Claims

1. A method for producing an RSV F protein trimer in a fed batch cell culture, said method comprising the steps of:
(i) providing mammalian cells that contain a gene encoding an RSV F protein in a cell culture medium to start a cell culture, and,
(ii) culturing the cells at a temperature between about 33.0°C and 35.0°C, and
(iii) providing glucose in a restricted manner to the cell culture by feeding glucose to the cell culture in response to rise of pH above a predetermined pH value.

2. The method according to claim 1, wherein the temperature is about 34.5°C.

3. The method according to claim 1 or 2 wherein the temperature is shifted to a lower temperature, ~~preferably~~ optionally between about 30.0°C and about 32.0°C, optionally about 31.0°C.

~~4. The method according to claim 3 wherein the lower temperature is about 31.0°C~~

~~5~~4. The method according to claim 3 ~~or 4~~ wherein the temperature is shifted to a lower temperature between day 3 and day 7, or on day 5 or on day 6.

~~6. The method according to any one of claims 3 to 5 wherein the temperature is shifted to a lower temperature on day 5 or on day 6.~~

~~7~~5. The method according to any one of the preceding claims, wherein a pH sensor is used to monitor pH of the cell culture.

~~8~~6. The method according to any one of the preceding claims, wherein the predetermined pH value corresponds to an increase of 0.01 to 0.10 above the pH set point of the culture, ~~or~~optionally wherein the predetermined pH corresponds to an increase of 0.05 above the pH set point of the culture.

~~9. The method according to any one of the preceding claims, wherein the predetermined pH value corresponds to an increase of 0.05 above the pH set point of the culture.~~

~~10~~7. The method according to any one of the preceding claims wherein the pH set point of the cell culture is between 6.70 and 7.30, between 6.90 and 7.20, between 7.00 and 7.10, ~~,~~ or 7.05.

~~11. The method according to any one of the preceding claims wherein the pH set point of the cell culture is between 6.90 and 7.20.~~

~~12. The method according to any one of the preceding claims wherein the pH set point of the cell culture is between 7.00 and 7.10.~~

~~13. The method according to any one of the preceding claims wherein the pH set point of the cell culture is 7.05.~~

~~14~~8. The method according to any one of claims 1 to ~~9~~ 6 wherein:
- the pH set point is 6.95, 7.01, 7.05, 7.07 or 7.20 during the phase of the cell culture where glucose is provided in a restricted manner and,
- the pH set point is 6.90 or 7.20 after the phase of the cell culture where glucose is provided in a restricted manner.

~~15~~9. The method according to any one of the preceding claims wherein feeding glucose to the cell culture in response to rise of pH above a predetermined pH value comprises feeding glucose until the pH decreases to reach the pH set point of the culture.

~~16~~10. The method according to any one of the preceding claims wherein glucose is provided in a restricted manner from day 1 to day 6.

~~17~~11. The method according to any one of the preceding claims, wherein the cell culture medium~~.~~:
(a) does not comprise glucocorticoid compounds;
(b) ~~not comprising~~does not comprise any of hydrocortisone, prednisone, prednisolone, methylprednisolone, dexamethasone, betamethasone, triamcinolone and fludrocortisone acetate;
(c) does not ~~comprising~~comprise hydrocortisone;
(d) ~~being~~is serum free;
(e) ~~being~~is protein free; and/or
(f) ~~being~~is defined.

~~18. The method according to any one of the preceding claims, wherein the cell culture medium does not comprise any of hydrocortisone, prednisone, prednisolone, methylprednisolone, dexamethasone, betamethasone, triamcinolone and fludrocortisone acetate.~~

~~19. The method according to any one of the preceding claims, wherein the cell culture medium does not comprise hydrocortisone.~~

~~20. The method according to any one of the preceding claims, wherein the cell culture medium is serum free.~~

~~21. The method according to any one of the preceding claims, wherein the cell culture medium is protein free.~~

~~22. The method according to any one of the preceding claims, wherein the cell culture medium is defined.~~

~~23~~12. The method according to any one of the preceding claims, wherein the cell culture is further provided with a feed medium.

~~24~~13. The method according to claim ~~23~~12, wherein the feed medium ~~meets one or more of the following criteria~~:

(a) is provided continuously or at multiple intervals;

(b) does not comprisinge glucocorticoid compounds;

(c) does not comprisinge any of hydrocortisone, prednisone, prednisolone, methylprednisolone, dexamethasone, betamethasone, triamcinolone and fludrocortisone acetate;

(d) does not comprising e hydrocortisone;

(e) beingis serum free;

(f) beingis protein free; and/or

(g) beingis defined.

