

[0006a] In one aspect, provided herein is a method of improving thaw recovery of cell banks comprising freezing CHO cells for banking in a freezing medium, wherein the freezing medium comprises a buffered solution and a cryoprotective agent, and wherein the pH of the freezing medium has been adjusted to 7.5 to 8.5 prior to freezing.

[0007] Also provided herein is a method of freezing eukaryotic cells (e.g., mammalian cells, insect cells, etc.) for storage comprising freezing the cells in a freezing medium, wherein the freezing medium comprises a buffered solution and a cryoprotective agent, and wherein the freezing medium has a pH of about 6.7 to about 8.5 prior to freezing or has been adjusted to a pH of about 6.7 to about 8.5 prior to freezing.

[0007a] In one aspect, provided herein is a method of freezing CHO cells for storage comprising freezing the CHO cells in a freezing medium, wherein the freezing medium comprises a buffered solution and a cryoprotective agent, and wherein the pH of the freezing medium has been adjusted to 7.5 to 8.5 prior to freezing.

[0008] In some embodiments of the methods described above or herein, the freezing medium has a pH of about 6.7 to about 8.3, about 6.8 to about 8.3, about 6.9 to about 8.3, about 7.0 to about 8.3, about 7.1 to about 8.3, about 7.2 to about 8.3, about 7.3 to about 8.3, about 7.4 to about 8.3, about 7.5 to about 8.3, about 7.2 to about 8.0, about 7.2 to about 7.8, or about 7.5 prior to freezing.

[0009] In some embodiments of the methods described above or herein, the pH of the freezing medium has been adjusted to a pH of about 6.7 to about 8.5, about 6.7 to about 8.3, about 6.8 to about 8.3, about 6.9 to about 8.3, about 7.0 to about 8.3, about 7.1 to about 8.3, about 7.2 to about 8.3, about 7.3 to about 8.3, about 7.4 to about 8.3, about 7.5 to about 8.3, about 7.2 to about 8.0, about 7.2 to about 7.8, or about 7.5. In some embodiments of the methods described above or herein, the cells (e.g., mammalian cells or insect cells) are combined with a freezing medium before and/or after pH adjustment. In some embodiments, the adjusted pH is a target pH or a measured pH. In some embodiments, the target pH is about 6.7 to about 8.5, or any of the pH or in any pH ranges described herein. In some embodiments, the measured pH is about 6.7 to about 8.5, or any of the pH or in any pH ranges described herein. In some embodiments of the methods described above or herein, the method further comprises a step of measuring an initial pH of the freezing medium containing the cells (e.g., mammalian cells or insect cells) prior to adjusting pH of the freezing medium. In some embodiments of the methods described above or herein, the method further comprises a step of measuring the adjusted pH of the freezing medium. In some embodiments of the methods described above or herein, if the measured pH of the freezing medium is below a target pH, the method comprises repeating the adjusting step and measuring step until the adjusted pH of the freezing medium is about 6.7 to about 8.5, or any of the pH or in any pH ranges described herein.

[0010] In some embodiments of the methods described herein or above, the pH is adjusted by adding a base. In some embodiments, the base is selected from the group consisting of sodium carbonate, sodium bicarbonate, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) sodium salt, sodium hydroxide, and potassium hydroxide. In some embodiments, the pH of the

[0015a] In one aspect, provided herein is a method of freezing CHO cells for storage comprising (a) adjusting the pH of a freezing medium containing CHO cells to a target pH of 7.5 to 8.5; and (b) freezing the CHO cells.

[0016] In some embodiments, the pH is adjusted to a pH of about 6.7 to about 8.3, about 6.8 to about 8.3, about 6.9 to about 8.3, about 7.0 to about 8.3, about 7.1 to about 8.3, about 7.2 to about 8.3, about 7.3 to about 8.3, about 7.4 to about 8.3, about 7.5 to about 8.3, about 7.2 to about 8.0, about 7.2 to about 7.8, or about 7.5. In some embodiments, the adjusted pH is a target pH or a measured pH. In some embodiments, the target pH is about 6.7 to about 8.5, or any of the pH or in any pH ranges described herein. In some embodiments, the target pH is about 7.5. In some embodiments, the measured pH is about 6.7 to about 8.5, or any of the pH or in any pH ranges described herein.

