.7% to about 3.0%.
37. The method of any one of claims 1 to 36, wherein the level of afucosylated glycans in the antibody composition is less than about 3.5%.

38. The method of claim 37, wherein the level of afucosylated glycans in the antibody composition is about 0.8% to about 2.8%.
39. The method of any one of claims 1 to 38, wherein the glycosylation-competent cells produce IgG antibodies.
40. The method of claim 39, wherein the glycosylation-competent cells produce IgG1 antibodies.
41. The method of claim 40, wherein the IgG1 antibodies are specific for a tumor-associated antigen.
42. The method of claim 41, wherein the tumor-associated antigen comprises SEQ ID NO. 3.
43. The method of any one of claims 1 to 42, wherein the culture medium does not comprise mannose.
44. An antibody composition produced by the method of any one of claims 1 to 43.
45. A pharmaceutical composition comprising the antibody composition of claim 44 and a pharmaceutically acceptable carrier, diluent or excipient.
46. A cell culture medium comprising:
 - a. glycosylation-competent cells comprising an exogenous nucleic acid encoding an antibody; and
 - b. a culture medium comprising fucose at a concentration of about 0.1 g/L to about 1.0 g/L or about 0.17 g/L to about 1.0 g/L.
47. The cell culture medium of claim 46, wherein the glycosylation-competent cells are not genetically modified to alter activity of an enzyme of the de novo pathway or the salvage pathway, optionally, wherein the glycosylation-competent cells are not genetically modified to knock-out a gene encoding GDP-keto-6-deoxymannose-3,5-epimerase, 4-reductase.
48. The cell culture medium of claim 46 or 47, wherein the culture medium further comprises glucose at a concentration less than about 10 g/L, optionally less than about 9 g/L.
49. The cell culture medium of claim 48, wherein the concentration of glucose is about 6 g/L or less.
50. The cell culture medium of claim 49, wherein the concentration of glucose is about 0.5 g/L to about 4 g/L.
51. The cell culture medium of any one of claims 46 to 50, wherein the pH of the culture medium is about 6.85 to about 7.05.
52. The cell culture medium of claim 51, wherein the pH of the cell culture medium is about 6.90 to about 7.00.

53. The cell culture medium of any one of claims 46 to 52, wherein the cell culture medium does not comprise mannose.
54. The cell culture medium of any one of claims 46 to 53, wherein the antibody is an IgG antibody.
55. The cell culture medium of claim 54, wherein the IgG antibody is an IgG1 antibody.
56. The cell culture medium of claim 55, wherein the IgG1 antibody is specific for a tumor-associated antigen.
57. The cell culture medium of claim 56, wherein the tumor-associated antigen comprises SEQ ID NO: 3.
58. A method of modulating the level of TAF glycans of an antibody composition produced by glycosylation-competent cells, comprising (A) adding fucose to a cell culture medium comprising the glycosylation-competent cells to achieve a fucose concentration of about 0.1 g/L to about 1.0 g/L to decrease the level of TAF glycans; or (B) adding glucose to a cell culture medium comprising the glycosylation-competent cells to achieve a glucose concentration less than about 10 g/L to increase the level of TAF glycans; or (C) both (A) and (B).
59. A method of modulating the level of afucosylated glycans of an antibody composition produced by glycosylation-competent cells, comprising (A) adding fucose to a cell culture medium comprising the glycosylation-competent cells to achieve a fucose concentration of about 0.1 g/L to about 1.0 g/L to decrease the level of afucosylated glycans; or (B) adding glucose to a cell culture medium comprising the glycosylation-competent cells to achieve a glucose concentration less than or about 10 g/L to increase the level of afucosylated glycans; or (C) both (A) and (B).
60. A method of modulating the level of high mannose glycans of an antibody composition produced by glycosylation-competent cells, comprising adding glucose to a cell culture medium comprising the glycosylation-competent cells to achieve a glucose concentration less than or about 10 g/L to increase the level of high mannose glycans.
61. The method of any one of claims 58-60, wherein the glycosylation-competent cells are not genetically modified to alter activity of an enzyme of the de novo pathway or the salvage pathway.
62. The method of claim 61, wherein the glycosylation-competent cells are not genetically modified to knock-out a gene encoding GDP-keto-6-deoxymannanase-3,5-epimerase, 4-reductase.
63. The method of any one of claims 58-62, wherein the level of TAF glycans in the antibody composition is less than or about 10%.

