

[0006a] In one aspect, provided herein is a method of improving thaw recovery of cell banks comprising CHO cells comprising cooling the CHO cells to a temperature of at or below 10°C and freezing the 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 cooling the CHO cells to a temperature of at or below 10°C and 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 examples 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 examples 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 examples 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 examples, the adjusted pH is a target pH or a measured pH. In some examples, 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 examples, 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 examples 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 examples of the methods described above or herein, the method further comprises a step of measuring the adjusted pH of the freezing medium. In some examples 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 examples of the methods described herein or above, the pH is adjusted by adding a base. In some examples, 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 examples, 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 examples, the pH of the freezing medium is adjusted by adding sodium carbonate to the freezing medium according to the following formula $V_{\text{Na}_2\text{CO}_3} = 0.0085 V_p (pH_t - pH_i)$, 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 examples, the target pH is a pH between 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. In some examples, the target pH is 7.5.

[0011] In some examples of the methods described above or herein, 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.

[0012] In some examples of the methods described above or herein, the cryoprotective agent is DMSO (dimethyl sulfoxide), glycerol, propanediol, ethylene glycol, a macromolecule, a sugar, or a combination thereof. In some examples, the DMSO or glycerol in the freezing medium prior to freezing is at a concentration of about 5% to about 12.5% by volume. In some examples, the DMSO or glycerol in the freezing medium prior to freezing the cells (e.g., mammalian cells or insect cells) is at a concentration of about 5% to about 10% by volume.

[0013] In some examples of the methods described above or herein, 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.

[0014] In some examples of the method described above or herein, 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 examples, the cell culture fluid is cooled to a temperature at or below about 20°C. In some examples, the cell culture fluid is cooled to a temperature at or below about 10°C.

[0015] Provided here 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 containing cells to a pH of about 6.7 to about 8.5, wherein the freezing medium comprises a buffered solution and a cryoprotective agent; and (b) freezing the cells.

[0015a] In one aspect, provided herein is a method of freezing CHO cells for storage comprising (a) cooling the CHO cells to a temperature of at or below 10°C (b) adjusting the pH of a freezing medium comprising a cryoprotective agent and a buffered solution and containing the CHO cells to a target pH of 7.5 to 8.5; and (c) freezing the CHO cells.

[0016] In some examples, 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 examples, the adjusted pH is a target pH or a measured pH. In some examples, 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 examples, the target pH is about 7.5. In some examples, 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 examples 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 examples, the method further comprises a step of measuring the adjusted pH of the freezing medium. In some examples, 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 examples of the methods described above or herein, the pH is adjusted by adding a base. In some examples, the base is selected from the group consisting of sodium carbonate, sodium bicarbonate, HEPES sodium salt, sodium hydroxide, and potassium hydroxide. In some examples, 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 examples, 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 examples, 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 examples, the target pH is about 7.5.

[0019] In some examples, 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 examples, the cryoprotective agent in the freezing medium is DMSO, glycerol, propanediol, ethylene glycol, a macromolecule, a sugar, or a combination thereof. In some examples, 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 examples, 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 examples, 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 examples, 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 examples, 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, wherein the CHO cells have been cooled to a temperature of at or below 10°C; and (c) freezing the CHO cells in the cell pool.

[0024] In some examples, 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 examples, the adjusted pH is a target pH or a measured pH. In some examples, 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 examples, 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 examples, the method further comprises a step of measuring the adjusted pH of the freezing medium. In some examples, 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.

[0026] In some examples of the methods described above or herein, the pH is adjusted by adding a base. In some examples, the base is selected from the group consisting of sodium carbonate, sodium bicarbonate, HEPES sodium salt, sodium hydroxide, and potassium hydroxide.

[0027] In some examples of the methods described above or herein, 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.

[0028] In some example of the methods described above or herein, the cryoprotective agent in the freezing medium is DMSO, glycerol, propanediol, ethylene glycol, a macromolecule, a sugar, or a combination thereof. In some example, the DMSO or glycerol in the cell pool is at a concentration of about 5% to about 12.5% by volume, or about 5% to about 10% by volume.

[0029] In some examples of the methods described above or herein, the cell pool contains the cells (e.g., mammalian cells or insect cells) at a cell density of about 8% to about 28% packed cell volume (PCV).