[0017] In some embodiments of the methods described above or herein, the method further comprises a step of measuring an initial pH of the freezing medium containing the cells (e.g., mammalian cells or insect cells) prior to adjusting pH of the freezing medium. In some embodiments, the method further comprises a step of measuring the adjusted pH of the freezing medium. In some embodiments, if the measured pH of the freezing medium is below a target pH, the method comprises repeating the adjusting step and measuring step until the adjusted pH of the freezing medium is about 6.7 to about 8.5, about 7.2 to about 8.3, or any of the pH or in any pH ranges described herein.

[0018] In some embodiments of the methods described above or herein, the pH is adjusted by adding a base. In some embodiments, the base is selected from the group consisting of sodium carbonate, sodium bicarbonate, HEPES sodium salt, sodium hydroxide, and potassium hydroxide. In some embodiments, the pH of the freezing medium is adjusted by adding a base to the freezing medium according to the following formula $V_{\text{base}} = C_{\text{base}} * V_p (pH_t - pH_i)$, wherein C_{base} is a base-specific coefficient, V_{base} is a volume of the base to add to the freezing medium, V_p is the volume of the freezing medium, pH_t is the target pH, and pH_i is the initial pH. In some embodiments, the pH of the freezing medium is adjusted by adding sodium carbonate to the freezing medium according to the following formula $V_{\text{Na}_2\text{CO}_3} = 0.0085V_p (pH_t - pH_i)$, wherein $V_{\text{Na}_2\text{CO}_3}$ is a volume of 1M sodium carbonate to add to the freezing medium, V_p is the volume of the freezing medium, pH_t is the target pH, and pH_i is the initial pH. In some embodiments, the target pH is about 6.7 to about 8.5, or any of the pH or in any pH ranges described herein. In some embodiments, the target pH is about 7.5.

[0019] In some embodiments, the cells (e.g., mammalian cells or insect cells) are in a medium having a pH of about 6.2 to about 6.6 before the cells are combined with a freezing medium.

[0020] In some embodiments, the cryoprotective agent in the freezing medium is DMSO, glycerol, propanediol, ethylene glycol, a macromolecule, a sugar, or a combination thereof. In some embodiments, the DMSO or glycerol in the freezing medium containing the cells (e.g., mammalian cells or insect cells) prior to freezing is at a concentration of about 5% to about 12.5% by volume. In some embodiments, the DMSO or glycerol in the freezing medium containing the cells (e.g., mammalian cells or insect cells) prior to freezing the cells is at a concentration of about 5% to about 10% by volume.

[0021] In some embodiments, the freezing medium containing the cells (e.g., mammalian cells or insect cells) has a cell density of about 8% to about 28% packed cell volume (PCV) prior to freezing.

[0022] In some embodiments, the method further comprises a step of cooling cell culture fluid during cell harvest and concentration process before the cells (e.g., mammalian cells or insect cells) are combined with a freezing medium. In some embodiments, the cell culture fluid is cooled to a temperature at or below about 20°C, or at or below about 10°C.

[0023] Provided herein is a method of freezing eukaryotic cells (e.g., mammalian cells, insect cells, etc.) for storage or improving thaw recovery of cell banks comprising (a) adjusting pH of a freezing medium to a pH of about 6.7 to about 8.5, wherein the freezing medium comprises a buffered solution and a cryoprotective agent; (b) combining the cells with the freezing medium to form a cell pool; and (c) freezing the cells in the cell pool.