~~25. The method according to claim 23 or 24, wherein the feed medium does not comprise glucocorticoid compounds.~~

~~26. The method according to any of claims 23 to 25, wherein the feed medium does not comprise any of hydrocortisone, prednisone, prednisolone, methylprednisolone, dexamethasone, betamethasone, triamcinolone and fludrocortisone acetate.~~

~~27. The method according to any of claims 23 to 26, wherein the cell culture medium does not comprise hydrocortisone.~~

~~28. The method according to any of claims 23 to 27, wherein the feed medium is serum free.~~

~~29. The method according to any of claims 23 to 28, wherein the feed medium is protein free.~~

~~30. The method according to any of claims 23 to 29, wherein the feed medium is defined.~~

~~31~~14. The method of any one of the preceding claims wherein the maximum viable cell density during the cell culture is above 1 x 10^6 cells/mL, 5 x 10^6cells/mL, 1 x 10^7cells /mL, 5 x 10^7 cells/mL, 1X10^8 cells/mL or 5X10^8 cells/mL.

~~32. The method of any one of the preceding claims wherein the maximum viable cell density is above 5 x 10^6cells/mL, 1 x 10^7cells /mL, 5 x 10^7 cells/mL, 1X10^8 cells/mL or 5X10^8 cells/mL.~~

~~33~~15. The method of any one of the preceding claims wherein the volume of the cell culture medium is at least 500L, or at least 3000L.

~~34. The method of claim 33, wherein the volume of the cell culture medium is at least 3000L.~~

~~35~~16. The method according to any one of the preceding claims wherein the mammalian cells are selected from BALB/c mouse myeloma line, human retinoblasts (PER.C6), monkey kidney cells, human embryonic kidney line (293), baby hamster kidney cells (BHK), Chinese hamster ovary cells (CHO), mouse sertoli cells, African green monkey kidney cells (VERO-76), human cervical carcinoma cells (HeLa), canine kidney cells, buffalo rat liver cells, human lung cells, human liver cells, mouse mammary tumor cells, TRI cells, MRC 5 cells, FS4 cells, or human hepatoma line (Hep G2), optionally wherein the mammalian cells are Chinese hamster ovary cells (CHO), optionally GS-CHO cells .

~~36. The method according to any one of the preceding claims wherein the mammalian cells are Chinese hamster ovary cells (CHO).~~

~~37. The method according to any one of the preceding claims wherein the mammalian cells are GS-CHO cells.~~

~~38~~17. The method according to any one of the preceding claims, wherein the RSV F protein is of subtype A or subtype B.

~~39. The method according to any one of the preceding claims, wherein the RSV F protein is of subtype B.~~

~~40~~18. The method according to any one of the preceding claims wherein the RSV F protein comprises mutations stabilizing the trimer in the pre-fusion conformation.

~~41~~19. The method according to any one of the preceding claims wherein the RSV F protein comprise a combination of mutations selected from the group consisting of:

(1) combination of T103C, I148C, S190I, and D486S;

(2) combination of T54H S55C L188C D486S;

(3) combination of T54H, T103C, I148C, S190I, V296I, and D486S;

(4) combination of T54H, S55C, L142C, L188C, V296I, and N371C;

(5) combination of S55C, L188C, and D486S;

(6) combination of T54H, S55C, L188C, and S190I;

(7) combination of S55C, L188C, S190I, and D486S;

(8) combination of T54H, S55C, L188C, S190I, and D486S;

(9) combination of S155C, S190I, S290C, and D486S;

(10) combination of T54H, S55C, L142C, L188C, V296I, N371C, D486S, E487Q, and D489S; and

(11) combination of T54H, S155C, S190I, S290C, and V296I.

~~42~~20. The method of any one of claims 1 to ~~41~~19, further comprising obtaining the RSV F protein trimer produced by the cells, and optionally purifying the RSV F protein trimer.

~~43. The method of claim 41, further comprising purifying the RSV F protein trimer.~~

~~44~~21. A pharmaceutical composition comprising a purified RSV F protein trimer obtained by the method according to claim ~~42~~ 20 in combination with a pharmaceutically acceptable carrier.