

preferred. Host cells that require such animal products can be adapted to utilize serum-free and other animal product-free medium. A method for adapting murine myeloma NS0 cells to grow in serum- and cholesterol-free medium is described in Hartman *et al.*, 2007, Biotech. & Bioeng. 96(2):294-306 and Burky *et al.*, 2007, Biotech. & Bioeng. 96(2):281-293. ~~A specific strain of NS0 cells adapted to grow in serum-free and cholesterol-free medium that has been stably transfected with a vector as described above that can be used to produce DAC HYP (clone 7A11-5H7-14-43, also referred to as Daclizumab dWCB IP072911) has been deposited with the American Type Culture Collection (ATCC), under accession no. PTA-12891, on 15 May 2012.~~

[0021] As will be recognized by skilled artisans, the basal and feed media used to culture cells for recombinant protein production, as well as other variables such as the feeding schedule, growth rate, temperature, and oxygen levels, can affect the yield and quality of the expressed protein. Methods of optimizing these conditions are within the purview of a skilled artisan; exemplary conditions are set forth in the Exemplary Embodiments of the disclosure. Preferably, cells are adapted to grow in media free of cholesterol-, serum-, and other animal-sourced components; in such instances the basal and feed media preferably include defined chemicals that substitute for such components. It has also been discovered that media containing high levels of glucose, *e.g.*, 10-35 g/L glucose, advantageously increase the cell culture productivity. In a specific embodiment, the basal medium has about 10-20 g/L, more preferably about 15 g/L, glucose and/or the feed medium has 22-35 g/L, more preferably around 28 g/L, glucose. The feed medium can be added to the cells according to an escalating feed schedule, as is known in the art, over a period of 8-15 days, 9-13 days, or, most preferably 10-13 days.

[0022] For a DAC HYP expressed in NS0 producer strain 7A11-5H7-14-43, the components of the growth and feed media, and other variables affecting expression and production have been optimized. Accordingly, the disclosure also provides optimized basal media, feed media, feeding schedules and other culturing methods and conditions useful for producing daclizumab in high yield and purity. These media and culturing parameters and methods are described in more detail in Section 5.3.

formulation, to inhibit proliferation of activated T- and B-cells, both in *in vitro* applications and *in vivo* as a therapeutic approach towards the treatment of diseases in which activated T- and/or B-cell proliferation play a role, such as the treatment and prevention of allograft rejection, the treatment of uveitis, and the treatment of multiple sclerosis.

[0039] The methods generally involve contacting an activated T- and/or B-cell with an amount of a daclizumab composition or formulation described herein sufficient to inhibit its proliferation.

[0040] For methods of treatment, the methods generally involve administering to a subject an amount of a daclizumab composition, for example a DAC HYP composition or a high concentration DAC formulation as described herein, to provide therapeutic benefit. In a specific embodiment, the daclizumab compositions and formulations can be used to treat multiple sclerosis, either alone or in combination with other agents such as interferon beta. The DAC compositions described herein can be administered subcutaneously to a patient from weekly to monthly (*e.g.*, weekly, every two weeks, twice a month, every four weeks or monthly) in doses ranging from 75 mg to 300 mg (*e.g.*, 75 mg, 100 mg, 125 mg, 150 mg, 175 mg, 200 mg, 225 mg, 250 mg, 275 mg or 300 mg) or ranging from 1 mg/kg to 4 mg/kg. The compositions can be provided in prefilled syringes convenient for subcutaneous use, preferably at nominal daclizumab concentrations of 100 mg/mL $\pm$ 10-15% or 150 mg/mL $\pm$ 10-15%. The concentrated DAC compositions can also be diluted for intravenous administration.

[0040a] In another aspect, the disclosure provides a composition comprising daclizumab, wherein the daclizumab has an N-linked glycosylation profile determined according to the method described in Section 7.6.14, in which the total AUC of the peaks in the profile corresponding to non-fucosylated mannose glycosyls is less than about 6% of the total AUC of all peaks in the profile.

