WHAT IS CLAIMED IS:

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

providing a shake tube containing a mammalian cell disposed in a first liquid culture medium, wherein the first liquid culture medium occupies about 10% to about 30% of the volume of the shake tube and contains a plurality of microcarriers at a concentration of about 1.0 g/L to about 15.0 g/L;

incubating the shake tube for a period of time at about 32 ºC to about 39 ºC and with a rotary agitation of about 120 revolutions per minute (RPM) to about 240 RPM; and

after about the first 48 to 96 hours of the period of time, continuously or periodically 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 about equal.

2. The method of claim 1, wherein the first volume of the first liquid culture medium is substantially free of the microcarriers.

3. The method of claim 1, wherein at the beginning of the period of time, the first liquid culture medium contains 0.1×10^6 cells/mL to 0.5×10^6 cells/mL.

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

5. The method of claim 4, wherein the CHO cell contains a nucleic acid encoding a recombinant protein.

6. The method of claim 5, wherein the recombinant protein is an immunoglobulin, an enzyme, a growth factor, a protein fragment, or an engineered protein.

7. The method of claim 1, 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.

8. The method of claim 1, 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.

9. The method of claim 1, 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.

10. The method of claim 1, 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.

11. The method of claim 1, wherein the first liquid culture medium is the same as the second liquid culture medium.

12. The method of claim 1, wherein the first liquid culture medium is different from the second liquid culture medium.

13. The method of claim 1, wherein the shake tube has a volume of between about 10 mL to about 100 mL.

14. The method of claim 1, wherein the mammalian cell is suspended in about 2 mL to about 20 mL of the first liquid culture medium.

15. The method of claim 1, 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.

16. The method of claim 1, wherein after about the first 48 to 96 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 about 30% to about 95% of the volume of the first liquid culture medium.

17. The method of claim 1, 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.

18. The method of claim 1, wherein the plurality of microcarriers has a mean diameter of between about 200 μm to about 800 μm.

19. The method of claim 18, wherein the plurality of microcarriers contains one or more pores.

20. The method of claim 19, wherein the one or more pores has a mean diameter of about 25 μm to about 35 μm.

21. The method of claim 1, wherein the shake tube is incubated at a reactor angle of about 25 degrees to about 90 degrees from horizontal.

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

(a) providing a shake tube containing a mammalian cell disposed in a first liquid culture medium that occupies about 10% to about 30% of the volume of the shake tube and contains a plurality of microcarriers in a concentration of about 1.0 g/L to about 15.0 g/L;

(b) incubating the shake tube for a first time period at about 35 ºC to about 39 ºC with a rotary agitation of about 120 revolutions per minute (RPM) to about 240 RPM,

and after about the first 48 to 96 hours of the first period of time, in each subsequent 24-hour period,

(i) continuously or periodically removing a first volume of the first liquid culture medium that is substantially free of microcarriers from the shake tube, wherein the first volume is about 10% to about 95% of the volume of the first liquid culture medium; and

(ii) adding to the shake tube a second volume of a second liquid culture medium, wherein the first and second volumes are about equal;

(c) incubating the shake tube after the cell concentration reaches about target cell density for a second time period of about 2 days to about 7 days, at about 32 ºC to about 39 ºC with the rotary agitation, and in each 24-hour period, performing steps (b)(i) and (b)(ii),wherein the first and second liquid culture media used in step (b) are of a substantially different type from those used in step (c); and

(d) incubating the shake tube for a third time period greater than 2 days, at about 35 ºC to about 39 ºC with the rotary agitation, and in each 24-hour period, performing steps (b)(i) and (b)(ii), wherein the first and second liquid culture media used in step (c) are of the same type as those used in step (d).

23. The method of claim 22, wherein at the beginning of the first period of time, the first liquid culture medium contains 0.1×10^6 cells/mL to 0.5×10^6 cells/mL.

24. The method of claim 22, wherein the mammalian cell is a Chinese hamster ovary (CHO) cell.

25. The method of claim 24, wherein the CHO cell contains a nucleic acid encoding a recombinant protein.

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

27. The method of claim 22, 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 in one or more of the first time period, the second time period, and the third time period is performed simultaneously.

28. The method of claim 22, 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 in one or more of the first time period, the second time period, and the third time period is performed continuously.

29. The method of claim 22, 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 in one of more of the first time period, the second time period, and the third time period is performed periodically.

30. The method of claim 22, wherein the shake tube has a volume of between about 10 mL to about 100 mL.

31. The method of claim 22, wherein the volume of the first liquid culture medium is about 2 mL to about 20 mL.

32. The method of claim 22, wherein the first liquid culture medium and second liquid culture medium used in the first time period is serum-containing liquid culture medium or an animal-derived component-containing liquid culture medium, and the first liquid culture medium and the second liquid culture medium used in the second time period and the third time period is a serum-free liquid culture medium, an animal-derived component-free liquid culture medium, or a protein-free medium.

33. The method of claim 22, wherein the agitation is ceased for at least 30 seconds prior to removing the first volume of the first liquid culture medium from the

shake tube during one or more of the first time period, the second time period, and the third time period.