[0023a] In one aspect, provided herein is a method of freezing CHO cells for storage comprising (a) adjusting the pH of a freezing medium to a pH of 7.5 to 8.5, wherein the freezing medium comprises a buffered solution and a cryoprotective agent; (b) combining the CHO cells with the freezing medium to form a cell pool; and (c) freezing the CHO cells in the cell pool.

[0024] In some embodiments, the pH is adjusted to a pH of about 6.7 to about 8.3, about 6.8 to about 8.3, about 6.9 to about 8.3, about 7.0 to about 8.3, about 7.1 to about 8.3, about 7.2 to about 8.3, about 7.3 to about 8.3, about 7.4 to about 8.3, about 7.5 to about 8.3, about 7.2 to about 8.0, about 7.2 to about 7.8, or about 7.5. In some embodiments, the adjusted pH is a target pH or a measured pH. In some embodiments, the target pH is about 6.7 to about 8.5, or any of the pH or in any pH ranges described herein. In some embodiments, the measured pH is about 6.7 to about 8.5, or any of the pH or in any pH ranges described herein.

[0025] In some embodiments, the method further comprises a step of measuring the adjusted pH of the freezing medium. In some embodiments, if the measured pH of the freezing medium is below a target pH, the method further comprises repeating the adjusting step and measuring step until the adjusted pH of the freezing medium is about 6.7 to about 8.5, or any of the pH or in any pH ranges described herein.

pH ranges described herein) prior to freezing the cells.

[0032a] In one aspect, provided herein is a CHO cell pool for freezing CHO cells comprising a buffered solution, a cryoprotective agent, and CHO cells comprising a nucleic acid encoding a polypeptide, wherein the medium has a pH of 7.5 to 8.5 prior to freezing the cells.

[0032b] In some embodiments, the cells are mammalian cells, such as Chinese hamster ovary (CHO) cells, NS0 murine myeloma cells, PER.C6® human cells, or hybridomas. In some embodiments, the cells are insect cells, such as High Five™, S2 (Schneider 2), Sf9, and Sf21. In some embodiments, the cells (e.g., mammalian cells or insect cells) comprise a nucleic acid encoding a polypeptide. In some embodiments, the polypeptide is a therapeutic protein. In some embodiments, the therapeutic protein is selected from the group consisting of an antibody, an antibody fragment, an enzyme, and a receptor fusion protein.

[0033] Provided herein is a cell bank comprising a plurality of containers and each container contains (a) a freezing medium comprising a buffer and a cryoprotective agent, and (b) eukaryotic cells (e.g., mammalian cells or insect cells) comprising a nucleic acid encoding a polypeptide, wherein the freezing medium has a pH of about 6.7 to about 8.5 or about 7.2 to about 8.3 (or any of the pH or in any pH ranges described herein) prior to freezing the cell. In some embodiments, the containers are ampoules.

[0033a] In one aspect, provided herein is a cell bank comprising a plurality of containers and each container contains (a) a freezing medium comprising a buffered solution and a cryoprotective agent, and (b) CHO cells comprising a nucleic acid encoding a polypeptide, wherein the freezing medium has a pH of 7.5 to 8.5 prior to freezing the cell.

[0033b] In some embodiments, the cells are mammalian cells, such as Chinese hamster ovary (CHO) cells, NS0 murine myeloma cells, PER.C6® human cells, or hybridomas. In some embodiments, the cells are insect cells, such as High Five™, S2 (Schneider 2), Sf9, and Sf21. In some embodiments, the cells (e.g., mammalian cells or insect cells) comprise a nucleic acid encoding a polypeptide. In some embodiments, the polypeptide is a therapeutic protein. In some embodiments, the therapeutic protein is selected from the group consisting of an antibody, an antibody fragment, an enzyme, and a receptor fusion protein.

[0034] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present invention. These and other aspects of the invention will become apparent to one of skill in the art.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] FIG. 1 shows an example of a cell banking process flow.

[0036] FIGs. 2A-2B show thaw passage day 1 viability (FIG. 2A) and day 4 growth (PCV) (FIG. 2B) results for cell banks generated from pH adjusted pools at initial pH of 6.6 or 6.3.

