Claims

1. A mammalian cell culture medium formulation comprising mannose at about 3.5 g/L or more and calcium at more than about 9.5 mM.

2. The formulation of claim 1, wherein the medium comprises mannose in a range from about 4 g/L to about 5 g/L.

3. The formulation of claim 1 or claim 2, wherein the formulation comprises DMEM/F12 in 1:1 ratio.

4. A method of producing a recombinant protein in cell culture, comprising:
providing recombinant protein expressing mammalian cells; and
culturing the mammalian cells in a cell culture medium including mannose at about 3.5 g/L or more and calcium in a range from about 1.5 mM to about 9.5 mM, wherein the mammalian cells are selected from BHK cells, CHO cells, HKB cells, and NS0 cells.

5. The method of claim 4, wherein the medium comprises mannose at about 3.5 g/L or more and calcium at less than about 1.5 mM or more than about 9.5 mM.

6. The method of claim 4, wherein the medium comprises mannose at less than about 3.5 g/L and calcium in a range from about 1.5 mM to about 9.5 mM.

7. The method of claim 4, wherein the medium comprises mannose in a range from about 4 g/L to about 5 g/L.

8. The method of claim 5, wherein the medium comprises mannose in a range from about 4 g/L to about 5 g/L.

9. The method of claim 4, wherein the medium comprises calcium in a range from about 2 mM to about 5 mM.

10. The method of claim 6, wherein the medium comprises calcium in a range from about 2 mM to about 5 mM.

11. The method of claim 4, wherein the cells express a blood-coagulation pathway recombinant protein.

12. The method of claim 11, wherein the cells express recombinant human factor VIII (rhFVIII), or a variant thereof.

13. The method of claim 12, wherein the variant factor VIII is a genetic variant.

14. The method of claim 13, wherein the genetic variant is a B-domain deleted FVIII or a pegylated FVIII.

15. The method of claim 4, wherein the cells are BHK cells expressing recombinant factor VIII, and wherein the medium comprises DMEM/F12 in 1:1 ratio, and includes at least one of mannose in a range from about 4 g/L to about 5 g/L and calcium in a range from about 2 mM to about 5 mM.

CLAIMS

1. A cell culture medium formulation for BHK cells expressing recombinant human factor VIII (rhFVIII), or a variant thereof comprising DMEM/F12 medium in 1:1 ratio, mannose in a range from 4 g/L to 5 g/L and calcium in a range from 2 mM to 5 mM.

2. A method of producing recombinant human factor VIII (rhFVIII), or a variant thereof in cell culture, comprising:

   providing recombinant human factor VIII (rhFVIII), or a variant thereof expressing BHK cells; and

   culturing the BHK cells in a cell culture medium comprising DMEM/F12 in 1:1 ratio, mannose in a range from 4 g/L to 5 g/L and calcium in a range from 2 mM to 5 mM.

3. The method of claim 2, wherein the variant factor VIII is a genetic variant.

4. The method of claim 3, wherein the genetic variant is a B-domain deleted FVIII or a pegylated FVIII.