34. The method of claim 22, wherein the plurality of microcarriers has a mean diameter of between about 200 μm to about 800 μm.

35. The method of claim 34, wherein the plurality of microcarriers contains one or more pores.

36. The method of claim 35, wherein the one or more pores has a mean diameter of about 25 μm to about 35 μm.

37. The method of claim 22, wherein the first volume of the first liquid culture medium removed during the third period of time contains a substantial number of microcarriers.

38. The method of claim 22, wherein the first volume of the first liquid culture medium removed during the third period of time is substantially free of microcarriers.

39. The method of claim 22, wherein the first volume of the first liquid culture medium removed and the second volume of the second liquid culture medium added in one or more of the first time period, the second time period, and the third time period is about 70% of the volume of the first liquid culture medium.

40. The method of claim 22, wherein the shake tube is incubated in (b), (c), and (d) at a reactor angle of about 25 degrees to about 90 degrees from horizontal.

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

providing a shake tube containing a mammalian cell containing a nucleic acid encoding a recombinant protein disposed in a first liquid culture medium, wherein the first liquid culture medium occupies about 10% to about 30% of the volume of the shake

tube and contains a plurality of microcarriers at a concentration of about 1.0 g/L to about 15.0 g/L;

incubating the shake tube for a period of time at about 32 ºC to about 39 ºC and with a rotary agitation of about 120 revolutions per minute (RPM) to about 240 RPM; and

after about the first 48 to 96 hours of the period of time, continuously or periodically 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 about equal; and

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

42. The method of claim 41, wherein the recombinant protein is recovered from the mammalian cell.

43. The method of claim 41, wherein the recombinant protein is recovered from the first and/or second liquid culture medium.

44. The method of claim 41, wherein the first volume of the first liquid culture medium removed is substantially free of microcarriers.

45. The method of claim 41, wherein at the beginning of the period of time, the first liquid culture medium contains 0.1×10^6 cells/mL to 0.5×10^6 cells/mL.

46. The method of claim 41, wherein the mammalian cell is a Chinese hamster ovary (CHO) cell.

47. The method of claim 41, wherein the recombinant protein is an immunoglobulin, an enzyme, a growth factor, a protein fragment, or an engineered protein.

48. The method of claim 47, wherein the recombinant protein is secreted into the first and/or second liquid culture medium.

49. The method of claim 41, 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.

50. The method of claim 41, 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.

51. The method of claim 41, 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.

52. The method of claim 41, 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.

53. The method of claim 41, wherein the first liquid culture medium is the same as the second liquid culture medium.

54. The method of claim 41, wherein the first liquid culture medium is different from the second liquid culture medium.

55. The method of claim 41, wherein the shake tube has a volume of between about 10 mL to about 100 mL.

56. The method of claim 41, wherein the mammalian cell is suspended in about 2 mL to about 20 mL of the first liquid culture medium.

57. The method of claim 41, 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.

58. The method of claim 41, wherein after about the first 48 to 96 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 about 30% to about 95% of the volume of the first liquid culture medium.

59. The method of claim 41, 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.

60. The method of claim 41, wherein the plurality of microcarriers has a mean diameter of between about 200 μm to about 800 μm.

61. The method of claim 60, wherein the plurality of microcarriers contains one or more pores.

62. The method of claim 61, wherein the one or more pores has a mean diameter of about 25 μm to about 35 μm.

63. The method of claim 41, wherein the shake tube is incubated at a reactor angle of about 25 degrees to about 90 degrees from horizontal.

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

(a) providing a shake tube containing a mammalian cell containing a nucleic acid encoding a recombinant protein disposed in a first liquid culture medium, wherein the first liquid culture medium occupies about 10% to about 30% of the volume of the shake

tube and contains a plurality of microcarriers in a concentration of about 1.0 g/L to about 15.0 g/L;

(b) incubating the shake tube for a first time period at about 35 ºC to about 39 ºC with a rotary agitation of about 120 revolutions per minute (RPM) to about 240 RPM, and after about the first 48 hours to 96 hours of the first period of time, in each subsequent 24-hour period,

(i) continuously or periodically removing a first volume of the first liquid culture medium that is substantially free of microcarriers from the shake tube, wherein the first volume is about 10% to about 95% of the volume of the first liquid culture medium; and

(ii) adding to the shake tube a second volume of a second liquid culture medium, wherein the first and second volumes are about equal;

(c) incubating the shake tube after the cell concentration reaches about target cell density for a second time period of about 2 days to about 7 days, at about 32 ºC to about 39 ºC with the rotary agitation, and in each 24-hour period, performing steps (b)(i) and (b)(ii), wherein the first and second liquid culture media used in step (b) are of a substantially different type from those used in step (c);

(d) incubating the shake tube for a third time period greater than 2 days, at about 35 ºC to about 39 ºC with the rotary agitation, and in each 24-hour period, performing steps (b)(i) and (b)(ii), wherein the first and second liquid culture media used in step (c) are of the same type as those used in step (d); and

(e) recovering the recombinant protein from the mammalian cell or the first and/or second liquid culture medium used during the first, second, and/or third period of time.

