Claims

1. A method for reducing the heterogeneity of a population of recombinant proteins produced in cell culture, said method comprising growing host cells producing a recombinant protein in a cell culture medium wherein the cell culture medium comprises (a) one or more cysteine/cystine analogs, and (b) cysteine and/or cystine, wherein the molar ratio of (a) to (b) is between 1:10 and 10:1, wherein the cysteine/cystine analogs are selected from the group consisting of N,N'-diacetyl-L-cystine-dimethylester, N-Acetyl-L-cysteine and N,N'-Diacetyl-L-cystine, wherein said reduction of heterogeneity is obtained without substantially reducing the titer of the recombinant protein at the end of the production process and wherein said reduction of heterogeneity comprises reducing:

   a. color or intensity of color;
   b. charge heterogeneity, preferably by reducing acidic peak group species (APG) and/or basic peak group species (BPG), whereby the main charge species does not decrease; and/or
   c. amino acid oxidation, preferably methionine oxidation.

2. The method according to claim 1, wherein the method comprises the steps of:

   (i) inoculating said host cells in a basal medium, wherein the basal medium optionally comprises an initial amount of:

   (a) cysteine/cystine analogs; and/or

   (b) cysteine and/or cystine,

   (ii) progressing the culture through a production phase wherein the recombinant protein is produced by the cells, wherein, during said production phase, the cell culture medium is supplemented with:

   (a) cysteine/cystine analogs; and/or

   (b) cysteine and/or cystine,

   wherein (a) and (b) may be added simultaneously or sequentially,

   wherein, when the contents of the basal medium and the total supplements added are added up, the molar ratio of (a) to (b) is between 1:10 and 10:1.

3. A method for producing a recombinant protein preparation comprising:

(i) inoculating said host cells in a basal medium, wherein the basal medium optionally comprises an initial amount of:

(a) cysteine/cystine analogs selected from the group consisting of N,N’-diacetyl-L-cystine-dimethylester, N-Acetyl-L-cysteine and N,N'-Diacetyl-L-cystine; and/or

(b) cysteine and/or cystine,

(ii) progressing the culture through a production phase wherein the recombinant protein is produced by the cells, wherein, during said production phase, the cell culture medium is supplemented with:

(a) cysteine/cystine analogs; and/or

(b) cysteine and/or cystine,

wherein (a) and (b) may be added simultaneously or sequentially,
wherein, when the contents of the basal medium and the total supplements added are added up, the molar ratio of (a) to (b) is between 1:10 and 10:1.

4. A method for producing a recombinant protein preparation comprising:

(i) inoculating said host cells in a basal medium, wherein the basal medium optionally comprises an initial amount of:

(a) cysteine/cystine analogs selected from the group consisting of N,N’-diacetyl-L-cystine-dimethylester, N-Acetyl-L-cysteine and N,N'-Diacetyl-L-cystine; and/or

(b) cysteine and/or cystine,

(ii) progressing the culture through a production phase wherein the recombinant protein is produced by the cells, wherein, during said production phase, the cell culture medium is supplemented with:

(a) cysteine/cystine analogs; and/or

(b) cysteine and/or cystine,

wherein (a) and (b) may be added simultaneously or sequentially,

wherein, when the contents of the basal medium and the total supplements added are added up, the molar amount of (a) in view of the total molar amount of (a) and (b) is from 10 to 90 percent, wherein in case a cysteine analog is used, the molar amount of (b) is calculated in view of cysteine equivalent; and

wherein in case a cystine analog is used, the molar amount of (b) is calculated in view of cystine equivalent.

5. The method according to any one of claims 1 to 4, wherein said molar ratio of (a) to (b) is between 1:8 and 8:1, such as between 1:6 and 6:1, e.g. between 1:4 and 4:1, such as between 1:3 and 3:1, e.g. between 1:2 and 2:1, such as between 1.5:1 and 1:1.5 or is 1:1.

6. The method according to any one of the preceding claims, wherein said cell culture medium comprises:

(a) cysteine/cystine analogs; and
(b) cysteine and/or cystine,

wherein, the molar amount of (a) is from 10 to 90 percent in view of the total molar amount of (a) and (b), wherein in case a cysteine analog is used, the molar amount of (b) is calculated in view of cysteine equivalents; and

wherein in case a cystine analog is used, the molar amount of (b) is calculated in view of cystine equivalents.

7. The method according to any one of the preceding claims, wherein the method comprises the steps of:

(i) inoculating said host cells in a basal medium, wherein the basal medium optionally comprises an initial amount of:

(a) cysteine/cystine analogs; and/or
(b) cysteine and/or cystine,

(ii) progressing the culture through a production phase wherein the recombinant protein is produced by the cells, wherein, during said production phase, the cell culture medium is supplemented with:

(a) cysteine/cystine analogs; and/or
(b) cysteine and/or cystine,

wherein (a) and (b) may be added simultaneously or sequentially,

wherein, when the contents of the basal medium and the total supplements added are added up, the molar amount of (a) in view of the total molar amount of (a) and (b) is from 10 to 90 percent, wherein in case a cysteine analog is used, the molar amount of (b) is calculated in view of cysteine equivalent; and

wherein in case a cystine analog is used, the molar amount of (b) is calculated in view of cystine equivalent.

8. The method according to any one of the preceding claims, wherein the concentration of (b) in said basal medium is equivalent to between 0.05 and 5 mmol/L of cysteine, such as between 0.1 and 1 mmol/L, e.g. between 0.2 and 0.6 mmol/L.

9. The method according to any one of the preceding claims, wherein, during the production phase, the medium is supplemented with (a) cysteine/cystine analogs and (b) cysteine and/or cystine, wherein the sum of (a) and (b) added to the culture over the entire production phase is equivalent to between 1 and 75 mmol/L of cysteine, such as between 2 and 20 mmol/L.

10. The method according to any one of the preceding claims, wherein, during the production phase, the medium is supplemented daily with (a) cysteine/cystine analogs and (b) cysteine and/or cystine, wherein the daily addition brings the concentration of (a) + (b) to a concentration equivalent to between 0.05 and 5 mmol/L of cysteine, such as between 0.1 and 1 mmol/L.

11. The method according to any one of the preceding claims, wherein the host cells are mammalian cells, preferably CHO cells.

12. The method according to any one of the preceding claims, wherein the recombinant protein is an antibody or an antigen-binding fragment thereof.

13. The method according to any one of the preceding claims, wherein the antibody or antigen-binding fragment thereof is:

i) 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 at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 7 and a heavy variable region having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 8;

d. 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

e. comprises a light variable region having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 7 and a heavy chain having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 11; or

ii) 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; or

iii) an antibody which comprises a light chain having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 9 and a heavy chain having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 10.

14. The method according to any one of the preceding claims, wherein the recombinant protein is a protein which is not colorless at a concentration of 10 mg/ml or more, such as 50 mg/ml or more, when produced by host cells grown in a cell culture medium not comprising cysteine/cystine analogs.

15. A recombinant protein preparation obtainable or obtained by the method according to any one of the preceding claims.