64. The method of claim 63, wherein the level of TAF glycans is about 2% to about 6%.
65. The method of claim 64, wherein the level of TAF glycans is about 2% to about 5%.
66. The method of claim 65, wherein the level of TAF glycans in the antibody composition is about 2% to about 4%.
67. The method of any one of claims 58-66, wherein the level of high mannose glycans in the antibody composition is less than or about 3.5%.
68. The method of claim 67, wherein the level of high mannose glycans in the antibody composition is about 0.7% to about 3.0%.
69. The method of any one of claims 58-68, wherein the level of afucosylated glycans in the antibody composition is less than or about 3.5%.
70. The method of claim 69, wherein the level of afucosylated glycans in the antibody composition is about 0.8% to about 2.8%.
71. The method of any one of claims 58-70, wherein the fucose concentration is about 0.17 g/L to about 1.0 g/L.
72. The method of claim 71, wherein the fucose concentration is about 0.2 g/L to about 0.5 g/L.
73. The method of any one of claims 58 to 72, further comprising adding glucose to the cell culture medium according to a glucose feeding schedule that achieves an average glucose concentration of about 10 g/L, optionally less.
74. The method of claim 73, wherein the average glucose concentration is less than about 6.0 g/L, optionally, less than about 4.0 g/L.
75. The method of claim 73 or 74, wherein the average glucose concentration is based on the fucose concentration of the cell culture medium.
76. The method of claim 75, wherein the average glucose concentration is calculated based on Formula I:

$$T = 3.354 - 1.388F + 0.111G + [F - 0.4375] \times [1.9527(F - 0.4375)]$$

Formula I

wherein T is the targeted % total afucosylated (TAF) glycans in an antibody composition and is about 3% to about 5%, F is the concentration (g/L) of fucose in the medium, and G is the average glucose concentration (g/L).

77. A method of modulating the level of afucosylated glycans of an antibody composition produced by glycosylation-competent cells comprising reducing the pH of the cell culture medium by about 0.03 to about 1.2 to reduce the level of afucosylated glycans of an antibody composition by about 0.5% to about 2% or increasing the pH of the cell culture medium by about 0.03 to about 1.2 to increase the level of afucosylated glycans of an antibody composition by about 0.5% to about 2%.

78. The method of claim 77, comprising reducing the pH of the cell culture medium by about 0.05 to about 1.2 to reduce the level of afucosylated glycans of the antibody composition by about 1% to about 2% or increasing the pH of the cell culture medium by about 0.05 to about 1.2 to increase the level of afucosylated glycans of the antibody composition by about 1% to about 2%.
79. The method of claim 77, comprising reducing the pH of the cell culture medium by about 0.03 to about 0.07 to reduce the level of afucosylated glycans of the antibody composition by about 0.5% to about 1.1% or increasing the pH of the cell culture medium by about 0.03 to about 0.07 to increase the level of afucosylated glycans of the antibody composition by about 0.5% to about 1.1%.
80. A method of reducing the level of afucosylated glycans of an antibody composition produced by glycosylation-competent cells by about 1% to about 2%, comprising reducing the pH of the cell culture medium by about 0.05 to about 1.2.
81. The method of claim 80, comprising reducing the pH by about 0.05 to about 0.07 for a reduction in afucosylated glycans of about 1%.
82. The method of claim 80, comprising reducing the pH by about 0.09 to about 1.2 for a reduction in afucosylated glycans of more than about 1.5%.
83. The method of claim 80, comprising culturing the cells at a pH between about 7.10 to about 7.20, optionally about 7.12 to about 7.19.
84. A method of reducing the level of afucosylated glycans of an antibody composition produced by glycosylation-competent cells by about 0.5% to about 1.1%, comprising reducing the pH of the cell culture medium by about 0.03-0.07.
85. The method of claim 84, comprising reducing the pH by about 0.03 to about 0.06 for a reduction in afucosylated glycans of about 0.8%.
86. The method of claim 84, comprising reducing the pH by about 0.05 to about 0.07 for a reduction in afucosylated glycans of about 1%.
87. The method of claim 84, comprising culturing the cells at a pH higher than 7.05 and less than or about 7.15, optionally about 7.07 to about 7.13.
88. A method of increasing the level of afucosylated glycans of an antibody composition produced by glycosylation-competent cells by about 1% to about 2%, comprising increasing the pH of the cell culture medium by about 0.05 to about 1.2.
89. The method of claim 88, comprising increasing the pH by about 0.05 to about 0.07 for a reduction in afucosylated glycans of about 1%.
90. The method of claim 88, comprising increasing the pH by about 0.09 to about 1.2 for a reduction in afucosylated glycans of more than about 1.5%.
91. The method of claim 88, comprising culturing the cells at a pH between about 7.10 to about 7.20, optionally about 7.12 to about 7.19.