65. The method of claim 64, wherein the recombinant protein is recovered from the mammalian cell.

66. The method of claim 64, wherein the recombinant protein is recovered from the first and/or second liquid culture medium used during one or more of the first, second, and third period of time.

67. The method of claim 64, wherein at the beginning of the first period of time, the first liquid culture medium contains 0.1 x 10^6 cells/mL to 0.5 x 10^6 cells/mL.

68. The method of claim 64, wherein the mammalian cell is a Chinese hamster ovary (CHO) cell.

69. The method of claim 64, wherein the recombinant protein is an immunoglobulin, an enzyme, a growth factor, a protein fragment, or an engineered protein.

70. The method of claim 69, wherein the recombinant protein is secreted into the first and/or second liquid culture medium used during one or more of the first period of time, the second period of time, and the third period of time.

71. The method of claim 64, 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 in one or more of the first time period, the second time period, and the third time period is performed simultaneously.

72. The method of claim 64, 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 in one or more of the first time period, the second time period, and the third time period is performed continuously.

73. The method of claim 64, 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 in one of more of the first time period, the second time period, and the third time period is performed periodically.

74. The method of claim 64, wherein the shake tube has a volume of between about 10 mL to about 100 mL.

75. The method of claim 64, wherein the volume of the first liquid culture medium is about 2 mL to about 20 mL.

76. The method of claim 64, wherein the first liquid culture medium and second liquid culture medium used in the first time period is serum-containing liquid culture medium or an animal-derived component-containing liquid culture medium, and the first liquid culture medium and second liquid culture medium used in the second time period and the third time period is a serum-free liquid culture medium, an animal-derived component free liquid culture medium, or a protein-free medium.

77. The method of claim 64, wherein the agitation is ceased for at least 30 seconds prior to removing the first volume of the first liquid culture medium, from the shake tube during one or more of the first time period, the second time period, and the third time period.

78. The method of claim 64, wherein the plurality of microcarriers has a mean diameter of between about 200 μm to about 800 μm.

79. The method of claim 78, wherein the plurality of microcarrier contains one or more pores.

80. The method of claim 79, wherein the one or more pores has a mean diameter of about 25 μm to about 35 μm.

81. The method of claim 64, wherein the first volume of the liquid culture medium removed during the third period of time contains a substantial number of microcarriers.

82. The method of claim 64, wherein the first volume of the liquid culture medium removed during the third period of time is substantially free of microcarriers.

83. The method of claim 64, wherein the first volume of the first liquid culture medium removed and the second volume of the second liquid culture medium added in one or more of the first time period, the second time period, and the third time period is about 70% of the volume of the first liquid culture medium.

84. The method of claim 64, the shake tube is incubated in (b), (c), and (d) at a reactor angle of about 25 degrees to about 90 degrees from horizontal.

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

providing a shake tube containing a mammalian cell containing a nucleic acid encoding a recombinant protein disposed in a first liquid culture medium, wherein the first liquid culture medium occupies about 10% to about 30% of the volume of the shake tube and contains a plurality of microcarriers at a concentration of about 1.0 g/L to about 15.0 g/L;

incubating the shake tube for a period of time at about 32 ºC to about 39 ºC and with a rotary agitation of about 120 revolutions per minute (RPM) to about 240 RPM;

after about the first 48 to 96 hours of the period of time, continuously or periodically 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 about equal;

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

comparing the amount of recombinant protein present in the cell or in the first and/or second culture medium to a reference level of recombinant protein.

86. The method of claim 85, wherein the first volume of the first liquid culture medium is substantially free of mammalian cells.

87. The method of claim 85, wherein the reference level of recombinant protein is a level of recombinant protein produced using a different culturing method.

88. The method of claim 87, 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, a different shake tube, or a different microcarrier.

89. The method of claim 87, wherein the different culturing method utilizes a different raw material, anti-clumping agent, or chemically-defined liquid culture medium.

90. The method of claim 85, wherein the method is used to perform high throughput cell culture experiments to perform a design-of-experiment (DOE) or a quality-by-design (QBD) study.

91. The method of claim 85, wherein the shake tube is has a volume of between about 10 mL to about 100 mL.

92. The method of claim 85, wherein the mammalian cell is suspended in about 2 mL to about 20 mL of the first liquid culture medium.

93. The method of claim 85, wherein the mammalian cell is a Chinese hamster ovary (CHO) cell.

94. The method of claim 85, wherein the recombinant protein is a secreted immunoglobulin, a secreted enzyme, a secreted growth factor, a secreted protein fragment, or a secreted engineered protein, and wherein the recombinant protein is recovered from the first or second culture medium.

95. The method of claim 85, wherein the recombinant protein is recovered from the mammalian cell.

96. The method of claim 95, wherein the recombinant protein is an immunoglobulin, an enzyme, a growth factor, a protein fragment, or an engineered protein.

97. The method of claim 85, 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.

98. The method of claim 85, 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.

99. The method of claim 85, 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.

100. The method of claim 85, 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.

101. The method of claim 85, 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.

102. The method of claim 85, wherein the shake tube is incubated at a reactor angle of about 25 degrees to about 90 degrees from horizontal.

