CLAIMS

1. A composition comprising an in vitro-transcribed (IVT) RNA and a pharmaceutically acceptable excipient, wherein the composition is substantially free of reverse transcription complement product

2. The composition of claim 1, wherein greater than about 80% of the mass of the RNA comprises single stranded full length transcripts.

3. The composition of claim 2, wherein greater than about 90% of the mass of the RNA comprises single stranded full length transcripts.

4. The composition of claim 2, wherein greater than about 95% of the mass of the RNA comprises single stranded full length transcripts.

5. The composition of claim 2, wherein greater than about 96% of the mass of the RNA comprises single stranded full length transcripts.

6. The composition of claim 2, wherein greater than about 97% of the mass of the RNA comprises single stranded full length transcripts.

7. The composition of claim 2, wherein greater than about 98% of the mass of the RNA comprises single stranded full length transcripts.

8. The composition of claim 2, wherein greater than about 99% of the mass of the RNA comprises single stranded full length transcripts.

9. The composition of claim 2, wherein greater than about 99.5% of the mass of the RNA comprises single stranded full length transcripts.

10. The composition of claim 2, wherein 100% of the mass of the single stranded RNA comprises single stranded full length transcripts.

11. The composition of any one of claims 2-10, wherein the composition is substantially free of RNAse III insensitive fragments.

13. The composition of any one of claims 2-10, wherein the composition comprises a population of single stranded partial RNA transcripts in a sense orientation and wherein greater than 80% of the population of single stranded partial RNA transcripts in a sense orientation has a nucleotide length of 100 nucleotides or less.

14. The composition of claim 13, wherein greater than 90% of the population of single stranded partial RNA transcripts in a sense orientation has a nucleotide length of 100 nucleotides or less.

15. The composition of claim 13, wherein greater than 95% of the population of single stranded partial RNA transcripts in a sense orientation has a nucleotide length of 100 nucleotides or less.

16. The composition of claim 13, wherein greater than 98% of the population of single stranded partial RNA transcripts in a sense orientation has a nucleotide length of 100 nucleotides or less.

17. The composition of claim 13, wherein greater than 99% of the population of single stranded partial RNA transcripts in a sense orientation has a nucleotide length of 100 nucleotides or less.

18. The composition of claim 13, wherein 100% of the population of single stranded partial RNA transcripts in a sense orientation has a nucleotide length of 100 nucleotides or less.

19. The composition of claim 1, wherein less than about 0.5% of the mass of the RNA in the composition is reverse complement transcription product.

20. The composition of claim 1, wherein less than about 0.25% of the mass of the RNA in the composition is reverse complement transcription product.

21. The composition of claim 1, wherein less than about 0.1% of the mass of the RNA in the composition is reverse complement transcription product.

22. The composition of claim 1, wherein less than about 0.05% of the mass of the RNA in the composition is reverse complement transcription product.

23. The composition of claim 1, wherein less than about 0.01% of the mass of the RNA in the composition is reverse complement transcription product.

24. The composition of claim 1, wherein less than about 0.005% of the mass of the RNA in the composition is reverse complement transcription product.

25. The composition of claim 1, wherein less than about 0.001% of the mass of the RNA in the composition is reverse complement transcription product.

26. The composition of claim 1, wherein less than about 5% of the mass of the RNA in the composition is reverse complement transcription product.

27. The composition of any one of claims 1 to 26, wherein the mass of the RNA in the composition is determined by LC or MS.

28. A composition comprising an in vitro-transcribed (IVT) RNA and a pharmaceutically acceptable excipient, wherein the composition is substantially free of cytokine-inducing RNA contaminant.

29. The composition of claim 28, wherein greater than about 80% of the mass of the RNA comprises single stranded full length transcripts.

30. The composition of claim 29, wherein greater than about 90% of the mass of the RNA comprises single stranded full length transcripts.

31. The composition of claim 29, wherein greater than about 95% of the mass of the RNA comprises single stranded full length transcripts.

32. The composition of claim 29, wherein greater than about 96% of the mass of the RNA comprises single stranded full length transcripts.

33. The composition of claim 29, wherein greater than about 97% of the mass of the RNA comprises single stranded full length transcripts.

34. The composition of claim 29, wherein greater than about 98% of the mass of the RNA comprises single stranded full length transcripts.