[0037] FIGs. 3A-3F show thaw passage day 1 viability (FIGs. 3A, 3C, and 3E) and overall growth rate by PCV (FIGs. 3B, 3D, and 3F) vs. banking pH and the effect of adjusting pH to a

CLAIMS

What is claimed is:

1. A method of improving thaw recovery of cell banks comprising freezing CHO cells for banking in a freezing medium, wherein the freezing medium comprises a buffered solution and a cryoprotective agent, and wherein the pH of the freezing medium has been adjusted to 7.5 to 8.5 prior to freezing.
2. A method of freezing CHO cells for storage comprising freezing the CHO cells in a freezing medium, wherein the freezing medium comprises a buffered solution and a cryoprotective agent, and wherein the pH of the freezing medium has been adjusted to 7.5 to 8.5 prior to freezing.
3. The method of claim 1 or 2, wherein the pH of the freezing medium has been adjusted to a pH of 7.5.
4. The method of any one of claims 1-3, wherein the cells are combined with the freezing medium before and/or after pH adjustment.
5. The method of any one of claims 1-4, wherein the adjusted pH is a target pH or a measured pH.
6. The method of claim 5, wherein the target pH is 7.5 to 8.3.
7. The method of claim 5, wherein the measured pH is 7.5 to 8.3.
8. The method of any one of claims 1-7, further comprising a step of measuring an initial pH of the freezing medium containing the cells prior to adjusting pH of the freezing medium.
9. The method of any one of claims 1-8, further comprising a step of measuring the adjusted pH of the freezing medium.
10. The method of claim 9, wherein if the measured pH of the freezing medium is below a target pH, repeating the adjusting step and measuring step until the adjusted pH of the freezing medium is 7.5 to 8.3.

11. The method of any one of claims 1-10, wherein the pH is adjusted by adding a base.
12. The method of claim 11, wherein the base is selected from the group consisting of sodium carbonate, sodium bicarbonate, HEPES sodium salt, sodium hydroxide, and potassium hydroxide.
13. The method of claim 11 or 12, wherein the pH of the freezing medium is adjusted by adding a base to the freezing medium according to the following formula $V_{\text{base}} = C_{\text{base}} * V_p (pH_t - pH_i)$, wherein C_{base} is a base-specific coefficient, V_{base} is a volume of the base to add to the freezing medium, V_p is the volume of the freezing medium, pH_t is the target pH, and pH_i is the initial pH.
14. The method of claim 13, wherein the pH of the freezing medium is adjusted by adding sodium carbonate to the freezing medium according to the following formula $V_{\text{Na}_2\text{CO}_3} = 0.0085V_p (pH_t - pH_i)$, wherein $V_{\text{Na}_2\text{CO}_3}$ is a volume of 1M sodium carbonate to add to the freezing medium, V_p is the volume of the freezing medium, pH_t is the target pH, and pH_i is the initial pH.
15. The method of claim 13 or 14, wherein the target pH is 7.5.
16. The method of any one of claims 1-15, wherein the CHO cells are in a medium having a pH of 6.2 to 6.6 before the CHO cells are combined with a freezing medium.
17. The method of any one of claims 1-16, wherein the cryoprotective agent is DMSO, glycerol, propanediol, ethylene glycol, or a sugar.
18. The method of claim 17, wherein cryoprotective agent is DMSO or glycerol, and the DMSO or glycerol in the freezing medium prior to freezing is at a concentration of 5% to 12.5% by volume.
19. The method of claim 18, wherein the DMSO or glycerol in the freezing medium prior to freezing the cells is at a concentration of 5% to 10% by volume.
20. The method of any one of claims 1-19, wherein the freezing medium containing the CHO cells has a cell density of 8% to 28% packed cell volume (PCV) prior to freezing.
21. The method of any of claims 1-20 further comprising a step of cooling cell culture fluid during cell harvest and concentration process before the CHO cells are combined with the freezing medium.