3. BRIEF DESCRIPTION OF THE FIGURES

[0041] FIG. 1 provides DAC-HYP light chain cDNA (SEQ ID NO:1) and translated amino acid (SEQ ID NO:2) sequences. The bold, underlined aspartate (D) residue is the first amino acid in the properly processed mature protein; the amino acid sequence upstream of this residue corresponds to the signal sequence.

[0042] FIG. 2 provides DAC-HYP heavy chain cDNA (SEQ ID NO:3) and translated amino acid sequences (SEQ ID NO:4). The bold, underlined glutamine (Q) residue is the first

8. The modified NS0 cell of embodiment 1 which is stably transfected with a nucleic acid useful for expressing an anti-CD25 monoclonal antibody.
9. The modified NS0 cell of embodiment 8 in which the anti-CD25 monoclonal antibody comprises a VL chain corresponding in sequence to positions 21-233 of SEQ ID NO:2 and a VH chain corresponding in sequence to positions 20 to 465 of SEQ ID NO:4.
10. The modified NS0 cell of embodiment 1 which is transformed with vector pAbX.gpt.
11. The modified NS0 cell of embodiment 1 which is transformed with vector pHAT.IgG1.rg.dE.
12. The modified NS0 cell of embodiment 1 which is designated as clone 7A11-5H7-14-43 (~~obtainable from ATCC under strain designation Daclizumab dWCB IP072911, accession no. PTA-12891~~).
13. A method of producing a recombinant protein, comprising culturing the modified NS0 cell of any one of embodiments 1-12.
14. The method of embodiment 13, wherein the modified NS0 cell is cultured under conditions that result in the production of at least 100 mg/L/day recombinant protein in a 100 L, 1,000 L or 16,000 L culture in a 10-day fed-batch process, or at least 200 mg/L/day recombinant protein in a 100 L, 1,000 L or 16,000 L culture in a 13-day fed-batch process.
15. The method of embodiment 13 or embodiment 14, wherein the modified NS0 cell is cultured in the absence of serum and cholesterol.
16. The method of embodiment 15 wherein the modified NS0 cell is cultured in the absence of tropolone and hydrocortisone.
17. The method of embodiment 13 or embodiment 14, wherein the modified NS0 cell is cultured in a basal and/or feed medium containing 10-35 g/L glucose.

the pH-adjusted eluate at specified temperature for a period of time sufficient to inactivate viruses;

(iii) neutralizing the virus-inactivated eluate to a pH in the range of about pH 7.7 – 7.9 (measured at 25°C);

(iv) flowing the neutralized eluate across a strong anion exchange chromatography resin; and

(v) eluting the adsorbed daclizumab from the weak cation exchange chromatography resin.

114. A purified daclizumab composition, obtained or obtainable by the process of any one of embodiments 74-80.

[0248] Deposit of strain: A strain of NS0 cells adapted to grow in serum-free and cholesterol-free medium that has been stably transfected with vector pHAT.IgG1.rg.dE and which can be used to produce DAC HYP, clone 7A11-5H7-14-43, also referred to as Daclizumab dWCB IP072911, was deposited with the American Type Tissue Collection ("ATCC") at 10801 University Blvd., Manassas, VA 20510-209, U.S., ~~on 15 May 2012, in compliance with the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure on behalf of Abbott Biotherapeutics Corp., having a place of business at 1500 Seaport Blvd, Redwood City, CA 94063. The strain is assigned ATCC accession no. PTA-12891.~~

[0249] All publications, patents, patent applications and other documents cited in this application are hereby incorporated by reference in their entireties for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference for all purposes.

[0250] While various specific embodiments have been illustrated and described, it will be appreciated that various changes can be made without departing from the spirit and scope of the invention(s).

WHAT IS CLAIMED IS:

1. A modified NS0 cell that has been adapted to grow in serum- and cholesterol-free media and that is engineered to express a recombinant protein, said cell being capable of achieving a volumetric productivity exceeding 100 mg/L/day recombinant protein in a culture of 100 L in a 10-day fed-batch process when grown in serum- and cholesterol-free media.

2. The modified NS0 cell of **claim 1** which is capable of achieving a volumetric productivity exceeding 100 mg/L/day recombinant protein in a culture of 1,000 L in a 10-day fed-batch process when grown in media free of cholesterol and animal-derived components.