[0030] In some examples of the methods described above or herein, 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 examples, the cell culture fluid is cooled to a temperature at or below about 20°C. In some examples, the cell culture fluid is cooled to a temperature at or below about 10°C.

[0031] In some examples of the methods described above or herein, the cells are mammalian cells, such as Chinese hamster ovary (CHO) cells, NS0 murine myeloma cells, PER.C6® human cells, or hybridomas. In some examples, the cells are insect cells, such as High Five™, S2 (Schneider 2), Sf9, and Sf21. In some examples of the methods described above or herein, the cells (e.g., mammalian cells or insect cells) comprise a nucleic acid encoding a polypeptide. In some examples, the polypeptide is a therapeutic protein. In some examples, the therapeutic protein is selected from the group consisting of an antibody, an antibody fragment, an enzyme, and a receptor fusion protein.

[0032] Provided herein is an eukaryotic cell pool (e.g., a mammalian cell pool, or an insect cell pool) for freezing cells comprising a buffered solution, a cryoprotective agent, and eukaryotic cells comprising a nucleic acid encoding a polypeptide, wherein the 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 cells.

[0032a] In one aspect, provided herein is a CHO cell pool for freezing the CHO cells comprising a buffered solution, a cryoprotective agent, and the CHO cells comprising a nucleic acid encoding a polypeptide, wherein the medium has a pH of 7.5 to 8.5 and temperature at or below about 10°C prior to freezing the cells.

[0032b] In some examples, the cells are mammalian cells, such as Chinese hamster ovary (CHO) cells, NS0 murine myeloma cells, PER.C6® human cells, or hybridomas. In some examples, the cells are insect cells, such as High Five™, S2 (Schneider 2), Sf9, and Sf21. In some examples, the cells (e.g., mammalian cells or insect cells) comprise a nucleic acid encoding a polypeptide. In some examples, the polypeptide is a therapeutic protein. In some examples, 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 examples, 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 and temperature at or below about 10°C prior to freezing the cell.

[0033b] In some examples, the cells are mammalian cells, such as Chinese hamster ovary (CHO) cells, NS0 murine myeloma cells, PER.C6® human cells, or hybridomas. In some examples, the cells are insect cells, such as High Five™, S2 (Schneider 2), Sf9, and Sf21. In some examples, the cells (e.g., mammalian cells or insect cells) comprise a nucleic acid encoding a polypeptide. In some examples, the polypeptide is a therapeutic protein. In some examples, 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 examples described herein may be combined to form other examples 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 CHO cells comprising cooling the CHO cells to a temperature of at or below 10°C and freezing the 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 cooling the CHO cells to a temperature of at or below 10°C and 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 claim 1 or claim 2, wherein the CHO cells are combined with the freezing medium before pH adjustment.
5. The method of any one of claims 1, 2, or 4, wherein the CHO cells are combined with the freezing medium after pH adjustment.
6. The method of any one of claims 1, 2, 4, or 5, wherein the adjusted pH is a target pH or a measured pH.
7. The method of claim 6, wherein the target pH is 7.5 to 8.3.
8. The method of claim 6, wherein the measured pH is 7.5 to 8.3.
9. The method of any one of claims 1, 2, or 5-8, further comprising a step of measuring an initial pH of the freezing medium containing the CHO cells prior to adjusting pH of the freezing medium.
10. The method of any one of claims 1, 2, or 5-9, further comprising a step of measuring the adjusted pH of the freezing medium.
11. The method of claim 10, 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.

12. The method of any one of claims 1, 2, or 4-11, wherein the pH is adjusted by adding a base.
13. The method of claim 12, wherein the base is selected from the group consisting of sodium carbonate, sodium bicarbonate, HEPES sodium salt, sodium hydroxide, and potassium hydroxide.
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 $V_{\text{Na}_2\text{CO}_3} = 0.0085V_p (\text{pH}_t - \text{pH}_i)$, 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.
15. The method of claim 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 CHO 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. A method of freezing CHO cells for storage comprising (a) cooling the CHO cells to a temperature of at or below 10°C (b) adjusting the pH of a freezing medium comprising a cryoprotective agent and a buffered solution and containing the CHO cells to a target pH of 7.5 to 8.5; and (c) freezing the CHO cells.
22. The method of claim 21 wherein the pH is adjusted to a pH of 7.5 to 8.3.
23. The method of claim 21, wherein the pH is adjusted to a pH of 7.5.
24. The method of claim 21 or claim 22, wherein the adjusted pH is a target pH or a measured pH.
25. The method of claim 24, wherein the target pH is 7.5 to 8.3.
26. The method of claim 24, wherein the measured pH is 7.5 to 8.3.