22. The method of claim 21, wherein the cell culture fluid is cooled to a temperature at or below about 20°C.
23. The method of claim 21, wherein the cell culture fluid is cooled to a temperature at or below about 10°C.
24. A method of freezing CHO cells for storage comprising (a) adjusting the pH of a freezing medium containing CHO cells to a target pH of 7.5 to 8.5; and (b) freezing the CHO cells.
25. The method of claim 24, wherein the pH is adjusted to a pH of 7.5 to 8.3.
26. The method of claim 25, wherein the pH is adjusted to a pH of 7.5.
27. The method of any one of claims 24-26, wherein the adjusted pH is a target pH or a measured pH.
28. The method of claim 27, wherein the target pH is 7.5 to 8.3.
29. The method of claim 27, wherein the measured pH is 7.5 to 8.3.
30. The method of any one of claims 24-29, further comprising a step of measuring an initial pH of the freezing medium containing the cells prior to adjusting pH of the freezing medium.
31. The method of any one of claims 24-30, further comprising a step of measuring the adjusted pH of the freezing medium.
32. The method of claim 31, wherein if the measured pH of the freezing medium is below a target pH, repeating the adjusting step and measuring step until the adjusted pH of the freezing medium is 7.5 to 8.5.
33. The method of any one of claims 24-32, wherein the pH is adjusted by adding a base.
34. The method of claim 33, wherein the base is selected from the group consisting of sodium carbonate, sodium bicarbonate, HEPES sodium salt, sodium hydroxide, and potassium hydroxide.
35. The method of claim 33 or 34, wherein the pH of the freezing medium is adjusted by adding a base to the freezing medium according to the following formula $V_{\text{base}} = \frac{C_{\text{base}} * V_p (pH_t - pH_i)}{pH_t - pH_i}$, wherein C_{base} is a base-specific coefficient, V_{base} is a volume of the base to add to the freezing medium, V_p is the volume of the freezing medium, pH_t is the target pH, and pH_i is the initial pH.

36. The method of claim 33 or 34, wherein the pH of the freezing medium is adjusted by adding sodium carbonate to the freezing medium according to the following formula $V_{\text{Na}_2\text{CO}_3} = 0.0085V_p (\text{pH}_t - \text{pH}_i)$, wherein $V_{\text{Na}_2\text{CO}_3}$ is a volume of 1M sodium carbonate to add to the freezing medium, V_p is the volume of the freezing medium, pH_t is the target pH, and pH_i is the initial pH.
37. The method of claim 35 or 36, wherein the target pH is 7.5 to 8.3.
38. The method of any one of claims 24-37, wherein the CHO cells are in a medium having a pH of 6.2 to 6.6 before the CHO cells are combined with a freezing medium.
39. The method of any one of claims 24-37, wherein the cryoprotective agent is DMSO, glycerol, propanediol, ethylene glycol, or a sugar.
40. The method of claim 39, wherein the cryoprotective agent is DMSO or glycerol and the DMSO or glycerol in the freezing medium containing the CHO cells prior to freezing is at a concentration of 5% to 12.5% by volume.
41. The method of claim 40, wherein the DMSO or glycerol in the freezing medium containing the CHO cells prior to freezing the cells is at a concentration of 5% to 10% by volume.
42. The method of any one of claims 24-41, wherein the freezing medium containing the CHO cells has a cell density of 8% to 28% packed cell volume (PCV) prior to freezing.
43. The method of any of claims 24-42 further comprising a step of cooling cell culture fluid during cell harvest and concentration process before the CHO cells are combined with the freezing medium.
44. The method of claim 43, wherein the cell culture fluid is cooled to a temperature at or below about 20°C.
45. The method of claim 43, wherein the cell culture fluid is cooled to a temperature at or below about 10°C.
46. A method of freezing CHO cells for storage comprising (a) adjusting the pH of a freezing medium to a pH of 7.5 to 8.5, wherein the freezing medium comprises a buffered solution and a cryoprotective agent; (b) combining the CHO cells with the freezing medium to form a cell pool; and (c) freezing the CHO cells in the cell pool.