3. The modified NS0 cell of **claim 1** which is capable of achieving a volumetric productivity exceeding 100 mg/L/day recombinant protein in a culture of 16,000 L in a 10-day fed-batch process when grown in media free of cholesterol and animal-derived components.

4. The modified NS0 cell of **any one of claims 1-3** wherein said fed-batch process comprises adding a feed medium which is added according to the following schedule, where the volume added represents the percentage of the initial cell culture volume:

Day	Volume added
0	0
1	0
2	4
3	7.8
4	7.8
5	7.8
6	11
7	13
8	15
9	15
10	0

5. The modified NS0 cell of **claim 1** that is capable of achieving a volumetric productivity exceeding 200 mg/L/day recombinant protein in a culture of at least 100 L in a 13-day fed-batch process.

6. The modified NS0 cell of **claim 5** which is capable of achieving a volumetric productivity exceeding 200 mg/L/day recombinant protein in a culture of 1,000 L in a 13-day fed-batch process when grown in cholesterol-free media.

7. The modified NS0 cell of **claim 5** which is capable of achieving a volumetric productivity exceeding 100 mg/L/day recombinant protein in a culture of 16,000 L in a 10-day fed-batch process when grown in serum- and cholesterol-free media.

8. The modified NS0 cell of **claim 1** which is stably transfected with a nucleic acid useful for expressing an anti-CD25 monoclonal antibody.

9. The modified NS0 cell of **claim 8** in which the anti-CD25 monoclonal antibody comprises a V_L chain corresponding in sequence to positions 21-233 of SEQ ID NO:2 and a V_H chain corresponding in sequence to positions 20 to 465 of SEQ ID NO:4.

10. The modified NS0 cell of **claim 1** which is transformed with vector **pAbX.gpt**.

11. The modified NS0 cell of **claim 1** which is transformed with vector **pHAT.IgG1.rg.dE**.

12. The modified NS0 cell of **claim 1** which is designated as clone 7A11-5H7-14-43 (obtainable from ATCC under strain designation Daclizumab dWCB IP072911, accession no.PTA-12891).

13. A method of producing a recombinant protein, comprising culturing the modified NS0 cell of **any one of claims 1-12**.

14. The method of **claim 13**, wherein the modified NS0 cell is cultured under conditions that result in the production of at least 100 mg/L/day recombinant protein in a 100 L, 1,000 L or 16,000 L culture in a 10-day fed-batch process, or at least 200 mg/L/day recombinant protein in a 100 L, 1,000 L or 16,000 L culture in a 13-day fed-batch process.

15. The method of **claim 13 or claim 14**, wherein the modified NS0 cell is cultured in the absence of serum and cholesterol.
16. The method of **claim 15** wherein the modified NS0 cell is cultured in the absence of tropolone and hydrocortisone.
17. The method of **claim 13 or claim 14**, wherein the modified NS0 cell is cultured in a basal and/or feed medium containing 10-35 g/L glucose.
18. The method of **claim 17**, wherein the modified NS0 cell is cultured in a basal medium containing 15 g/L glucose and/or a feed medium containing 28 g/L glucose.
19. The method of **claim 18**, wherein the basal medium is composed of the components of PFBM2 $\pm$ 10%.
20. The method of **claim 18**, wherein the feed medium is composed of the components of PFFM3 $\pm$ 10%.
21. The method of **claim 19 or claim 20**, wherein the cell is cultured in basal medium for 1-3 days, and then in feed medium for 10-13 days.
22. The method of **claim 18**, wherein the feed medium is added according to the schedule outlined in Table 7 $\pm$ 10%.
23. A vector useful for recombinantly expressing a protein of interest, comprising a weak promoter driving expression of a selectable marker operable in mammalian cells and a strong promoter driving expression of a protein of interest.
24. The vector of **claim 23**, wherein the protein of interest is a therapeutic antibody.
25. The vector of **claim 24**, wherein the therapeutic antibody is an anti-CD25 antibody.

