

CLAIMS

1. A process for producing a recombinant protein comprising:
 - a. culturing host cells capable of producing a recombinant protein in a medium free of animal-derived products, wherein the host cells are CHO cells;
 - b. progressing the culture through a production phase wherein the recombinant protein is produced by the cells, wherein, during said production phase, the culture is supplemented with
 - cysteine or cystine in a total amount of from 10 wt% to 30 wt% of the expected total amount of recombinant protein produced; and
 - tryptophan in a total amount of from 8 wt% to 35 wt% of the expected total amount of recombinant protein produced,
 - c. and recovering the recombinant protein from the cell culture medium,

wherein one or more initial experiments are performed to determine the expected total amount of recombinant protein produced.
2. A process for producing a recombinant protein comprising:
 - a. culturing host cells capable of producing a recombinant protein in a medium free of animal-derived products, wherein the host cells are CHO cells;
 - b. progressing the culture through a production phase wherein the recombinant protein is produced by the cells and the cell culture medium is supplemented with cysteine or cystine and tryptophan, wherein
 - the total amount of cysteine or cystine provided during the process is from 2.9 to 7 g/10¹² cells or from 5.6 to 7 g/10¹² cells, wherein cells refers to the expected integral viable cell count at the end of the production phase, and
 - the total amount of tryptophan provided during the process is from 2.5 to 3.5 g/10¹² cells, wherein cells refers to the expected integral viable cell count at the end of the production phase,
 - c. and recovering the recombinant protein from the cell culture medium,

wherein one or more initial experiments are performed to determine the expected integral viable cell count at the end of the production phase.
3. A method for reducing the heterogeneity of the population of recombinant proteins in a batch produced in production phase by recombinant host cells, wherein the host cells are CHO cells, comprising :

- a. culturing host cells capable of producing a recombinant protein in a medium free of animal-derived products
- b. progressing the culture through a production phase wherein the recombinant protein is produced by the cells, wherein, during said production phase, the culture is supplemented with
 - i. cysteine or cystine in a total amount of from 10 wt% to 30 wt% of the expected total amount of recombinant protein produced; and
 - ii. tryptophan in a total amount of from 8 wt% to 35 wt% of the expected total amount of recombinant protein produced

and recovering the recombinant protein from the cell culture medium, wherein one or more initial experiments are performed to determine the expected total amount of recombinant protein produced.

- 4. The process according to any one of the preceding claims, wherein the culture is supplemented with cysteine or cystine in a total amount of from 12 wt% to 28 wt% of the expected total amount of recombinant protein produced, from 12 wt% to 25 wt% or from 12 wt% to 20 wt% of the expected total amount of recombinant protein produced.
- 5. The process according to any one of the preceding claims, wherein the culture is supplemented with tryptophan in a total amount of from 8 wt% to 30 wt% of the expected total amount of recombinant protein produced, from 8 wt% to 25 wt% or from 8 wt% to 20 wt% of the expected total amount of recombinant protein produced.
- 6. The process according to any one of the preceding claims, wherein the total amount of cysteine or cystine provided during the process is from 2.9 to 12 g/10¹² cells, from 2.9 to 7 g/10¹² cells or from 5.6 to 7 g/10¹² cells, wherein cells refers to the expected integral viable cell count at the end of the production phase.
- 7. The process according to any one of the preceding claims, wherein the total amount of tryptophan provided during the process is from 2.5 to 7 g/10¹² cells or from 2.5 to 3.5 g/10¹² cells, wherein cells refers to the expected integral viable cell count at the end of the production phase.
- 8. The process according to any one of the preceding claims, wherein the total amount of cysteine or cystine and tryptophan in the culture is reached by adding cysteine or cystine and tryptophan to the cell culture medium:
 - a. at the beginning of the production phase,
 - b. once or multiple times at any time point during the production phase,
 - c. through continuous addition during the production phase, or
 - d. in any combination of a., b. and c.

