

CLAIMS

WHAT IS CLAIMED:

1. A method for selecting a cell culture medium with reduced impurities for use in cell culture to improve cell culture performance, the method comprising:

(a) providing a cell culture medium comprising a 4-hydroxyethyl piperazine ethanesulfonic acid (HEPES) buffer;

(b) analyzing the cell culture medium comprising the HEPES buffer to determine the amount of HEPES related impurities HEPES+[O₂]-[H₂] and HEPES-[CH₄] present in the cell culture medium;

(c) selecting the cell culture medium comprising the HEPES buffer for use in cell culture if the cell culture medium comprising the HEPES buffer is determined to have less than about 4000 μ mol of HEPES+[O₂]-[H₂] per mole of total HEPES, and less than about 400 μ mol of HEPES-[CH₄] per mole of total HEPES;

wherein the use of the cell culture medium comprising the HEPES buffer having less than about 4000 μ mol of HEPES+[O₂]-[H₂] per mole of total HEPES, and less than about 400 μ mol of HEPES-[CH₄] per mole of total HEPES improves cell culture performance, as compared to cell culture performance in non-HEPES related impurity reduced media.

2. The method of claim 1, wherein the improved cell culture performance comprises at least one selected from the group consisting of improved protein titer and cell growth.

3. A cell culture comprising (i) at least one recombinant eukaryotic cell that can express a recombinant protein and (ii) a cell culture medium with reduced impurities, wherein the cell culture is produced by a method comprising the steps of:

(a) providing a cell culture medium comprising a 4-hydroxyethyl piperazine ethanesulfonic acid (HEPES) buffer having reduced HEPES related impurities, wherein the cell culture medium has less than about 4000 μ mol of HEPES+[O₂]-[H₂] per mole of total HEPES,

and less than about 400 μmol of HEPES-[CH₄] per mole of total HEPES, wherein the cell culture medium is provided by analysis of cell culture medium to determine the amount of HEPES+[O₂]-[H₂] and HEPES-[CH₄] present in the cell culture medium, and selection of cell culture medium having less than about 4000 μmol of HEPES+[O₂]-[H₂] per mole of total HEPES, and less than about 400 μmol of HEPES-[CH₄] per mole of total HEPES;

(b) culturing said recombinant eukaryotic cells in said cell culture medium having reduced impurities; and

(c) expressing a recombinant protein of interest from said recombinant eukaryotic cells.

4. The cell culture of claim 3, wherein the eukaryotic cell is selected from the group consisting of mammalian cell, avian cell, insect cell, and yeast cell.

5. The cell culture of claim 4, wherein the eukaryotic cell is selected from the group consisting of CHO, COS, retinal cell, Vero, CV1, kidney, HeLa, HepG2, WI38, MRC 5, Colo25, HB 8065, HL-60, lymphocyte, A431, CV-1, U937, 3T3, L cell, C127 cell, SP2/0, NS-0, MMT cell, stem cell, tumor cell, and a cell line derived from an aforementioned cell.

6. The cell culture of claim 5, wherein the eukaryotic cell is a CHO cell.

7. The cell culture of claim 3, wherein said expressing a recombinant protein of interest occurs during production phase, growth phase, or both.

8. The cell culture of claim 3, wherein said culturing of the recombinant eukaryotic cells in said cell culture medium having reduced impurities occurs during production phase, growth phase, or both.

9. The cell culture of claim 3, wherein the cell growth during said culturing of the recombinant eukaryotic cells is higher than the cell growth of similar or identical recombinant eukaryotic cells in non-impurity reduced media.

10. The cell culture of claim 3, wherein the higher titer of the recombinant protein is increased as compared to that of similar or identical cells cultured in non-reduced impurity media.

11. The cell culture of claim 3, wherein the recombinant protein is an antibody, a human antibody, a humanized antibody, a chimeric antibody, a monoclonal antibody, a multispecific antibody, a bispecific antibody, an antigen binding antibody fragment, a single chain antibody, a diabody, triabody or tetrabody, a Fab fragment or a F(ab')₂ fragment, an IgD antibody, an IgE antibody, an IgM antibody, an IgG antibody, an IgG1 antibody, an IgG2 antibody, an IgG3 antibody, or an IgG4 antibody.

12. The cell culture of claim 3, wherein the recombinant protein comprises an Fc domain.

13. The cell culture of claim 12, wherein the recombinant protein is selected from the group consisting of an Fc-fusion protein, a receptor-Fc-fusion protein (TRAP), an antibody, an antibody fragment, and a ScFv-Fc fusion protein.

