

CLAIMS

1. **A compartmentalized serum-free cell culture perfusion medium** comprising the medium components subgrouped into at least three separate aqueous concentrated feeds and a diluent, wherein the first concentrated feed is an alkaline concentrated feed, the second concentrated feed is an acidic concentrated feed and the third concentrated feed is a near neutral concentrated feed; wherein

the compartmentalized serum-free cell culture perfusion medium is pH-adjusting to neutral pH upon mixing of the at least three separate aqueous concentrated feeds and the diluent in the resulting serum-free cell culture perfusion medium.
2. The compartmentalized serum-free cell culture perfusion medium of claim 1, wherein the resulting serum-free cell culture perfusion medium has a pH of between 6.7 and 7.5, between 6.9 and 7.4, preferably between 6.9 and 7.2, upon mixing of the at least three separate aqueous concentrated feeds and the diluent.
3. The compartmentalized serum-free cell culture perfusion medium of claim 1 or 2, wherein the diluent is sterile water.
4. The compartmentalized serum-free cell culture perfusion medium of any one of claims 1 to 3, wherein the compartmentalized serum-free cell culture perfusion medium is for
 - (a) separate addition of the alkaline concentrated feed, the acidic concentrated feed and the near neutral concentrated feed to a cell culture and/or a reaction vessel of a bioreactor;
 - (b) direct addition of the alkaline concentrated feed, the acidic concentrated feed and the near neutral concentrated feed to a cell culture and/or a reaction vessel of a bioreactor without prior pre-mixing; and/or
 - (c) direct mixing of the at least three separate aqueous concentrated feeds in a cell culture and/or a reaction vessel of a bioreactor.

5. The compartmentalized serum-free cell culture perfusion medium of any one of the preceding claims, wherein the alkaline concentrated feed is a 2x to 80x concentrated feed, the acidic concentrated feed is a 2x to 40x concentrated feed and the near neutral concentrated feed is a 2x to 50x concentrated feed.
6. The compartmentalized serum-free cell culture perfusion medium of any one of the preceding claims, wherein the alkaline concentrated feed has a pH of 9 or higher, the acidic concentrated feed has a pH of 5 or lower and the near neutral concentrated feed has a pH of 7 to 8.5.
7. **An alkaline aqueous concentrated feed** for combination with an acidic aqueous concentrated feed, a near neutral aqueous concentrated feed and a diluent to form a serum-free cell culture perfusion medium, wherein the pH of the resulting serum-free cell culture perfusion medium is automatically adjusted to a neutral pH.
8. **An acidic aqueous concentrated feed** for combination with an alkaline aqueous concentrated feed, a near neutral aqueous concentrated feed and a diluent to form a serum-free cell culture perfusion medium, wherein the pH of the resulting serum-free cell culture perfusion medium is automatically adjusted to a neutral pH.
9. **A near neutral aqueous concentrated feed** for combination with an alkaline aqueous concentrated feed, an acidic aqueous concentrated feed and a diluent to form a serum-free cell culture perfusion medium, wherein the pH of the resulting serum-free cell culture perfusion medium is automatically adjusted to a neutral pH.
10. **A method of preparing a serum-free cell culture perfusion medium** comprising
 - (a) providing the components of a cell culture media in at least three subgroups of components based on solubility at alkaline, acidic and neutral pH,
 - (b) dissolving
 - (i) the subgroup of components soluble at alkaline pH in an alkaline aqueous solution to form an alkaline concentrated feed;

- (ii) the subgroup of components soluble at acidic pH in an acidic aqueous solution to form an acidic concentrated feed; and
 - (iii) the subgroup of components soluble at neutral pH in a neutral aqueous solution to form a near neutral concentrated feed;
- (c) optionally storing the prepared alkaline concentrated feed, acidic concentrated feed and near neutral concentrated feed in separate containers; and
- (d) adding the prepared alkaline concentrated feed, acidic concentrated feed and near neutral concentrated feed and the diluent to the cell culture and/or the reaction vessel of the bioreactor, wherein
- (i) the alkaline concentrated feed, the acidic concentrated feed and the near neutral concentrated feed are added separately to the cell culture and/or the reaction vessel of the bioreactor; and
 - (ii) the diluent is added separately to the cell culture and/or the reaction vessel of the bioreactor or the diluent is premixed with one of the at least three separate aqueous concentrated feeds immediately before addition to the cell culture and/or the reaction vessel of the bioreactor;

wherein the pH of the resulting serum-free cell culture perfusion medium is automatically pH adjusted to a neutral pH upon mixing of the at least three separate aqueous concentrated feeds and the diluent.

11. **A serum-free cell culture perfusion medium** obtainable by the method according to claim 10.
12. **A method of culturing mammalian cells** expressing a heterologous protein in perfusion culture, comprising:
- (a) inoculating a bioreactor with mammalian cells expressing a heterologous protein in a serum-free cell culture medium;
 - (b) culturing the mammalian cells in a perfusion culture by continuously feeding the mammalian cells with a serum-free cell culture perfusion medium feed and removing

spent media while keeping the cells in culture, wherein the serum-free cell culture perfusion medium feed is (i) a compartmentalized serum-free cell culture perfusion medium comprising the medium components subgrouped into at least three separate aqueous concentrated feeds and a diluent, wherein the first concentrated feed is an alkaline concentrated feed, the second concentrated feed is an acidic concentrated feed and the third concentrated feed is a near neutral concentrated feed; and wherein the compartmentalized serum-free cell culture perfusion medium is pH-adjusting to neutral pH upon mixing of the at least three separate aqueous concentrated feeds and the diluent in the resulting serum-free cell culture perfusion medium; and/or (ii) the serum-free cell culture perfusion medium according to claim 11, and

wherein the alkaline concentrated feed, the acidic concentrated feed and the near neutral concentrated feed of the compartmentalized serum-free cell culture perfusion medium feed are added separately to the cell culture and/or the reaction vessel of the bioreactor and wherein the diluent is added separately to the cell culture and/or the reaction vessel of the bioreactor or the diluent is premixed with one of the at least three separate aqueous concentrated feeds immediately before addition to the cell culture and/or the reaction vessel of the bioreactor.

13. **A method of producing a therapeutic protein** using the method of claim 12.
14. **Use** of the compartmentalized serum-free cell culture perfusion medium of any one of claims 1-6 or the serum-free cell culture perfusion medium of claim 11 **for culturing mammalian cells**.
15. **Use** of the compartmentalized serum-free cell culture perfusion medium of any one of claims 1-6 or the serum-free cell culture perfusion medium of claim 11 **for culturing mammalian cells in a perfusion culture**.
16. **Use** of the compartmentalized serum-free cell culture perfusion medium of any one of claims 1-6 or the serum-free cell culture perfusion medium of claim 11 **for controlling osmolality in a perfusion cell culture**.

17. **Use** of the compartmentalized serum-free cell culture perfusion medium of any one of claims 1-6 **for separate addition of the at least three separate aqueous concentrated feeds to a cell culture and/or a reaction vessel of a bioreactor.**