103. A method of testing the efficacy of a first or second liquid culture medium, a raw ingredient or supplement present in a first or second liquid culture medium, or a

source of a mammalian cell for use in a method of producing a recombinant protein, the method comprising:

providing a shake tube containing a mammalian cell disposed in a first liquid culture medium, wherein the first liquid culture medium occupies about 10% to about 30% of the volume of the shake tube and contains a plurality of microcarriers at a concentration of about 1.0 g/L to about 15.0 g/L;

incubating the shake tube for a period of time at about 32 ºC to about 39 ºC and with a rotary agitation of about 120 revolutions per minute (RPM) to about 240 RPM; and

after about the first 48 to 96 hours of the period of time, continuously or periodically 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 about equal;

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

comparing the amount of recombinant protein present in the cell or in the first and/or second culture medium to a reference level of recombinant protein produced by a different method that uses one or more of a different first or second liquid culture medium, a different raw ingredient or supplement present in the first or second liquid culture medium, or a different source of a mammalian cell; and

identifying the first or second liquid culture medium, the raw ingredient or supplement present in the first or second liquid culture medium, or the source of the mammalian cell that is associated with an increased amount of recombinant protein as compared to the reference level as being efficacious for use in a method of producing a recombinant protein.

104. The method of claim 103, wherein the shake tube is incubated at a reactor angle of about 25 degrees to about 90 degrees from horizontal.

105. A method of optimizing a manufacturing process of producing a recombinant protein, the method comprising:

providing a shake tube containing a mammalian cell disposed in a first liquid culture medium, wherein the first liquid culture medium occupies about 10% to about 30% of the volume of the shake tube and contains a plurality of microcarriers at a concentration of about 1.0 g/L to about 15.0 g/L;

incubating the shake tube for a period of time at about 32 ºC to about 39 ºC and with a rotary agitation of about 120 revolutions per minute (RPM) to about 240 RPM; and

after about the first 48 to 96 hours of the period of time, continuously or periodically 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 about equal;

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

comparing the amount of recombinant protein present in the cell or in the first and/or second culture medium to a reference level of recombinant protein produced by a different method; and

identifying and removing or altering in a manufacturing process any culture components or parameters that are associated with a decrease in the amount of recombinant protein produced as compared to the reference level, or identifying and adding to a manufacturing process any culture components or parameters that are associated with an increase in the amount of recombinant protein produced as compared to the reference level.

106. The method of claim 105, wherein the shake tube is incubated at a reactor angle of about 25 degrees to about 90 degrees from horizontal.

107. A method of testing for the presence of a contaminant in a first or second liquid culture medium, a raw material used to generate a first or second liquid culture medium, or a source of a mammalian cell, the method comprising:

providing a shake tube containing a mammalian cell disposed in a first liquid culture medium, wherein the first liquid culture medium occupies about 10% to about

30% of the volume of the shake tube and contains a plurality of microcarriers at a concentration of about 1.0 g/L to about 15.0 g/L;

incubating the shake tube for a period of time at about 32 ºC to about 39 ºC and with a rotary agitation of about 120 revolutions per minute (RPM) to about 240 RPM; and

after about the first 48 to 96 hours of the period of time, continuously or periodically 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 about equal;

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

comparing the amount of recombinant protein present in the cell or in the first and/or second culture medium to a reference level of recombinant protein produced by a different method that uses one or more of a different first or second liquid culture medium, a different raw material to generate the first or second liquid culture medium, or a different source of the mammalian cell; and

identifying the first or second liquid culture medium, the raw material used to generate the first or second liquid culture medium, or the source of a mammalian cell as containing a contaminant when the level of recombinant protein produced is less than the reference level.

108. The method of claim 107, wherein the contaminant is a biological contaminant.

109. The method of claim 108, wherein the biological contaminant is selected from the group consisting of: mycobacterium, a fungus, a bacterium, a virus, and an undesired mammalian cell.

110. The method of claim 107, wherein the shake tube is incubated at a reactor angle of about 25 degrees to about 90 degrees from horizontal.

WHAT IS CLAIMED IS:

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

providing a shake tube containing an adherent mammalian cell disposed in a first liquid culture medium, wherein the first liquid culture medium occupies about 10% to about 30% of the volume of the shake tube and contains a plurality of microcarriers at a concentration of about 1.0 g/L to about 15.0 g/L;

incubating the shake tube for a period of time at about 32 ºC to about 39 ºC ~~and~~ with a rotary agitation of about 130 revolutions per minute (RPM) to about 150 RPM, and at a reactor angle of about 25 degrees to about 90 degrees from horizontal; and

after about the first 48 to 96 hours of the period of time, continuously or periodically 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 about equal.

2. The method of claim 1, wherein the first volume of the first liquid culture medium is substantially free of the microcarriers.

3. The method of claim 1, wherein at the beginning of the period of time, the first liquid culture medium contains 0.1×10^6 cells/mL to 0.5×10^6 cells/mL.

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

5. The method of claim 4, wherein the CHO cell contains a nucleic acid encoding a recombinant protein.

6. The method of claim 5, wherein the recombinant protein is an immunoglobulin, an enzyme, a growth factor, a protein fragment, or an engineered protein.