26. The vector of **claim 25**, wherein the anti-CD25 antibody comprises the CDRs of daclizumab.

27. The vector of **claim 26**, wherein the anti-CD25 antibody is daclizumab.

28. A method for obtaining a mammalian host cell that has a high volumetric productivity of a protein of interest, comprising transfecting the cell with the vector of **any one of claims 23-27**, and selecting a cell that is capable of producing at least 100 mg/L/day protein of interest in a 100 L, 1,000 L or 16,000 L culture in a 10-day fed-batch process or at least 200 mg/L/day recombinant protein in a 100 L, 1,000 L or 16,000 L culture in a 13-day fed-batch process.

29. A composition comprising daclizumab, where the daclizumab is characterized by the presence of a pE/Q heavy chain N-linked isoform and/or a Q/VHS heavy chain N-terminal isoform.

30. The composition of **claim 29** in which the pE/Q heavy chain N-terminal isoform constitutes approximately 3-17% or 6-15% of the daclizumab.

31. The composition of **claim 29** in which the pE/Q heavy chain N-terminal isoform constitutes approximately 5-12% or 7-12% of the daclizumab.

32. The composition of **any one of claims 29-31** in which the Q/VHS heavy chain N-terminal isoform constitutes approximately 1-15% of the daclizumab.

33. The composition of **claim 32** in which the Q/VHS heavy chain N-terminal isoform constitutes approximately 3-12% of the daclizumab.

34. The composition of **claim 29** in which the heavy chain of daclizumab exists in the following N-terminal isoforms:

Isoform	Prevalence
pE/pE	25%-50%
pE/Q	3%-15%
pE/VHS	25%-48%
Q/VHS	1%-15%

VHS/VHS	0.5%-25%
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35. The composition of **claim 29** in which the heavy chain of daclizumab exists in the following N-terminal isoforms:

Isoform	Prevalence
pE/pE	31-46%
pE/Q	5%-12%
pE/VHS	31%-42%
Q/VHS	3%-12%
VHS/VHS	1%-17%

36. The composition of **claim 29**, where the daclizumab is characterized by a cation exchange chromatography isoform profile substantially similar to that of FIG. 18.

37. The composition of **claim 29**, where the daclizumab is DAC HYP.

38. A composition comprising daclizumab, where the daclizumab is characterized by an N-linked glycosylation HPLC profile containing two main peaks, one corresponding to oligosaccharide G0-GlcNAc and one corresponding to oligosaccharide G0, where the combined AUC of these two peaks constitutes about 75-100%, about 80-100%, about 85-100%, or about 88-99.5% of the total AUC of all peaks.

39. The composition of **claim 38**, in which the AUC of the G0-GlcNAc peak constitutes about 5-20%, about 5-18% of the total AUC of all peaks and the AUC of the G0 peak constitutes about 70-99%, about 75-92% or about 75-90% of the total AUC of all peaks.

40. The composition of **claim 39**, in which the AUC of the G0-GlcNAc peak constitutes about 6-16% or about 7-15% of the total AUC of all peaks and the AUC of the G0 peak constitutes about 78-90% or about 81-88% of the total AUC of all peaks.

41. The composition of **any one of claims 38-40** in which the N-linked glycosylation profile has less than about 3% of Man5.

42. The composition of **claim 41** in which the N-linked glycosylation profile has less than about 0.5% G2, Man6 and/or Man7.

43. The composition of **claim 38**, in which the N-linked glycosylation profile contains a third peak corresponding to sialylated oligosaccharides, and the AUC of the sialylated oligosaccharide peak constitutes 1% or less of the total AUC of all peaks.

44. The composition of **claim 38**, in which the N-linked glycosylation profile contains a third peak corresponding to oligosaccharide G1, and the AUC of the G1 peak constitutes less than about 10%, less than about 5%, less than about 4%, less than about 3%, or about 1-5% , about 1-4%, or about 1-3% of the total AUC of all peaks.

45. The composition of **claim 44**, in which the AUC of the G1 peak constitutes about 1-2% of the total AUC of all peaks.

46. The composition of **claim 38**, in which the daclizumab has an N-linked glycosylation HPLC profile substantially similar to that of FIG. 19 or to that of the lower panel of FIG. 21.

