

WHAT IS CLAIMED IS:

1. A method of culturing a mammalian cell, the method comprising:

providing a multi-well plate comprising at least one well comprising a mammalian cell

5 disposed in a first liquid culture medium, wherein the first liquid culture medium occupies 5% to 70% of the volume of the well;

incubating the multi-well plate for a period of time at 31°C to 40 °C and with a rotary agitation of 320 revolutions per minute (RPM) to 500 RPM; and

10 continuously or periodically, during the period of time, removing a first volume of the first liquid culture medium and adding to the first liquid culture medium a second volume of a second liquid culture medium, wherein the first and second volumes are equal.

2. The method of claim 1, wherein the mammalian cell is a Chinese hamster ovary (CHO) cell.

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3. The method of claim 2, wherein the CHO cell contains a nucleic acid encoding a recombinant protein.

4. The method of claim 3, wherein the recombinant protein is an immunoglobulin, an enzyme, a growth factor, a protein fragment, or an engineered protein.

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5. A method of producing a recombinant protein, the method comprising:

providing a multi-well plate comprising at least one well comprising a mammalian cell disposed in a first liquid culture medium, wherein the first liquid culture medium occupies 5% to 70% of the volume of the well and the mammalian cell contains a nucleic acid that encodes a recombinant protein;

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incubating the multi-well plate for a period of time at 31 °C to 40 °C and with a rotary agitation of 320 revolutions per minute (RPM) to 500 RPM;

continuously or periodically, during the period of time, removing a first volume of the first liquid culture medium and adding to the first liquid culture medium a second volume of a second liquid culture medium, wherein the first and second volumes are equal; and

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recovering the recombinant protein from the mammalian cell or from the first or second culture medium.

5 6. The method of claim 5, wherein the recombinant protein is recovered from the mammalian cell.

7. The method of claim 6, wherein the recombinant protein is an immunoglobulin, an enzyme, a growth factor, a protein fragment, or an engineered protein.

10 8. The method of claim 5, wherein the recombinant protein is recovered from the first or second liquid culture medium.

15 9. The method of claim 8, wherein the recombinant protein is a secreted immunoglobulin, a secreted enzyme, a secreted growth factor, a secreted protein fragment, or a secreted engineered protein.

10. A method for testing a manufacturing process for making a recombinant protein, the methods comprising:

20 providing a multi-well plate comprising at least one well comprising a mammalian cell disposed in a first liquid culture medium, wherein the first liquid culture medium occupies 5% to 70% of the volume of the well and the mammalian cell contains a nucleic acid that encodes a recombinant protein;

incubating the multi-well plate for a period of time at 31 °C to 40 °C and with a rotary agitation of 320 revolutions per minute (RPM) to 500 RPM;

25 continuously or periodically, during the period of time, removing a first volume of the first liquid culture medium and adding to the first liquid culture medium a second volume of a second liquid culture medium, wherein the first and second volumes are equal;

detecting the recombinant protein in the mammalian cell or in the first or second liquid culture medium; and

30 comparing the amount of recombinant protein present in the mammalian cell or in the first or second liquid culture medium to a reference level of recombinant protein.

11. The method of claim 10, wherein the reference level of recombinant protein is a level of recombinant protein produced using a different culturing method.

5 12. The method of claim 11, wherein the different culturing method utilizes a different first or second liquid culture medium, a different mammalian cell, a different temperature, a different level of agitation, or a different multi-well plate.

10 13. The method of claim 11, wherein the different culturing method utilizes different raw materials, anti-clumping agents, or chemically-defined liquid culture media.

14. The method of claim 10, wherein the method is used to perform high throughput cell culture experiments to perform a design-of-experiment (DOE) or a quality-by-design (QDB) study.

15 15. The method of claim 10, wherein the recombinant protein is detected in the first or second liquid culture medium.

20 16. The method of claim 15, wherein the recombinant protein is a secreted immunoglobulin, a secreted enzyme, a secreted growth factor, a secreted protein fragment, or a secreted engineered protein.

17. The method of claim 10, wherein the recombinant protein is detected in the cell.

25 18. The method of claim 17, wherein the recombinant protein is an immunoglobulin, an enzyme, a growth factor, a protein fragment, or an engineered protein.

19. The method of any one of claims 1, 5, and 10, wherein the first volume of the first liquid culture medium is at least 90% free of mammalian cells.

30 20. The method of any one of claims 1, 5, and 10, wherein the first liquid culture medium occupies 10% to 60% of the volume of the well.

21. The method of claim 5 or 10, wherein the mammalian cell is a Chinese hamster ovary (CHO) cell.

22. The method of any one of claims 1, 5, and 10, wherein the rotary agitation is 320
5 RPM to 400 RPM.

23. The method of any one of claims 1, 5, and 10, wherein the removing of the first
volume of the first liquid culture medium and the adding of the second volume of the second
liquid culture medium is performed simultaneously.

10 24. The method of any one of claims 1, 5, and 10, wherein the removing of the first
volume of the first liquid culture medium and the adding of the second volume of the second
liquid culture medium is performed continuously.

15 25. The method of any one of claims 1, 5, and 10, wherein the removing of the first
volume of the first liquid culture medium and the adding of the second volume of the second
liquid culture medium is performed periodically.