7. The method of claim 1, 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.

8. The method of claim 1, 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.

9. The method of claim 1, 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.

10. The method of claim 1, 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.

11. The method of claim 1, wherein the first liquid culture medium is the same as the second liquid culture medium.

12. The method of claim 1, wherein the first liquid culture medium is different from the second liquid culture medium.

13. The method of claim 1, wherein the shake tube has a volume of between about 10 mL to about 100 mL.

14. The method of claim 1, wherein the adherent mammalian cell is suspended in about 2 mL to about 20 mL of the first liquid culture medium.

15. The method of claim 1, 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.

16. The method of claim 1, wherein after about the first 48 to 96 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 about 30% to about 95% of the volume of the first liquid culture medium.

17. The method of claim 1, 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.

18. The method of claim 1, wherein the plurality of microcarriers has a mean diameter of between about 200 μm to about 800 μm.

19. The method of claim 18, wherein the plurality of microcarriers contains one or more pores.

20. The method of claim 19, wherein the one or more pores has a mean diameter of about 25 μm to about 35 μm.

21. The method of claim 1, wherein the shake tube is incubated at a reactor angle of about 25 degrees to about 60 degrees from horizontal.

22. The method of any one of claims 1-21, wherein a viable cell concentration in the first and/or second liquid culture medium of greater than 2×10^6 cells/mL is achieved at some point during the period of time.

23. A method of culturing an adherent mammalian cell, the method comprising:

(a) providing a shake tube containing an adherent mammalian cell disposed in a first liquid culture medium that occupies about 10% to about 30% of the volume of the shake tube and contains a plurality of microcarriers in a concentration of about 1.0 g/L to about 15.0 g/L;

(b) incubating the shake tube for a first time period at about 35 ºC to about 39 ºC with a rotary agitation of about 130 revolutions per minute (RPM) to about 150 RPM, and at a reactor angle of about 25 degrees to about 90 degrees, and after about the first 48 to 96 hours of the first period of time, in each subsequent 24-hour period,

(i) continuously or periodically removing a first volume of the first liquid culture medium that is substantially free of microcarriers from the shake tube, wherein the first volume is about 10% to about 95% of the volume of the first liquid culture medium; and

(ii) adding to the shake tube a second volume of a second liquid culture medium, wherein the first and second volumes are about equal;

(c) incubating the shake tube after the adherent mammalian cell concentration reaches about target cell density for a second time period of about 2 days to about 7 days, at about 32 ºC to about 39 ºC with the rotary agitation, and in each 24-hour period, performing steps (b)(i) and (b)(ii), wherein the first and second liquid culture media used in step (b) are of a substantially different type from those used in step (c); and

(d) incubating the shake tube for a third time period greater than 2 days, at about 35 ºC to about 39 ºC with the rotary agitation, and in each 24-hour period, performing steps (b)(i) and (b)(ii), wherein the first and second liquid culture media used in step (c) are of the same type as those used in step (d).

24. The method of claim 23, wherein at the beginning of the first period of time, the first liquid culture medium contains 0.1×10^6 cells/mL to 0.5×10^6 cells/mL.

25. The method of claim 23, wherein the adherent mammalian cell is a Chinese hamster ovary (CHO) cell.

26. The method of claim 25, wherein the CHO cell contains a nucleic acid encoding a recombinant protein.

27. The method of claim 26, wherein the recombinant protein is a secreted immunoglobulin, a secreted enzyme, a secreted growth factor, a secreted protein fragment, or a secreted engineered protein.

28. The method of claim 23, 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 in one or more of the first time period, the second time period, and the third time period is performed simultaneously.

29. The method of claim 23, 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 in one or more of the first time period, the second time period, and the third time period is performed continuously.

30. The method of claim 23, 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 in one of more of the first time period, the second time period, and the third time period is performed periodically.

31. The method of claim 23, wherein the shake tube has a volume of between about 10 mL to about 100 mL.

32. The method of claim 23, wherein the volume of the first liquid culture medium is about 2 mL to about 20 mL.

33. The method of claim 23, wherein the first liquid culture medium and second liquid culture medium used in the first time period is serum-containing liquid culture medium or an animal-derived component-containing liquid culture medium, and the first liquid culture medium and the second liquid culture medium used in the second time period and the third time period is a serum-free liquid culture medium, an animal-derived component-free liquid culture medium, or a protein-free medium.

34. The method of claim 23, wherein the agitation is ceased for at least 30 seconds prior to removing the first volume of the first liquid culture medium from the shake tube during one or more of the first time period, the second time period, and the third time period.

35. The method of claim 23, wherein the plurality of microcarriers has a mean diameter of between about 200 μm to about 800 μm.

36. The method of claim 35, wherein the plurality of microcarriers contains one or more pores.

37. The method of claim 36, wherein the one or more pores has a mean diameter of about 25 μm to about 35 μm.

38. The method of claim 23, wherein the first volume of the first liquid culture medium removed during the third period of time contains a substantial number of microcarriers.

39. The method of claim 23, wherein the first volume of the first liquid culture medium removed during the third period of time is substantially free of microcarriers.

40. The method of claim 23, wherein the first volume of the first liquid culture medium removed and the second volume of the second liquid culture medium added in one or more of the first time period, the second time period, and the third time period is about 70% of the volume of the first liquid culture medium.