27. The method of any one of claims 21, 22, or 24-26, further comprising a step of measuring an initial pH of the freezing medium containing the CHO cells prior to adjusting pH of the freezing medium.
28. The method of any one of claims 21, 22, or 24-27, further comprising a step of measuring the adjusted pH of the freezing medium.
29. The method of claim 28, 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.
30. The method of any one of claims 21, 22, or 24-29, wherein the pH is adjusted by adding a base.
31. The method of claim 30, wherein the base is selected from the group consisting of sodium carbonate, sodium bicarbonate, HEPES sodium salt, sodium hydroxide, and potassium hydroxide.
32. The method of claim 30 or 31, 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.
33. The method of claim 32, wherein the target pH is 7.5 to 8.3.
34. The method of any one of claims 21-33, 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.
35. The method of any one of claims 21-34, wherein the cryoprotective agent is DMSO, glycerol, propanediol, ethylene glycol, or a sugar.
36. The method of claim 35, 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.
37. The method of claim 35, wherein the DMSO or glycerol in the freezing medium containing the CHO cells prior to freezing the CHO cells is at a concentration of 5% to 10% by volume.
38. The method of any one of claims 21-37, wherein the freezing medium containing the CHO cells has a cell density of 8% to 28% packed cell volume (PCV) prior to freezing.
39. 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, wherein the CHO cells have been cooled to a temperature of at or below 10°C; and (c) freezing the CHO cells in the cell pool.

40. The method of claim 39, wherein the pH is adjusted to a pH of 7.5.
41. The method of claim 39, wherein the adjusted pH is a target pH or a measured pH.
42. The method of claim 41, wherein the target pH is 7.5 to 8.3.
43. The method of claim 41, wherein the measured pH is 7.5 to 8.3.
44. The method of any one of claims 39 or 41-43, further comprising a step of measuring the adjusted pH of the freezing medium.
45. The method of claim 44, 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.
46. The method of any one of claims 39 or 41-45, wherein the pH is adjusted by adding a base.
47. The method of claim 46, wherein the base is selected from the group consisting of sodium carbonate, sodium bicarbonate, HEPES sodium salt, sodium hydroxide, and potassium hydroxide.
48. The method of any one of claims 39-47, 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.
49. The method of any one of claims 39-48, wherein the cryoprotective agent is DMSO, glycerol, propanediol, ethylene glycol, or a sugar.
50. The method of claim 49, 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.
51. The method of claim 50, wherein the DMSO or glycerol in the cell pool is at a concentration of 5% to 10% by volume.
52. The method of any one of claims 39-51, wherein the cell pool contains the CHO cells at a cell density of 8% to 28% packed cell volume (PCV).
53. The method of any one of claims 1-52, wherein the CHO cells comprise a nucleic acid encoding a polypeptide.
54. The method of claim 53, wherein the polypeptide is a therapeutic protein.
55. The method of claim 54, wherein the therapeutic protein is selected from the group consisting of an antibody, an antibody fragment, an enzyme, and a receptor fusion protein.

56. A CHO cell pool for freezing the CHO cells comprising a buffered solution, a cryoprotective agent, and the CHO cells comprising a nucleic acid encoding a polypeptide, wherein the medium has a pH of 7.5 to 8.5 and temperature at or below about 10°C prior to freezing the cells.
57. The cell pool of claim 56, wherein the CHO cells comprise a nucleic acid encoding a polypeptide.
58. The cell pool of claim 57, wherein the polypeptide is a therapeutic protein.
59. The cell pool of claim 58, wherein the therapeutic protein is selected from the group consisting of an antibody, an antibody fragment, an enzyme, and a receptor fusion protein.
60. 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 and temperature at or below about 10°C prior to freezing the cell.
61. The cell bank of claim 60, wherein the containers are ampoules.
62. The cell bank of claim 60 or 61, wherein the CHO cells comprise a nucleic acid encoding a polypeptide.
63. The cell bank of claim 62, wherein the polypeptide is a therapeutic protein.
64. The cell bank of claim 63, wherein the therapeutic protein is selected from the group consisting of an antibody, an antibody fragment, an enzyme, and a receptor fusion protein.