47. The composition of **claim 38**, in which the daclizumab is DAC HYP.

48. A composition comprising daclizumab which exhibits less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, or less than 5% ADCC average cytotoxicity as measured in an *in vitro* cellular assay using effector cells from at least 3 healthy donors and Kit 225 K6 cells as target cells, at a daclizumab concentration of 1 µg/mL and an effector to target cell ratio of about 25:1.

49. The composition of **claim 48** wherein the daclizumab exhibits 5% to 30%, 5% to 25%, 10% to 30%, 10% to 25% or 15% to 25% ADCC average cytotoxicity in said assay.

50. The composition of **claim 48 or claim 49** wherein said assay uses effector cells from at least 6 healthy donors.

51. The composition of **claim 48 or claim 49** wherein said assay uses effector cells from at least 10 healthy donors.

52. The composition of **any one of claims 48-51**, in which the daclizumab is DAC HYP.

53. A composition useful for making a daclizumab drug formulation, comprising about 150-190 mg/mL daclizumab and quantities of excipients such that dilution of the composition with a dilution buffer yields a diluted composition that contains about 85-165 mg/mL daclizumab and has an osmolality in the range of about 267-327 mOsm/kg and a pH in the range of about pH 5.8 - 6.2 at 25 °C, and in which at least about 95% of the daclizumab is in monomer form, as measured by size exclusion chromatography.

54. The composition of **claim 53** which contains quantities of excipients such that when diluted with a dilution buffer the diluted composition contains about 85-115 mg/mL daclizumab.

55. The composition of **claim 53** which contains quantities of excipients such that when diluted with a dilution buffer the diluted composition contains about 150 ± 15 mg/mL daclizumab.

56. A composition comprising about 4 to 15 mg/mL daclizumab, where 0.1% or less of the daclizumab is in aggregate form.

57. The composition of **claim 56** which is obtained by purifying a daclizumab composition comprising about 4 to 15 mg/mL daclizumab, wherein up to 2.5% of the daclizumab is in aggregate form, via column chromatography on a weak cation exchange resin.

58. The composition of **claim 57**, wherein the weak cation exchange resin is CM-650M.

59. The composition of **claim 58**, wherein the CM-650M resin is equilibrated with an equilibration buffer containing about 20 mM sodium citrate, pH 4.4 - 4.6, and the daclizumab is eluted with an elution buffer containing about 20 mM sodium citrate and about 75 mM sodium sulfate, pH 4.4 - 4.6.

60. The composition of **claim 59**, wherein the chromatography is carried out in a cylindrical column using a resin bed having a height of about 10-30 cm or about 17-19 cm, and the daclizumab is eluted at a temperature in the range of about 4-22°C or about 18-22 °C, and a flow rate in the range of about 50-200 cm/hr or about 90-110 cm/hr.

61. A composition suitable for administration to humans, comprising about 85-165 mg/mL daclizumab; and about 0.02 - 0.04% (w/v) polysorbate 80, wherein the composition has an osmolality in the range of about 267 - 327 mOsm/kg and a pH in the range of about pH 5.8 - 6.2 at 25 °C, and at least about 95% of the daclizumab is in monomer form, as measured by size exclusion chromatography.

62. The composition of **claim 61**, in which at least about 99% of the daclizumab is in monomer form, as measured by size exclusion chromatography.

63. The composition of **claim 61** which comprises about 85-115 mg/mL daclizumab.

64. The composition of **claim 63**, which consists essentially of about 100 mg/mL daclizumab, about 40 mM sodium succinate, about 100 mM sodium chloride, and about 0.03% (w/v) polysorbate 80, and has a pH of about 6.0 at 25 °C.

65. The composition of **claim 61** which comprises about 135-165 mg/mL daclizumab.

66. The composition of **claim 65**, which consists essentially of about 150 mg/mL daclizumab, about 40 mM sodium succinate, about 100 mM sodium chloride, and about 0.03% (w/v) polysorbate 80, and has a pH of about 6.0 at 25 °C.

67. The composition of **claim 65** which is obtained by a process comprising the steps of concentrating a daclizumab composition comprising about 4 to 15 mg/mL daclizumab via ultrafiltration in a suitable buffer to achieve a daclizumab concentration in the range of about 85-180 mg/mL and optionally diluting the concentrated composition with a dilution buffer.