41. The method of claim 23, wherein the shake tube is incubated in (b), (c), and (d) at a reactor angle of about 25 degrees to about 60 degrees from horizontal.

42. The method of any one of claims 23-41, wherein a viable mammalian cell concentration in the first and/or second liquid culture medium of greater than 2×10^6 cells/mL is achieved at some point during the first, second, or third time period.

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

providing a shake tube containing an adherent mammalian cell containing a nucleic acid encoding a recombinant protein disposed in a first liquid culture medium, wherein the first liquid

culture medium occupies about 10% to about 30% of the volume of the shake tube and contains a plurality of microcarriers at a concentration of about 1.0 g/L to about 15.0 g/L;

incubating the shake tube for a period of time at about 32 ºC to about 39 ºC, with a rotary agitation of about 130 revolutions per minute (RPM) to about 150 RPM, and at a reactor angle of about 25 degrees to about 90 degrees; and

after about the first 48 to 96 hours of the period of time, continuously or periodically 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 about equal; and

recovering the recombinant protein from the adherent mammalian cell or from the first and/or second liquid culture medium.

44. The method of claim 43, wherein the recombinant protein is recovered from the adherent mammalian cell.

45. The method of claim 43, wherein the recombinant protein is recovered from the first and/or second liquid culture medium.

46. The method of claim 43, wherein the first volume of the first liquid culture medium removed is substantially free of microcarriers.

47. The method of claim 43, wherein at the beginning of the period of time, the first liquid culture medium contains 0.1×10^6 cells/mL to 0.5×10^6 cells/mL.

48. The method of claim 43, wherein the adherent mammalian cell is a Chinese hamster ovary (CHO) cell.

49. The method of claim 43, wherein the recombinant protein is an immunoglobulin, an enzyme, a growth factor, a protein fragment, or an engineered protein.

50. The method of claim 49, wherein the recombinant protein is secreted into the first and/or second liquid culture medium.

51. The method of claim 43, 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.

52. The method of claim 43, 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.

53. The method of claim 43, 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.

54. The method of claim 43 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.

55. The method of claim 43, wherein the first liquid culture medium is the same as the second liquid culture medium.

56. The method of claim 43, wherein the first liquid culture medium is different from the second liquid culture medium.

57. The method of claim 43, wherein the shake tube has a volume of between about 10 mL to about 100 mL.

58. The method of claim 43, wherein the adherent mammalian cell is suspended in about 2 mL to about 20 mL of the first liquid culture medium.

59. The method of claim 43, 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.

60. The method of claim 43, wherein after about the first 48 to 96 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 about 30% to about 95% of the volume of the first liquid culture medium.

61. The method of claim 43 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.

62. The method of claim 43, wherein the plurality of microcarriers has a mean diameter of between about 200 μm to about 800 μm.

63. The method of claim 62, wherein the plurality of microcarriers contains one or more pores.

64. The method of claim 63, wherein the one or more pores has a mean diameter of about 25 μm to about 35 μm.

65. The method of claim 43, wherein the shake tube is incubated at a reactor angle of about 25 degrees to about 60 degrees from horizontal.

66. The method of any one of claims 43-65, wherein a viable cell concentration in the first and/or second liquid culture medium of greater than 2×10^6 cells/mL is achieved at some point during the period of time.

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

(a) providing a shake tube containing an adherent mammalian cell containing a nucleic acid encoding a recombinant protein disposed in a first liquid culture medium, wherein the first liquid culture medium occupies about 10% to about 30% of the volume of the shake tube and contains a plurality of microcarriers in a concentration of about 1.0 g/L to about 15.0 g/L;

(b) incubating the shake tube for a first time period at about 35 ºC to about 39 ºC with a rotary agitation of about 130 revolutions per minute (RPM) to about 150 RPM, and at a reactor angle of about 25 degrees to about 90 degrees, and after about the first 48 hours to 96 hours of the first period of time, in each subsequent 24-hour period,

(i) continuously or periodically removing a first volume of the first liquid culture medium that is substantially free of microcarriers from the shake tube, wherein the first volume is about 10% to about 95% of the volume of the first liquid culture medium; and

(ii) adding to the shake tube a second volume of a second liquid culture medium, wherein the first and second volumes are about equal;

(c) incubating the shake tube after the adherent mammalian cell concentration reaches about target cell density for a second time period of about 2 days to about 7 days, at about 32 ºC to about 39 ºC with the rotary agitation, and in each 24-hour period, performing steps (b)(i) and (b)(ii), wherein the first and second liquid culture media used in step (b) are of a substantially different type from those used in step (c);

(d) incubating the shake tube for a third time period greater than 2 days, at about 35 ºC to about 39 ºC with the rotary agitation, and in each 24-hour period, performing steps (b)(i) and (b)(ii), wherein the first and second liquid culture media used in step (c) are of the same type as those used in step (d); and

(e) recovering the recombinant protein from the adherent mammalian cell or the first and/or second liquid culture medium used during the first, second, and/or third period of time.