14. The cell culture of claim 13, wherein the recombinant protein is selected from the group consisting of an anti-PD1 antibody, an anti-PDL-1 antibody, an anti-Dll4 antibody, an anti-ANG2 antibody, an anti-AngPt13 antibody, an anti-PDGFR antibody, an anti-Erb3 antibody, an anti-PRLR antibody, an anti-TNF antibody, an anti-EGFR antibody, an anti-PCSK9 antibody, an anti-GDF8 antibody, an anti-GCGR antibody, an anti-VEGF antibody, an anti-IL1R antibody, an anti-IL4R antibody, an anti-IL6R antibody, an anti-IL1 antibody, an anti-IL2 antibody, an anti-IL3 antibody, an anti-IL4 antibody, an anti-IL5 antibody, an anti-IL6 antibody, an anti-IL7 antibody, an anti-RSV antibody, an anti-NGF antibody, an anti-CD3 antibody, an anti-CD20 antibody, an anti-CD19 antibody, an anti-CD28 antibody, an anti-CD48 antibody, an anti-CD3/anti-CD20 bispecific antibody, an anti-CD3/anti-MUC16 bispecific antibody, and an anti-CD3/anti-PSMA bispecific antibody.

15. The cell culture of claim 13, wherein the recombinant protein is selected from the group consisting of alirocumab, atoltivimab, maftivimab, odesivimab, odesivimab-ebgn,

casirivimab, imdevimab, cemiplitmab, cemplitmab-rwlc, dupilumab, evinacumab, evinacumab-dgnb, fasimumab, nesvacumab, trevogrumab, rinucumab and sarilumab.

16. The cell culture of claim 15, wherein the recombinant protein is dupilumab.
17. The cell culture according to claim 13, wherein the recombinant protein is aflibercept.
18. The method of claim 1, wherein the eukaryotic cell is selected from the group consisting of mammalian cell, avian cell, insect cell, and yeast cell.
19. The method of claim 1, wherein the eukaryotic cell is selected from the group consisting of CHO, COS, retinal cell, Vero, CV1, kidney, HeLa, HepG2, WI38, MRC 5, Colo25, HB 8065, HL-60, lymphocyte, A431, CV-1, U937, 3T3, L cell, C127 cell, SP2/0, NS-0, MMT cell, stem cell, tumor cell, and a cell line derived from an aforementioned cell.
20. The method of claim 1, wherein the eukaryotic cell is a CHO cell.
21. The method of claim 1, wherein the recombinant protein is an antibody, a human antibody, a humanized antibody, a chimeric antibody, a monoclonal antibody, a multispecific antibody, a bispecific antibody, an antigen binding antibody fragment, a single chain antibody, a diabody, triabody or tetrabody, a Fab fragment or a F(ab')₂ fragment, an IgD antibody, an IgE antibody, an IgM antibody, an IgG antibody, an IgG1 antibody, an IgG2 antibody, an IgG3 antibody, or an IgG4 antibody.
22. The method of claim 1, wherein the recombinant protein comprises an Fc domain.
23. The method of claim 1, wherein the recombinant protein is selected from the group consisting of an Fc-fusion protein, a receptor-Fc-fusion protein (TRAP), an antibody, an antibody fragment, and a ScFv-Fc fusion protein.
24. The method of claim 23, wherein the recombinant protein is selected from the group consisting of an anti-PD1 antibody, an anti-PDL-1 antibody, an anti-Dll4 antibody, an anti-ANG2 antibody, an anti-AngPt13 antibody, an anti-PDGFR antibody, an anti-Erb3 antibody,

an anti-PRLR antibody, an anti-TNF antibody, an anti-EGFR antibody, an anti-PCSK9 antibody, an anti-GDF8 antibody, an anti-GCGR antibody, an anti-VEGF antibody, an anti-IL1R antibody, an anti-IL4R antibody, an anti-IL6R antibody, an anti-IL1 antibody, an anti-IL2 antibody, an anti-IL3 antibody, an anti-IL4 antibody, an anti-IL5 antibody, an anti-IL6 antibody, an anti-IL7 antibody, an anti-RSV antibody, an anti-NGF antibody, an anti-CD3 antibody, an anti-CD20 antibody, an anti-CD19 antibody, an anti-CD28 antibody, an anti-CD48 antibody, an anti-CD3/anti-CD20 bispecific antibody, an anti-CD3/anti-MUC16 bispecific antibody, and an anti-CD3/anti-PSMA bispecific antibody.

25. The method of claim 23, wherein the recombinant protein is selected from the group consisting of alirocumab, atoltivimab, maftivimab, odesivimab, odesivivmab-ebgn, casirivimab, imdevimab, cempiplimab, cemiplimab-rwlc, dupilumab, evinacumab, evinacumab-dgnb, fasimumab, nesvacumab, trevogrumab, rinucumab and sarilumab.

26. The method of claim 25, wherein the recombinant protein is dupilumab.

27. The method of claim 23, wherein the recombinant protein is aflibercept.

28. The method of claim 1, wherein the analyzing is performed by at least one selected from the group consisting of HPLC and LC-MS.

29. The cell culture of claim 3, wherein the analysis is performed by at least one selected from the group consisting of HPLC and LC-MS.