35. The composition of claim 29, wherein greater than about 99% of the mass of the RNA comprises single stranded full length transcripts.

36. The composition of claim 29, wherein greater than about 99.5% of the mass of the RNA comprises single stranded full length transcripts.

37. The composition of claim 29, wherein 100% of the mass of the single stranded RNA comprises single stranded full length transcripts.

38. The composition of any one of claims 29-37, wherein the composition is substantially free of RNAse III insensitive fragments.

39. The composition of any one of claims 29-38, wherein the composition comprises a population of single stranded partial RNA transcripts in a sense orientation and wherein greater than 80% of the population of single stranded partial RNA transcripts in a sense orientation has a nucleotide length of 100 nucleotides or less.

40. The composition of claim 39, wherein greater than 90% of the population of single stranded partial RNA transcripts in a sense orientation has a nucleotide length of 100 nucleotides or less.

41. The composition of claim 39, wherein greater than 95% of the population of single stranded partial RNA transcripts in a sense orientation has a nucleotide length of 100 nucleotides or less.

42. The composition of claim 39, wherein greater than 98% of the population of single stranded partial RNA transcripts in a sense orientation has a nucleotide length of 100 nucleotides or less.

43. The composition of claim 39, wherein greater than 99% of the population of single stranded partial RNA transcripts in a sense orientation has a nucleotide length of 100 nucleotides or less.

44. The composition of claim 39, wherein 100% of the population of single stranded partial RNA transcripts in a sense orientation has a nucleotide length of 100 nucleotides or less.

45. The composition of claim 28, wherein less than about 0.5% of the mass of the RNA in the composition is cytokine-inducing RNA contaminant.

46. The composition of claim 28, wherein less than about 0.25% of the mass of the RNA in the composition is cytokine-inducing RNA contaminant.

47. The composition of claim 28, wherein less than about 0.1% of the mass of the RNA in the composition is cytokine-inducing RNA contaminant.

48. The composition of claim 28, wherein less than about 0.05% of the mass of the RNA in the composition is cytokine-inducing RNA contaminant.

49. The composition of claim 28, wherein less than about 0.01% of the mass of the RNA in the composition is cytokine-inducing RNA contaminant.

50. The composition of claim 28, wherein less than about 0.005% of the mass of the RNA in the composition is cytokine-inducing RNA contaminant.

51. The composition of claim 28, wherein less than about 0.001% of the mass of the RNA in the composition is cytokine-inducing RNA contaminant.

52. The composition of any one of claims 12 to 18, wherein the mass of the RNA in the composition is determined by LC or MS.

53. The composition of claim 1, wherein the reverse complement transcription product is a dsRNA comprising a strand comprising a sequence which is a reverse complement of at least a portion of the IVT RNA or a dsRNA comprising a strand comprising a polyU containing sequence.

54. The composition of claim 53, wherein the strand comprising the sequence which is the reverse complement of the IVT RNA or the strand comprising the polyU sequence initiates with a 5' triphosphate (5'-PPP).

55. The composition of claim 1, wherein the reverse complement transcription product comprises a reverse complement of the 5'-end of the IVT RNA and/or a reverse complement of the 3'-end of the RNA encoding the polypeptide of interest.

56. The composition of claim 1, wherein the IVT RNA is an RNA encoding a polypeptide of interest and wherein the reverse complement of the RNA encoding the polypeptide of interest comprises a sequence complementary to all or a portion of an open reading frame of the RNA encoding the polypeptide of interest.

57. The composition of claim 54, wherein the reverse complement of the 5'-end of the IVT RNA comprises a sequence complementary to all or a portion of a 5' UTR of the RNA encoding the polypeptide of interest.

58. The composition of claim 54, wherein the reverse complement of the 3'-end of the IVT RNA comprises a sequence complementary to all or a portion of a polyA tail of the RNA encoding the polypeptide of interest.

59. The composition of claim 28, wherein the cytokine-inducing RNA-contaminant is a dsRNA comprising a strand comprising a reverse sequence which is a reverse complement of the IVT RNA or a dsRNA comprising a strand comprising a polyU sequence.

60. The composition of claim 59, wherein the strand comprising the sequence which is the reverse complement of the IVT RNA or the strand comprising the polyU sequence initiates with a 5' triphosphate (5'-PPP).