20 26. The method of any one of claims 1, 5, and 10, wherein the first volume of the first
liquid culture medium removed and the second volume of the second liquid culture medium
added are increased over time.

27. The method of any one of claims 1, 5, and 10, wherein:
the multi-well plate is incubated for a period of time greater than 7 days, and on days 1
25 through 3 of incubation, in each 24-hour period, the first volume of the first liquid culture
medium removed and the second volume of the second liquid culture medium added is between
30% to 50% of the volume of the first liquid culture medium;
on days 4 through 6 of the incubation, in each 24-hour period, the first volume of the first
liquid culture medium removed and the second volume of the second liquid culture medium
30 added is between 40% and 70% of the volume of the first liquid culture medium; and

on day 7 and onwards of incubation, in each 24-hour period, the first volume of the first liquid culture medium removed and the second volume of the second liquid culture medium added is 90% to 150% of the volume of the first liquid culture medium.

5 28. The method of any one of claims 1, 5, and 10, wherein the well has a volume of between 1 mL to 18 mL.

29. The method of any one of claims 1, 5, and 10, wherein the well has a volume of between 1 mL and 7 mL.

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30. The method of any one of claims 1, 5, and 10, wherein the well has a volume of between 1 mL and 3.5 mL.

15 31. The method of any one of claims 1, 5, and 10, wherein the multi-well plate is a 6-well plate, a 12-well plate, a 24-well plate, a 48-well plate, or a 96-well plate.

32. The method of any one of claims 1, 5, and 10, wherein the multi-well plate is a deep-well plate.

20 33. The method of any one of claims 1, 5, and 10, wherein the diameter of the bottom of the well is between 6.0 mm and 35 mm.

34. The method of any one of claims 1, 5, and 10, wherein the height of the well is 12 mm to 50 mm.

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35. The method of any one of claims 1, 5, and 10, wherein the mammalian cell is suspended in 150 TL to 15 mL of the first culture medium.

30 36. The method of claim 35, wherein the mammalian cell is suspended in 150 TL to 10 mL of the first culture medium.

37. The method of claim 36, wherein the mammalian cell is suspended in 150 TL to 5 mL of the first culture medium.

5 38. The method of claim 37, wherein the mammalian cell is suspended in 150 TL to 1 mL of the first culture medium.

39. The method of any one of claims 1, 5, and 10, wherein the first liquid culture medium and/or second liquid culture medium is selected from the group consisting of: a chemically-defined liquid culture medium, a serum-free liquid culture medium, a serum-containing liquid culture medium, an animal-derived component free liquid culture medium, and a protein-free medium.

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40. The method of any one of claims 1, 5, and 10, wherein after the first 24 to 48 hours of the period of time, in each 24-hour period, the first volume of the first liquid culture medium removed and the second volume of the second liquid culture medium added is 30% to 150% of the volume of the first liquid culture medium.

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41. The method of any one of claims 1, 5, and 10, wherein the agitation is ceased for a period of time of at least 30 seconds prior to removing the first volume of the first liquid culture medium.

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42. The method of any one of claims 1, 5, and 10, wherein the multi-well plate is sealed with a gas-permeable disposable membrane.

25 43. The method of any one of claims 1, 5, and 10, wherein the multi-well plate is sealed with a gas-permeable silicone layer.

44. The method of any one of claims 1, 5, and 10, wherein the well has a flat bottom.

30 45. The method of any one of claims 1, 5, and 10, wherein the well has a round bottom.

46. The method of any one of claims 1, 5, and 10, further comprising periodically adding an additional volume of second liquid culture medium to each of the plurality of wells in order to offset any decrease in the volume of the first liquid culture medium due to evaporation.

5 47. The method of any one of claims 1, 5, and 10, wherein removing of the first volume of the first liquid culture medium and adding to the first liquid culture medium the second volume of the second liquid culture medium is performed using an automated device.

10 48. The method of any one of claims 1, 5, and 10, wherein the method results in a viable cell density of between 15×10^6 cells/mL and 60×10^6 cells/mL in the well.

49. The method of claim 1, 5, or 10, wherein the rotary agitation is 320 RPM to 400 RPM.

15 50. The method of claim 49, wherein the rotary agitation is 320 RPM to 380 RPM.

51. The method of claim 1, 5, or 10, wherein the method results in a viable cell density of greater than 15×10^6 cells/mL in the well.

20 52. The method of claim 51, wherein the method results in a viable cell density of greater than 15×10^6 cells/mL in the well at 8 days or more after the beginning of the period of time.

53. The method of claim 1, 5, or 10, wherein the method results in a viable cell density of greater than 20×10^6 cells/mL in the well.

25 54. The method of claim 53, wherein the method results in a viable cell density of greater than 20×10^6 cells/mL in the well at 8 days or more after the beginning of the period of time.

30 55. The method of claim 1, 5, or 10, wherein the method results in a viable cell density of greater than 25×10^6 cells/mL in the well.

56. The method of claim 55, wherein the method results in a viable cell density of greater than 25×10^6 cells/mL in the well at 8 days or more after the beginning of the period of time.