68. A pharmaceutical composition suitable for subcutaneous administration comprising about 85-165 mg/mL daclizumab, where the percentage of daclizumab in aggregate form does not exceed about 3% following storage for a period of about 12 months at a temperature in the range of about 2-8°C.

69. The pharmaceutical composition of **claim 68** which comprises about 85-115 mg/mL daclizumab.

70. The pharmaceutical composition of **claim 69** which comprises about 135-165 mg/mL daclizumab.

71. The pharmaceutical composition of **claim 69 or claim 70** in which the percentage of daclizumab in aggregate form does not exceed about 2% following storage for a period of about 12 months at a temperature in the range of about 2-8°C.

72. The pharmaceutical composition of **claim 69 or claim 70** in which the percentage of daclizumab in aggregate form does not exceed about 3% following storage for a period of about 18 months at a temperature in the range of about 2-8°C.

73. A process for harvesting a recombinant protein from a cell culture, comprising the steps of:

- (i) adjusting the pH of a cell culture that expresses and secretes a recombinant protein to a pH in the range of about pH 4.5 - 5.5;
- (ii) incubating the pH-adjusted cell culture for about 30 - 90 minutes at a temperature in the range of about 4 to 15 °C; and
- (iii) centrifuging the incubated pH-adjusted cell culture to remove cell debris.

74. A process for producing a purified daclizumab composition, comprising the steps of:

- (i) adsorbing daclizumab from a crude daclizumab preparation onto affinity chromatography resin;
- (ii) washing the affinity chromatography resin with a wash buffer to remove contaminants;
- (iii) eluting the adsorbed daclizumab with an elution buffer;
- (iv) inactivating viruses in the eluate by adjusting the pH to a pH in the range of about pH 3 - 4 and incubating the pH-adjusted eluate at specified temperature for a period of time sufficient to inactivate viruses;
- (v) neutralizing the virus-inactivated eluate to a pH in the range of about pH 7.7 - 7.9 (measured at 25 °C);
- (vi) flowing the neutralized eluate across a strong anion exchange chromatography resin;
- (vii) adsorbing the daclizumab of the eluate of step (vi) onto a weak cation exchange chromatography resin; and
- (viii) eluting the adsorbed daclizumab from the weak cation exchange chromatography resin.

75. The process of **claim 74** in which the crude daclizumab preparation is harvested from a cell culture.

76. The process of **claim 74** in which the crude daclizumab preparation is obtained by culturing host cell 7A11-5H7-14-43 under conditions in which daclizumab is secreted into the culture medium and harvesting the secreted daclizumab.

77. The process of **claim 76** in which the daclizumab is harvested using the method of **claim 73**.

78. The process of **claim 74**, which further comprises the steps of:

- (ix) filtering the eluted daclizumab composition of step (viii) to remove viruses; and
- (x) concentrating the filtered solution via ultrafiltration to yield a purified, daclizumab composition comprising about 85 - 180 mg/mL daclizumab.

79. The process of **claim 78**, which further comprises the step of diluting the purified daclizumab composition with a dilution buffer so as to obtain a composition comprising about 85 - 165 mg/mL daclizumab; and about 0.02 - 0.04% (w/v) polysorbate 80, where the composition has an osmolality in the range of about 267 - 327 mOsm/kg and a pH in the range of about pH 5.8 - 6.2 at 25 °C, and at least about 95% of the daclizumab is in monomer form, as measured by size exclusion chromatography.

80. The process of **claim 79**, wherein the composition obtained has less than 50 ppm of host cell proteins from a recombinant source of daclizumab, less than 10 ppm of protein A, and no more than 3% of the daclizumab in the composition is in aggregate form.

81. Basal medium PFBM2.

82. Feed medium PFFM3.

83. A daclizumab composition, which is obtained by a process comprising the step of culturing a host cell according to **any one of claims 1-12** under conditions in which daclizumab is secreted into the culture medium.

84. The daclizumab composition of **claim 83**, in which the process further comprises the step of isolating the secreted daclizumab from the cell culture medium.

85. A buffer useful for sanitizing a protein A affinity chromatography resin, comprising about 100-500 mM sodium citrate, about 10-30 mM NaOH and about 0.5 - 3% (v/v) benzyl alcohol.