92. A method of increasing the level of afucosylated glycans of an antibody composition produced by glycosylation-competent cells by about 0.5% to about 1.1%, comprising increasing the pH of the cell culture medium by about 0.03-0.07.
93. The method of claim 92, comprising increasing the pH by about 0.03 to about 0.06 for a reduction in afucosylated glycans of about 0.8%.
94. The method of claim 92, comprising increasing the pH by about 0.05 to about 0.07 for a reduction in afucosylated glycans of about 1%.
95. The method of claim 92, comprising culturing the cells at a pH higher than 7.05 and lower than or about 7.15, optionally about 7.07 to about 7.13.
96. The method of any one of the preceding claims, wherein the pH of the cell culture medium throughout the culture is greater than 7.0, optionally, higher than 7.05 and lower than 7.2.
97. The method of any one of the preceding claims, wherein the level of afucosylated glycans in the antibody composition is less than about 10%.
98. The method of claim 97, wherein the level of afucosylated glycans in the antibody composition is about 6.2% to about 8.4%.
99. The method of any one of the preceding claims, wherein the temperature changes by less than 2 degrees C during the culture period.
100. The method of claim 99, wherein the temperature of the culture changes by not more than 1.5 or 1.0 degrees C.
101. The method of any one of the preceding claims, wherein the cell culture medium does not comprise any detectable amounts of manganese or betaine.
102. The method of any one of the preceding claims, wherein the cell culture medium comprises about 0.10 g/L to about 1.0 g/L fucose, optionally, about 0.17 to about 1.0 g/L fucose.
103. The method of claim 102, wherein fucose is present in the culture medium at a concentration less than about 0.75 g/L, less than about 0.6 g/L, or about 0.2 g/L to about 0.5 g/L.
104. The method of any one of the preceding claims, wherein the glycosylation-competent cells are not genetically modified to alter activity of an enzyme of the de novo pathway or the salvage pathway.
105. The method of any one of the preceding claims, wherein glucose is added to the cell culture medium according to a glucose feeding schedule that achieves an average glucose concentration of about 10 g/L or less.
106. A method of modulating the level of TAF glycans of an antibody composition produced by glycosylation-competent cells, comprising modulating the level of afucosylated glycans of the antibody composition in accordance with a method of

any one of claims 77-79, reducing the level of afucosylated glycans of the antibody composition in accordance with a method of any one of claims 80-87, or increasing the level of afucosylated glycans of the antibody composition in accordance with a method of any one of claims 88-105.

107. A method of producing an antibody composition, wherein the level of afucosylated glycans in the antibody composition is about 6.2% to about 8.4%, the method comprising maintaining glycosylation-competent cells in a cell culture medium at a pH higher than 7.05 and lower than 7.2,

wherein:

- (A) the pH of the cell culture medium changes by less than 0.15 (optionally by less than 0.10) during the culture period or
- (B) the temperature of the cell culture medium changes by not more than 2 degrees C or
- (C) the method does not comprise culturing the cells in a cell culture medium comprising manganese or betaine or
- (D) a combination of two or three of (A), (B), and (C).

108. The method of claim 107, wherein the pH is maintained at a pH of about 7.07 to about 7.19 during the culture period, optionally, wherein the pH is maintained at about 7.07 or higher and below 7.10, or about 7.10 or higher and below 7.15, or about 7.15 or higher up to about 7.19.