9. The process according to any one of the preceding claims, wherein the process is a batch process or a fed-batch process.
10. The process according to any one of the preceding claims, wherein cysteine or cystine and tryptophan are provided through daily addition during the production phase.
11. The process according to claim 10, wherein cysteine or cystine is depleted in the culture before cysteine or cystine is added on the next day, e.g. by reducing cysteine or cystine addition to a level between 5.6 and 7 g/10¹²cells, wherein cells refers to the expected integral viable cell count at the end of the production phase.
12. The process according to claim 10 or 11, wherein during the late stage of production, i.e. when the cells have already reached the maximum viable cell density, tryptophan is depleted in the culture before tryptophan is added on the next day.
13. The process according to any one of the preceding claims, wherein the cysteine or cystine concentration in the cell culture medium does not exceed 0.9 g/L at any time point during the production phase, or wherein the cysteine or cystine concentration in the cell culture medium does not exceed 0.3 g/L at any time point during the production phase.
14. The process according to any one of the preceding claims, wherein the tryptophan concentration in the cell culture medium does not exceed 0.6 g/L at any time point during the production phase, or wherein the tryptophan concentration in the cell culture does not exceed 0.3 g/L medium at any time point during the production phase.
15. The process according to any one of the preceding claims, wherein the production phase is performed for at least 7 days, or at least 14 days.
16. The process according to any one of the preceding claims, wherein at any time point during the 2nd half of the production phase:
 - the amount of cysteine or cystine in the culture is from 10 wt% to 30% of the expected amount of recombinant protein produced; and
 - the amount of tryptophan in the culture is from 8 wt% to 35% of the expected amount of recombinant protein produced.
17. The process according to any one of the preceding claims, wherein at any time point during the production phase:
 - the amount of cysteine or cystine in the culture is from 10 wt% to 30% of the expected amount of recombinant protein produced; and
 - the amount of tryptophan in the culture is from 8 wt% to 35% of the expected amount of recombinant protein produced.

18. The process according to any one of the preceding claims, wherein the recombinant protein is an antibody or an antigen-binding fragment thereof.
19. The process according to claim 18, wherein the antibody or antigen-binding fragment thereof is:
 - 1) an antibody or antigen-binding fragment thereof which
 - a. comprises CDR-H1 having the sequence as defined in SEQ ID NO:1; CDR-H2 having the sequence as defined in SEQ ID NO:2; CDR-H3 having the sequence as defined in SEQ ID NO:3; CDR-L1 having the sequence as defined in SEQ ID NO:4; CDR-L2 having the sequence as defined in SEQ ID NO:5 and CDR-L3 having the sequence as defined in SEQ ID NO:6; or
 - b. comprises a light variable region having the sequence as defined in SEQ ID NO: 7 and a heavy variable region having the sequence as defined in SEQ ID NO: 8; or
 - c. comprises a light variable region having the sequence as defined in SEQ ID NO: 7 and a heavy chain having the sequence as defined in SEQ ID NO: 11; or
 - 2) an antibody which comprises a light chain having the sequence as defined in SEQ ID NO: 9 and a heavy chain having the sequence as defined in SEQ ID NO: 10.
20. The process according to any one of the preceding claims, wherein the production phase is carried out in a bioreactor with a volume of equal or more than 50 L, equal or more than 100 L, equal or more than 500 L, equal or more than 1000 L, equal or more than 2,000 L, equal or more than 5,000 L, equal or more than 10,000 L or equal or more than 20,000 L.
21. The process according to any one of the preceding claims, wherein the process comprises the step of recovering the recombinant protein from the cell culture medium and a further step of purifying the recombinant protein.
22. The process according to claim 21, wherein the purification comprises Protein A chromatography.
23. The process according to claim 21 or 22, further comprising the step of formulating the purified recombinant protein, wherein the protein is an antibody or antigen-binding fragment thereof as defined in claim 19.
24. The process according to claim 23, wherein the recombinant protein is formulated in a liquid formulation comprising one or more amino acids and a surfactant.
25. The process according to claim 24, wherein the formulation comprises histidine and/or proline.
26. The process according to claim 25, wherein the formulation comprises histidine in a concentration of 5 mM to 100 mM or a concentration of 10 mM to 50 mM, and/or proline

in a concentration of 100 mM to 500 mM, at a pH between 5 and 7.4, between 5 and 6.5 or between 5 and 6.

27. The process according to claim 26, wherein the formulation comprises histidine in a concentration of 30 mM and proline in a concentration of 250 mM, at a pH between 5.2 and 6.0
28. The process according to any one of claims 24 to 27, wherein the surfactant is polysorbate 80 in a concentration of 0.001% to 0.1% (w/v), 0.005% to 0.1%, 0.01% to 0.1% or 0.01% to 0.05%, such as 0.03%.
29. The processing according to any one of claims 24 to 28, wherein the recombinant protein is formulated at a concentration of 10 mg/ml to 250 mg/ml 20 mg/ml to 250 mg/ml, 50 mg/ml to 250 mg/ml or 120 mg/ml to 160 mg/ml, such as 140 mg/ml.
30. The process according to any one of the preceding claims, wherein the process reduces the heterogeneity of the recombinant proteins produced, wherein said reduction of heterogeneity comprises reducing:
 - a. charge heterogeneity, preferably acidic peak group (APG); and/or
 - b. amino acid oxidation, isomerization, fragmentation, other covalent adducts glycation, deamidation, cysteinylation; and/or
 - c. colour or intensity of colour, e.g. between different batches of the recombinant protein; and/or
 - d. high molecular weight species (HMWS); and/or
 - e. recombinant protein instability.