68. The method of claim 67, wherein the recombinant protein is recovered from the adherent mammalian cell.

69. The method of claim 67, wherein the recombinant protein is recovered from the first and/or second liquid culture medium used during one or more of the first, second, and third period of time.

70. The method of claim 67, wherein at the beginning of the first period of time, the first liquid culture medium contains 0.1×10^6 cells/mL to 0.5×10^6 cells/mL.

71. The method of claim 67, wherein the adherent mammalian cell is a Chinese hamster ovary (CHO) cell.

72. The method of claim 67, wherein the recombinant protein is an immunoglobulin, an enzyme, a growth factor, a protein fragment, or an engineered protein.

73. The method of claim 72, wherein the recombinant protein is secreted into the first and/or second liquid culture medium used during one or more of the first period of time, the second period of time, and the third period of time.

74. The method of claim 67, 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 in one or more of the first time period, the second time period, and the third time period is performed simultaneously.

75. The method of claim 67, 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 in one or more of the first time period, the second time period, and the third time period is performed continuously.

76. The method of claim 67, 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 in one of more of the first time period, the second time period, and the third time period is performed periodically.

77. The method of claim 67, wherein the shake tube has a volume of between about 10 mL to about 100 mL.

78. The method of claim 67, wherein the volume of the first liquid culture medium is about 2 mL to about 20 mL.

79. The method of claim 67, wherein the first liquid culture medium and second liquid culture medium used in the first time period is serum-containing liquid culture medium or an animal-derived component-containing liquid culture medium, and the first liquid culture medium and second liquid culture medium used in the second time period and the third time period is a serum-free liquid culture medium, an animal-derived component free liquid culture medium, or a protein-free medium.

80. The method of claim 67, wherein the agitation is ceased for at least 30 seconds prior to removing the first volume of the first liquid culture medium, from the shake tube during one or more of the first time period, the second time period, and the third time period.

81. The method of claim 67, wherein the plurality of microcarriers has a mean diameter of between about 200 μm to about 800 μm.

82. The method of claim 81, wherein the plurality of microcarrier contains one or more pores.

83. The method of claim 82, wherein the one or more pores has a mean diameter of about 25 μm to about 35 μm.

84. The method of claim 67, wherein the first volume of the liquid culture medium removed during the third period of time contains a substantial number of microcarriers.

85. The method of claim 67, wherein the first volume of the liquid culture medium removed during the third period of time is substantially free of microcarriers.

86. The method of claim 67, wherein the first volume of the first liquid culture medium removed and the second volume of the second liquid culture medium added in one or more of the first time period, the second time period, and the third time period is about 70% of the volume of the first liquid culture medium.

87. The method of claim 67, the shake tube is incubated in (b), (c), and (d) at a reactor angle of about 25 degrees to about 60 degrees from horizontal.

88. The method of any one of claims 67-87, wherein a viable cell concentration in the first and/or second liquid culture medium of greater than 2×10^6 cells/mL is achieved at some point during the first, second, or third period of time.

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

providing a shake tube containing an adherent mammalian cell containing a nucleic acid encoding a recombinant protein disposed in a first liquid culture medium, wherein the first liquid culture medium occupies about 10% to about 30% of the volume of the shake tube and contains a plurality of microcarriers at a concentration of about 1.0 g/L to about 15.0 g/L;

incubating the shake tube for a period of time at about 32 ºC to about 39 ºC, ~~and~~ with a rotary agitation of about 130 revolutions per minute (RPM) to about 150 RPM, and at a reactor angle of about 25 degrees to about 90 degrees; and

after about the first 48 to 96 hours of the period of time, continuously or periodically 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 about equal;

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

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

90. The method of claim 89, wherein the first volume of the first liquid culture medium is substantially free of mammalian cells.

91. The method of claim 89, wherein the reference level of recombinant protein is a level of recombinant protein produced using a different culturing method.

92. The method of claim 89, 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, a different shake tube, or a different microcarrier.

93. The method of claim 89, wherein the different culturing method utilizes a different raw material, anti-clumping agent, or chemically-defined liquid culture medium.

94. The method of claim 89, wherein the method is used to perform high throughput cell culture experiments to perform a design-of-experiment (DOE) or a quality-by-design (QBD) study.

95. The method of claim 89, wherein the shake tube has a volume of between about 10 mL to about 100 mL.

96. The method of claim 89, wherein the adherent mammalian cell is suspended in about 2 mL to about 20 mL of the first liquid culture medium.

97. The method of claim 89, wherein the adherent mammalian cell is a Chinese hamster ovary (CHO) cell.

98. The method of claim 89, wherein the recombinant protein is a secreted immunoglobulin, a secreted enzyme, a secreted growth factor, a secreted protein fragment, or a secreted engineered protein, and wherein the recombinant protein is recovered from the first or second culture medium.

99. The method of claim 89, wherein the recombinant protein is recovered from the adherent mammalian cell.

100. The method of claim 99, wherein the recombinant protein is an immunoglobulin, an enzyme, a growth factor, a protein fragment, or an engineered protein.

101. The method of claim 89, 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.

102. The method of claim 89, 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.

103. The method of claim 89, 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.

104. The method of claim 89, 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.

105. The method of claim 89, 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.

106. The method of claim 89, wherein the shake tube is incubated at a reactor angle of about 25 degrees to about 60 degrees from horizontal.