47. The method of claim 46, wherein the pH is adjusted to a pH of 7.5.
48. The method of claim 46 or 47, wherein the adjusted pH is a target pH or a measured pH.
49. The method of claim 48, wherein the target pH is 7.5 to 8.3.
50. The method of claim 48, wherein the measured pH is 7.5 to 8.3.
51. The method of any one of claims 46-50, further comprising a step of measuring the adjusted pH of the freezing medium.
52. The method of any one of claims 48-50, wherein if the measured pH of the freezing medium is below a target pH, repeating the adjusting step and measuring step until the adjusted pH of the freezing medium is 7.5 to 8.5.
53. The method of any one of claims 46-52, wherein the pH is adjusted by adding a base.
54. The method of claim 53, wherein the base is selected from the group consisting of sodium carbonate, sodium bicarbonate, HEPES sodium salt, sodium hydroxide, and potassium hydroxide.
55. The method of any one of claims 46-54, wherein the CHO cells are in a medium having a pH of 6.2 to 6.6 before the CHO cells are combined with the freezing medium.
56. The method of any one of claims 46-55, wherein the cryoprotective agent is DMSO, glycerol, propanediol, ethylene glycol, or a sugar.
57. The method of claim 56, wherein the cryoprotective agent is DMSO or glycerol and the DMSO or glycerol in the cell pool is at a concentration of 5% to 12.5% by volume.
58. The method of claim 57, wherein the DMSO or glycerol in the cell pool is at a concentration of 5% to 10% by volume.
59. The method of any one of claims 46-58, wherein the cell pool contains the CHO cells at a cell density of 8% to 28% packed cell volume (PCV).
60. The method of any of claims 46-59 further comprising a step of cooling cell culture fluid during cell harvest and concentration process before the CHO cells are combined with the freezing medium.

61. The method of claim 60, wherein the cell culture fluid is cooled to a temperature at or below about 20°C.
62. The method of claim 60, wherein the cell culture fluid is cooled to a temperature at or below about 10°C.
63. The method of any one of claims 1-62, wherein the CHO cells comprise a nucleic acid encoding a polypeptide.
64. The method of claim 63, wherein the polypeptide is a therapeutic protein.
65. The method of claim 64, wherein the therapeutic protein is selected from the group consisting of an antibody, an antibody fragment, an enzyme, and a receptor fusion protein.
66. A CHO cell pool for freezing CHO cells comprising a buffered solution, a cryoprotective agent, and CHO cells comprising a nucleic acid encoding a polypeptide, wherein the medium has a pH of 7.5 to 8.5 prior to freezing the cells.
67. The cell pool of claim 66, wherein the CHO cells comprise a nucleic acid encoding a polypeptide.
68. The cell pool of claim 67, wherein the polypeptide is a therapeutic protein.
69. The cell pool of claim 68, wherein the therapeutic protein is selected from the group consisting of an antibody, an antibody fragment, an enzyme, and a receptor fusion protein.
70. A cell bank comprising a plurality of containers and each container contains (a) a freezing medium comprising a buffered solution and a cryoprotective agent, and (b) CHO cells comprising a nucleic acid encoding a polypeptide, wherein the freezing medium has a pH of 7.5 to 8.5 prior to freezing the cell.
71. The cell bank of claim 70, wherein the containers are ampoules.

72. The cell bank of claim 70 or 71, wherein the CHO cells comprise a nucleic acid encoding a polypeptide.
73. The cell bank of claim 72, wherein the polypeptide is a therapeutic protein.
74. The cell bank of claim 73, wherein the therapeutic protein is selected from the group consisting of an antibody, an antibody fragment, an enzyme, and a receptor fusion protein.