61. The composition of claim 59, wherein the cytokine-inducing RNA-contaminant comprises a reverse complement of the 5'-end of the IVT RNA and/or a reverse complement of the 3'-end of the IVT RNA.

62. The composition of claim 61, wherein the reverse complement of the 5'-end of the IVT RNA comprises a sequence complementary to all or a portion of a 5' UTR of the IVT RNA.

63. The composition of 61, wherein the reverse complement of the 3'-end of the RNA encoding the polypeptide of interest comprises a sequence complementary to all or a portion of a polyA tail of the IVT RNA .

64. The composition of claim 53 or 59, wherein the dsRNA comprises strands of between 10 and 250 nucleotides in length.

65. The composition of claim 53 or 59, wherein the dsRNA is of duplex of between about 20 and about 50 bp in length.

66. The composition of claim 55 or 61, wherein the reverse complement comprises a sequence complementary the first 10-15 nucleotides of the 5' UTR.

67. The composition of claim 1 or 2, wherein less than about 0.5% of the mass of the RNA in the composition is dsRNA of a size greater than 40 base pairs.

68. The composition of any one of the preceding claims, wherein the amount of reverse complement transcription product or cytokine-inducing RNA contaminant is determined by high-

performance liquid chromatography (such as reverse-phase chromatography, size-exclusion chromatography), Bioanalyzer chip-based electrophoresis system, ELISA, flow cytometry, a reconstitution or surrogate type assay.

69. The composition of any one of the preceding claims, wherein the mass of RNA is determined by mass spectrometry such as MALDI-TOF (matrix-assisted laser desorption ionization time of flight).

70. The composition of any one of claims 1 to 69, wherein the composition comprises abortive transcripts.

71. The composition of any one of the preceding claims, wherein the RNA is produced by a process comprising

(a) forming a reaction mixture comprising a DNA template and NTPs including adenosine triphosphate (ATP), cytidine triphosphate (CTP), uridine triphosphate (UTP), guanosine triphosphate (GTP), and a magnesium-containing buffer,

(b) incubating the reaction mixture under conditions such that the RNA is transcribed, wherein:

1) the concentration of at least one of GTP, CTP, ATP, and UTP is at least 2X greater than the concentration of any one or more of ATP, CTP or UTP or
2) the reaction further comprises a nucleotide diphosphate (NDP) or a nucleotide analog and wherein the concentration of the NDP or nucleotide analog is at least 2X greater than the concentration of any one or more of ATP, CTP or UTP.

72. The composition of claim 71, wherein the ratio of the concentration of at least one of GTP, CTP, ATP, and UTP to the concentration of any one or more of ATP, CTP or UTP is at least 2:1, at least 3:1, at least 4:1, at least 5:1, at least 6:1, at least 7:1, at least 8:1, at least 9:1, or at least 10:1.

73. The composition of claim 71, wherein the reaction further comprises a nucleotide diphosphate (NDP) or a nucleotide analog and wherein the ratio of the concentration of the NDP or nucleotide analog to the concentration of any one or more of ATP, CTP or UTP is at least 2:1,

at least 3:1, at least 4:1, at least 5:1, at least 6:1, at least 7:1, at least 8:1, at least 9:1, or at least 10:1.

74. The composition of claim 71, wherein the ratio of concentration of GTP to concentration of ATP, CTP and UTP is 2:1, 4:1 and 4:1, respectively.

75. The composition of claim 71, wherein the ratio of concentration of GTP to concentration of ATP, CTP and UTP is 3:1, 6:1 and 6:1, respectively.

76. The composition of claim 71, wherein the reaction mixture comprises GTP and GDP and wherein the ratio of concentration of GTP plus GDP to the concentration of any one of ATP, CTP or UTP is at least 2:1, at least 3:1, at least 4:1, at least 5:1 or at least 6:1

77. The composition of claim 76, wherein the ratio of concentration of GTP plus GDP to concentration of ATP, CTP and UTP is 3:1, 6:1 and 6:1, respectively.

