CLAIMS

1. A method of culturing mammalian cells expressing a heterologous protein in a perfusion cell culture comprising:

(a) inoculating mammalian cells expressing a heterologous protein in a serum-free culture medium;

(b) culturing the mammalian cells during growth phase by perfusion with a serum-free perfusion medium; and

(c) culturing the mammalian cells during production phase by perfusion with a serum-free perfusion medium comprising potassium ions at a concentration of 30 mM to 250 mM and a molar ratio of sodium to potassium ions of less than 1,

wherein step (b) is optional.

2. A method of reducing cell bleeding in a perfusion cell culture expressing a heterologous protein comprising:

(a) inoculating mammalian cells expressing a heterologous protein in a serum-free culture medium;

(b) culturing the mammalian cells during growth phase by perfusion with a serum-free perfusion medium; and

(c) culturing the mammalian cells during production phase by perfusion with a serum-free perfusion medium comprising potassium ions at a concentration of 30 mM to 250 mM and a molar ratio of sodium to potassium ions of less than 1,

wherein step (b) is optional.

3. A method of increasing protein production in a perfusion cell culture expressing a heterologous protein comprising:

(a) inoculating mammalian cells expressing a heterologous protein in a serum-free culture medium;

(b) culturing the mammalian cells during growth phase by perfusion with a serum-free perfusion medium; and

(c) culturing the mammalian cells during production phase by perfusion with a serum-free perfusion medium comprising potassium ions at a concentration of 30 mM to 250 mM and a molar ratio of sodium to potassium ions of less than 1,

wherein step (b) is optional.

4. The method of any one of claims 1 to 3, wherein step (c) further comprises maintaining a cell density by cell bleeding, preferably wherein cell bleeding is reduced compared to a perfusion cell culture using the same serum-free perfusion medium comprising potassium ions at a concentration of less than 30 mM and a molar ratio of sodium to potassium of more than 2 and cultured under the same conditions.

5. The method of any one of the preceding claims, wherein the potassium ion concentration is about 40 mM to about 200 mM, preferably about 60 mM to about 150 mM and more preferably about 80 mM to about 100 mM.

6. The method of any one of the preceding claims, wherein the molar ratio of sodium to potassium is between about 0.9 and 0.1, between about 0.8 and 0.2, between about 0.7 and 0.2, preferably between about 0.6 and 0.3 and more preferably between about 0.5 and 0.4.

7. The method of any one of the preceding claims, wherein the potassium ion is provided as one or more potassium salt, preferably wherein the one or more potassium salt is selected from the group consisting of potassium bicarbonate, potassium chloride, potassium hydroxide, L-tyrosine dipotassium salt, potassium phosphate dibasic, potassium phosphate monobasic, potassium selenite, potassium pyruvic acid, potassium glutathione, potassium D-gluconate, potassium succinate and potassium ascorbate, more preferred wherein the one or more potassium salt substitutes the corresponding sodium salt in the serum-free perfusion culture medium, most preferred wherein the potassium ion is provided as one or more potassium salt and the one or more potassium salt substitutes the corresponding sodium salt in the serum-free perfusion medium of step b).

8. The method of any one of the preceding claims, wherein the mammalian cell is a Chinese hamster ovary (CHO) cell, preferably a CHO-DG44 cell, a CHO-K1 cell, a CHO DXB11 cell, a CHO-S cell, a CHO GS deficient cell or a derivative of any of these cells.

9. The method of any one of the preceding claims, wherein the heterologous protein is a therapeutic protein, preferably selected from the group consisting of an antibody, a fusion protein, a cytokine and a growth factor.

10. A method of producing a therapeutic protein using the method of any one of claims 1 to 9.

11. A serum-free perfusion medium comprising potassium ions at a concentration of 30 mM to 250 mM and a molar ratio of sodium to potassium ions of less than 1.

12. Use of the serum-free perfusion medium of claim 11 for culturing mammalian cells in a perfusion culture during production phase.

13. Use of the serum-free perfusion medium of claim 11 for reducing the total cell bleed volume in a perfusion culture.

14. Use of the serum-free perfusion medium of claim 11 for increasing protein production in a perfusion cell culture.

15. Use of the serum-free perfusion medium of claim 11 for increasing cell specific protein production in a perfusion cell culture.