86. A method of sanitizing a protein A affinity chromatography column, comprising washing the column with the sanitization buffer of **claim 85** at a flow rate and for a period of time sufficient to sanitize the column.

87. The method of **claim 86**, in which the column is washed with approximately 1.8 column volumes of sanitization buffer at a flow rate of about 150 cm/hr, the washed column incubated without flow for a period of about 30 - 45 min., and then equilibrated with an equilibration buffer.

88. The method of **claim 87**, in which the equilibration buffer comprises about 20 mM sodium citrate and 150 mM NaCl, and has a pH of about pH 7 (at 25 °C).

89. A method of treating a patient suffering from multiple sclerosis, comprising administering to the patient an amount of a DAC HYP composition sufficient to provide therapeutic benefit.

90. The method of **claim 89** in which the DAC HYP composition is administered intravenously.

91. The method of **claim 90** in which the DAC HYP composition is administered in amount corresponding to about 0.8-0.9 mg/kg DAC HYP.

92. The method of **claim 91** in which the DAC HYP composition is administered in an amount corresponding to about 1 mg/kg DAC HYP.

93. The method of **any one of claims 89-92** in which the DAC HYP is administered once per week for a period of at least 6 weeks, at least 12 weeks, at least 24 weeks.

94. The method of **any one of claims 89-93** in which the DAC HYP is administered as monotherapy.

95. The method of **claim 94** in which the patient has either failed to respond to prior treatment with interferon-beta or has discontinued prior treatment with interferon-beta.

96. The method of **any one of claims 89-93** in which the DAC HYP is administered adjunctively to interferon-beta.

97. The method of **claim 89** in which the DAC HYP composition is administered subcutaneously.

98. The method of **claim 97** in which the DAC HYP composition is administered in an amount corresponding to about 1 mg/kg DAC HYP.

99. The method of **claim 98** in which the DAC HYP composition is administered once every two weeks.

100. The method of **claim 99** in which the DAC HYP composition is administered for a total of about 24 weeks.

101. The method of **claim 97** in which the DAC HYP composition is administered in an amount corresponding to about 2 mg/kg DAC HYP.

102. The method of **claim 101** in which the DAC HYP composition is administered once every 4 weeks.

103. The method of **claim 102** in which the DAC HYP composition is administered for a total of about 24 weeks.

104. The method of **claim 103**, in which the DAC HYP composition is administered in an amount corresponding to 75 mg to 300 mg DAC HYP.

105. The method of **claim 104** in which the DAC HYP composition is administered in an amount corresponding to 150 mg.

106. The method of **claim 104** in which the DAC HYP composition is administered in an amount corresponding to 300 mg.

107. The method of **any one of claims 103-106** wherein the DAC HYP composition is administered once every 4 weeks.

108. The method of **claim 107** wherein the DAC HYP composition is administered for a total of at least 48 weeks.

109. The method of **any one of claims 103-108** in which the DAC HYP is administered as monotherapy.

110. The method of **claim 109** in which the patient has either failed to respond to prior treatment with interferon-beta or has discontinued prior treatment with interferon-beta.

111. The method of **any one of claims 103-108** in which the DAC HYP is administered adjunctively to interferon-beta.

Claims

1. A composition comprising daclizumab, wherein the daclizumab has an N-linked glycosylation profile determined according to the method described in Section 7.6.14, in which the total AUC of the peaks in the profile corresponding to non-fucosylated mannose glycosyls is less than about 6% of the total AUC of all peaks in the profile.
2. The composition of claim 1, wherein the non-fucosylated mannose glycosyls are Man5, Man 6, and Man7.
3. The composition of claim 2, wherein the N-linked glycosylation profile comprises a Man5 peak wherein the AUC of the Man5 peak constitutes about 3% or less of the total AUC of all peaks in the profile.
4. The composition of claim 1, wherein the composition exhibits less than 30% ADCC average cytotoxicity as measured in an in vitro cellular assay using effector cells from at least 3 healthy donors and KIT225/K6 cells as target cells, at a daclizumab concentration of 1 µg/mL and an effector to target cell ratio of about 25:1.