78. The composition of any one of the preceding claims, wherein the RNA is produced by a process comprising

(a) forming a reaction mixture comprising a DNA template and NTPs including adenosine triphosphate (ATP), cytidine triphosphate (CTP), uridine triphosphate (UTP), guanosine triphosphate (GTP), and a buffer magnesium-containing buffer,

(b) incubating the reaction mixture under conditions such that the RNA is transcribed, wherein the effective concentration of phosphate in the reaction is at least 150 mM phosphate, at least 160 mM, at least 170 mM, at least 180 mM, at least 190 mM, at least 200 mM, at least 210 mM or at least 220 mM.

79. The composition of claim 78, wherein the effective concentration of phosphate in the reaction is 180 mM.

80. The composition of claim 78, wherein the effective concentration of phosphate in the reaction is 195 mM.

81. The composition of claim 78, wherein the effective concentration of phosphate in the reaction is 225 mM.

82. The composition of any one of the preceding claims, wherein the RNA is produced by a process comprising

(a) forming a reaction mixture comprising a DNA template and NTPs including adenosine triphosphate (ATP), cytidine triphosphate (CTP), uridine triphosphate (UTP), guanosine triphosphate (GTP) and optionally guanosine diphosphate (GDP), and a buffer magnesium-containing buffer,

(b) incubating the reaction mixture under conditions such that the RNA is transcribed, wherein the magnesium-containing buffer comprises Mg2+ and wherein the molar ratio of concentration of ATP plus CTP plus UTP pus GTP and optionally GDP to concentration of Mg2+ is at least 1.0, at least 1.25, at least 1.5, at least 1.75, at least 1.85 or higher.

83. The composition of claim 82, wherein the molar ratio of concentration of ATP plus CTP plus UTP pus GTP and optionally GDP to concentration of Mg2+ is 1.5.

84. The composition of claim 82, wherein the molar ratio of concentration of Mg2+ to concentration of ATP plus CTP plus UTP pus GTP and optionally GDP to concentration of Mg2+ is 1.88.

85. The composition of any one of claims 1 to 84, produced by a process which does not comprise an dsRNase (e.g., RNaseIII) treatment step.

86. The composition of any one of claims 1 to 84, produced by a process which does not comprise a reverse phase (RP) chromatography purification step.

87. The composition of any one of claims 1 to 84, produced by a process which does not comprise a high-performance liquid chromatography (HPLC) purification step.

88. The composition of any one of claims 1 to 84, produced by a process which further comprises RP purification and/or RNase III treatment.

89. The composition of any of claims 1-84, wherein the RNA is modified mRNA.

90. The composition of any of claims 1-84, wherein the RNA includes a UTP and the UTP is modified UTP.

91. The composition of claim 90, wherein the RNA is 100% modified.

92. The composition of claim 90, wherein only a portion of the RNA is modified.

93. The composition of any one of claims 1-84, wherein the amount of reverse complement transcription product or cytokine-inducing species is determined indirectly by a process comprising:

(a) producing a composition comprising a model RNA from a DNA template encoding the model RNA under identical IVT conditions as used to produce the IVT RNA , and

(b) determining the amount of reverse complement transcription product or cytokine-inducing species by LC-MS in the composition comprising the model RNA,

wherein the amount of reverse complement transcription product or cytokine-inducing species by LC-MS in the composition comprising the model RNA indicates the amount of reverse complement transcription product or cytokine-inducing species in the composition comprising the IVT RNA.

94. An in vitro-transcribed (IVT) RNA composition wherein the RNA is not subject to RNaseIII treatments and/or is not subject to RP purification.

95. A composition comprising an in vitro-transcribed (IVT) single stranded RNA encoding a polypeptide of interest and a pharmaceutically acceptable excipient, wherein greater than 98% of the RNA is single stranded and wherein the single stranded RNA comprises transcripts of

different lengths, wherein greater than about 80% of the single stranded RNA comprises single stranded full length transcripts.

96. The composition of claim 95, wherein greater than about 90% of the single stranded RNA comprises single stranded full length transcripts.

97. The composition of claim 95, wherein greater than about 95% of the single stranded RNA comprises single stranded full length transcripts.

98. The composition of claim 95, wherein greater than about 96% of the single stranded RNA comprises single stranded full length transcripts.

99. The composition of claim 95, wherein greater than about 97% of the single stranded RNA comprises single stranded full length transcripts.

100. The composition of claim 95, wherein greater than about 98% of the single stranded RNA comprises single stranded full length transcripts.

