

WHAT IS CLAIMED IS:

1. A method of perfusion culturing a mammalian cell, the method comprising:
providing a vessel containing a mammalian cell disposed in a first liquid culture medium having an osmolality of about 270 mOsm/kg to about 380 mOsm/kg;
incubating the mammalian cell for a period of time at about 32 °C to about 39 °C; and
during the period of time, continuously or periodically removing a first volume of the first liquid culture medium and adding to the first liquid culture medium a second volume of a second liquid culture medium, wherein the first and second volumes are about equal and the osmolality of the first and second liquid culture medium in the vessel is maintained at about 270 mOsm/kg to about 380 mOsm/kg over the period of time.
2. The method of claim 1, wherein the first liquid culture medium has an osmolality of about 270 mOsm/kg to about 320 mOsm/kg.
3. The method of claim 1, wherein the second liquid culture medium comprises greater than 8 g/L of a poloxamer.
4. The method of claim 3, wherein the poloxamer is Pluronic F68.
5. The method of any one of claims 1-4, wherein the second liquid culture medium comprises sodium chloride.
6. The method of any one of claims 1-5, wherein the second liquid culture medium has an osmolality of about 800 mOsm/kg to about 2,500 mOsm/kg.
7. The method of any one of claims 1-6, wherein maintaining the osmolality of the first and second liquid culture medium in the vessel comprises adjusting the flow rate of the second liquid culture medium at some point during the period of time.
8. The method of claim 7, wherein the adjusting step comprises increasing the flow rate of the second liquid culture medium at some point during the period of time.

9. The method of claim 8, wherein the method further comprises adjusting one or both of the pH and the pCO₂ of the first and second liquid culture medium in the vessel at some point during the period of time.

10. The method of claim 9, wherein the adjusting step comprises decreasing the flow rate of the second liquid culture medium at some point during the period of time.

11. The method of any one of claims 1-10, wherein the method further comprises increasing the pCO₂ and increasing the pH of the first and the second liquid culture medium in the vessel over the period of time.

12. The method of claim 11, wherein the pCO₂ and the pH of the first and the second liquid culture medium in the vessel are increased based on the viable cell density over the period of time.

13. The method of any one of claims 1-12, wherein the method further comprises continuously monitoring the osmolality of the first and second liquid culture medium in the vessel during the period of time.

14. The method of any one of claims 1-13, wherein the method further comprises periodically adding a third volume of an aqueous solution to the first and the second liquid culture medium in the vessel during the period of time, wherein the first volume and the sum of the second and third volumes are about equal and the osmolality of the first, second, and third liquid culture medium in the vessel is maintained at about 270 mOsm/kg to about 380 mOsm/kg over the period of time.

15. The method of any one of claims 1-13, wherein the method further includes continuously or periodically adding to the first liquid culture medium a third volume of a third liquid culture medium, wherein the first volume and the sum of the second and third volumes are

about equal and the osmolality of the first, second, and third liquid culture medium in the vessel is maintained at about 270 mOsm/kg to about 380 mOsm/kg over the period of time.

16. A method of producing a recombinant protein, the method comprising:

providing a vessel containing a mammalian cell containing a nucleic acid encoding a recombinant protein disposed in a first liquid culture medium having an osmolality of about 270 mOsm/kg to about 380 mOsm/kg;

incubating the mammalian cell for a period of time at about 32 °C to about 39 °C; and

during the period of time, continuously or periodically removing a first volume of the first liquid culture medium and adding to the first liquid culture medium a second volume of a second liquid culture medium, wherein the first and second volumes are about equal and the osmolality of the first and second liquid culture medium in the vessel is maintained at about 270 mOsm/kg to about 380 mOsm/kg over the period of time; and

recovering the recombinant protein from the mammalian cell or from the first and/or second liquid culture medium.

17. The method of claim 16, wherein the first liquid culture medium has an osmolality of about 270 mOsm/kg to about 320 mOsm/kg.

18. The method of claim 16 or claim 17, wherein the second liquid culture medium has an osmolality of about 800 mOsm/kg to about 2,500 mOsm/kg.

19. The method of any one of claims 16-18, wherein the method further comprises isolating the recombinant protein.

20. The method of claim 19, wherein the method further comprises formulating the isolated recombinant protein.