107. The method of any one of claims 89-106, wherein a viable cell concentration in the first and/or second liquid culture medium of greater than 2 x 10^6 cells/mL is achieved at some point during the period of time.

108. A method of testing the efficacy of a first or second liquid culture medium, a raw ingredient or supplement present in a first or second liquid culture medium, or a source of an adherent mammalian cell for use in a method of producing a recombinant protein, the method comprising:

providing a shake tube containing an adherent mammalian cell disposed in a first liquid culture medium, wherein the first liquid culture medium occupies about 10% to about 30% of the volume of the shake tube and contains a plurality of microcarriers at a concentration of about 1.0 g/L to about 15.0 g/L;

incubating the shake tube for a period of time at about 32 ºC to about 39 ºC, ~~and~~ with a rotary agitation of about 130 revolutions per minute (RPM) to about 150 RPM, and at a reactor angle of about 25 degrees to about 90 degrees; and

after about the first 48 to 96 hours of the period of time, continuously or periodically 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 about equal;

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

comparing the amount of recombinant protein present in the adherent mammalian cell or in the first and/or second culture medium to a reference level of recombinant protein produced by a different method that uses one or more of a different first or second liquid culture medium, a different raw ingredient or supplement present in the first or second liquid culture medium, or a different source of an adherent mammalian cell; and

identifying the first or second liquid culture medium, the raw ingredient or supplement present in the first or second liquid culture medium, or the source of the adherent mammalian cell that is associated with an increased amount of recombinant protein as compared to the reference level as being efficacious for use in a method of producing a recombinant protein.

109. The method of claim 108, wherein the shake tube is incubated at a reactor angle of about 25 degrees to about 60 degrees from horizontal.

110. The method of claim 108 or 109, wherein a viable mammalian cell concentration in the first and/or second liquid culture medium of greater than 2 x 10^6 cells/mL is achieved at some point during the period of time.

111. A method of optimizing a manufacturing process of producing a recombinant protein, the method comprising:

providing a shake tube containing an adherent mammalian cell disposed in a first liquid culture medium, wherein the first liquid culture medium occupies about 10% to about 30% of the volume of the shake tube and contains a plurality of microcarriers at a concentration of about 1.0 g/L to about 15.0 g/L;

incubating the shake tube for a period of time at about 32 ºC to about 39 ºC, with a rotary agitation of about 130 revolutions per minute (RPM) to about 150 RPM, and at a reactor angle of about 25 degrees to about 90 degrees; and

after about the first 48 to 96 hours of the period of time, continuously or periodically 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 about equal;

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

comparing the amount of recombinant protein present in the adherent mammalian cell or in the first and/or second culture medium to a reference level of recombinant protein produced by a different method; and

identifying and removing or altering in a manufacturing process any culture components or parameters that are associated with a decrease in the amount of recombinant protein produced as compared to the reference level, or identifying and adding to a manufacturing process any culture components or parameters that are associated with an increase in the amount of recombinant protein produced as compared to the reference level.

112. The method of claim 111, wherein the shake tube is incubated at a reactor angle of about 25 degrees to about 60 degrees from horizontal.

113. The method of claim 111 or 112, wherein a viable cell concentration in the first and/or second liquid culture medium of greater than 2 x 10^6 cells/mL is achieved at some point during the period of time.

114. A method of testing for the presence of a contaminant in a first or second liquid culture medium, a raw material used to generate a first or second liquid culture medium, or a source of an adherent mammalian cell, the method comprising:

providing a shake tube containing an adherent mammalian cell disposed in a first liquid culture medium, wherein the first liquid culture medium occupies about 10% to about 30% of the volume of the shake tube and contains a plurality of microcarriers at a concentration of about 1.0 g/L to about 15.0 g/L;

incubating the shake tube for a period of time at about 32 ºC to about 39 ºC, with a rotary agitation of about 130 revolutions per minute (RPM) to about 150 RPM, and at a reactor angle of about 25 degrees to about 90 degrees; and

after about the first 48 to 96 hours of the period of time, continuously or periodically 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 about equal;

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

comparing the amount of recombinant protein present in the adherent mammalian cell or in the first and/or second culture medium to a reference level of recombinant protein produced by a different method that uses one or more of a different first or second liquid culture medium, a different raw material to generate the first or second liquid culture medium, or a different source of the adherent mammalian cell; and

identifying the first or second liquid culture medium, the raw material used to generate the first or second liquid culture medium, or the source of adherent mammalian cell as containing a contaminant when the level of recombinant protein produced is less than the reference level.

115. The method of claim 114, wherein the contaminant is a biological contaminant.

116. The method of claim 115, wherein the biological contaminant is selected from the group consisting of: mycobacterium, a fungus, a bacterium, a virus, and an undesired mammalian cell.

117. The method of claim 114, wherein the shake tube is incubated at a reactor angle of about 25 degrees to about 60 degrees from horizontal.

118. The method of any one of claims 114-117, wherein a viable cell concentration in the first and/or second liquid culture medium of greater than 2×10^6 cells/mL is achieved at some point during the period of time.