101. The composition of claim 95, wherein greater than about 99% of the single stranded RNA comprises single stranded full length transcripts.

102. The composition of claim 95, wherein greater than about 99.5% of the single stranded RNA comprises single stranded full length transcripts.

103. The composition of any one of claims 95-102, wherein the composition is substantially free of RNAse III insensitive fragments.

104. The composition of any one of claims 95-102, wherein the single stranded RNA comprises a population of single stranded partial RNA transcripts in a sense orientation and wherein greater than 80% of the population of single stranded partial RNA transcripts in a sense orientation has a nucleotide length of 100 nucleotides or less.

105. The composition of claim 104, wherein greater than 90% of the population of single stranded partial RNA transcripts in a sense orientation has a nucleotide length of 100 nucleotides or less.

106. The composition of claim 104, wherein greater than 95% of the population of single stranded partial RNA transcripts in a sense orientation has a nucleotide length of 100 nucleotides or less.

107. The composition of claim 104, wherein greater than 98% of the population of single stranded partial RNA transcripts in a sense orientation has a nucleotide length of 100 nucleotides or less.

108. The composition of claim 104, wherein greater than 99% of the population of single stranded partial RNA transcripts in a sense orientation has a nucleotide length of 100 nucleotides or less.

109. The composition of claim 104, wherein 100% of the population of single stranded partial RNA transcripts in a sense orientation has a nucleotide length of 100 nucleotides or less.

110. The composition of claim 95, wherein the single stranded RNA comprising transcripts of different lengths includes full length transcript and abortive transcripts.

111. The composition of claim 95, wherein 80-98% of the population of single stranded partial RNA transcripts in a sense orientation comprise abortive transcripts.

112. The composition of claim 95, wherein 95-98% of the population of single stranded partial RNA transcripts in a sense orientation comprise abortive transcripts.

113. A unit of use composition comprising an in vitro-transcribed (IVT) single stranded RNA encoding a polypeptide of interest and a pharmaceutically acceptable excipient, wherein the composition is free of residual organic solvents or alkylammonium ions.

114. A composition comprising an in vitro-transcribed (IVT) single stranded RNA encoding a polypeptide of interest and a pharmaceutically acceptable excipient, wherein the composition is non-immunogenic and wherein the single stranded RNA comprises transcripts of different lengths.

115. The composition of claim 114, wherein the single stranded RNA comprising transcripts of different lengths includes full length transcript and abortive transcripts.

116. A method of preparing RNA comprising

(a) forming a reaction mixture comprising a DNA template and and NTPs including adenosine triphosphate (ATP), cytidine triphosphate (CTP), uridine triphosphate (UTP), guanosine triphosphate (GTP) and optionally guanosine diphosphate (GDP) or an analog of each respective nucleotide, and a buffer magnesium-containing buffer, and

(b) incubating the reaction mixture under conditions such that the RNA is transcribed, wherein:

1) the concentration of at least one of GTP, CTP, ATP, and UTP is at least 2X greater than the concentration of any one or more of ATP, CTP or UTP or

2) the reaction further comprises a nucleotide diphosphate (NDP) or a nucleotide analog and wherein the concentration of the NDP or nucleotide analog is at least 2X greater than the concentration of any one or more of ATP, CTP or UTP,

117. The method of claim 116, wherein the ratio of concentration of GTP to the concentration of any one ATP, CTP or UTP is at least 2:1, at least 3:1, at least 4:1, at least 5:1 or at least 6:1 to produce the RNA.

118. The method of claim 116, wherein the ratio of concentration of GTP to concentration of ATP, CTP and UTP is 2:1, 4:1 and 4:1, respectively.

119. The method of claim 116, wherein the ratio of concentration of GTP to concentration of ATP, CTP and UTP is 3:1, 6:1 and 6:1, respectively.

120. The method of claim 116, wherein the reaction mixture comprises GTP and GDP and wherein the ratio of concentration of GTP plus GDP to the concentration of any one of ATP, CTP or UTP is at least 2:1, at least 3:1, at least 4:1, at least 5:1 or at least 6:1

121. The method of claim 120, wherein the ratio of concentration of GTP plus GDP to concentration of ATP, CTP and UTP is 3:1, 6:1 and 6:1, respectively.