CLAIMS

What is claimed is:

1. A messenger ribonucleic acid (mRNA) comprising an open reading frame (ORF) that encodes a SARS-CoV-2 spike (S) protein having a double proline stabilizing mutation.

2. The mRNA of claim 1, wherein the double proline stabilizing mutation is at positions corresponding to K986 and V987 of a wild-type SARS-CoV-2 S protein.

3. The mRNA of claim 1, wherein the coronavirus antigen comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% identity to the amino acid sequence of SEQ ID NO: 29.

4. The mRNA of claim 1, wherein the SARS-CoV-2 S protein comprises an amino acid sequence of SEQ ID NO: 29.

5. The mRNA of claim 1, wherein the mRNA comprises a 5′ untranslated region (UTR) and a 3′ UTR.

6. The mRNA of claim 5, wherein the 5′ UTR comprises the nucleotide sequence of SEQ ID NO: 2 or SEQ ID NO: 36 and/or the 3′ UTR comprises the nucleotide sequence of SEQ ID NO: 4 or SEQ ID NO: 37.

7. A composition comprising a lipid nanoparticle and a messenger RNA (mRNA) comprising an open reading frame (ORF) that encodes a SARS-CoV-2 spike (S) protein having a double proline stabilizing mutation of a wild-type SARS-CoV-2 S protein.

8. The composition of claim 7, wherein the lipid nanoparticle comprises a PEG-modified lipid, a non-cationic lipid, a sterol, an ionizable cationic lipid, or any combination thereof.

9. The composition of claim 8, wherein the lipid nanoparticle comprises 0.5-15 mol% PEG-modified lipid; 5-25 mol% non-cationic lipid; 25-55 mol% sterol; and 20-60 mol% ionizable cationic lipid.

10. A messenger ribonucleic acid (mRNA) comprising an open reading frame (ORF) that comprises a nucleotide sequence having at least 80% identity to the nucleotide sequence of SEQ ID NO: 28 and encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 29.

11. The mRNA of claim 10, wherein the ORF comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, or at least 98% identity to the nucleotide sequence of SEQ ID NO: 28.

12. The mRNA of claim 10, wherein the ORF comprises a nucleotide sequence of SEQ ID NO: 28.

13. The mRNA of claim 10, wherein the mRNA comprises a nucleotide sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% identity to the nucleotide sequence of SEQ ID NO: 27.

14. The mRNA of claim 10, wherein the mRNA comprises a nucleotide sequence of SEQ ID NO: 27.

15. The mRNA of claim 10, wherein the RNA comprises a 5′ untranslated region (UTR) and a 3′ UTR.

16. The mRNA of claim 15, wherein the 5′ UTR comprises the nucleotide sequence of SEQ ID NO: 2 or SEQ ID NO: 36.

17 The mRNA of claim 15, wherein the 3′ UTR comprises the nucleotide sequence of SEQ ID NO: 4 or SEQ ID NO: 37.

18. The mRNA of any one of claims 10-17, wherein the mRNA comprises a chemical modification.

19. The mRNA of claim 18, wherein the mRNA is chemically modified with 1-methyl-pseudouridine, such that each U in the sequence is a 1-methyl-pseudouridine.

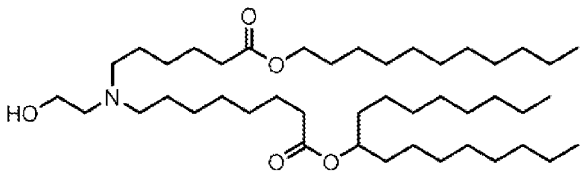
20. A composition comprising a lipid nanoparticle and a messenger RNA (mRNA) comprising an open reading frame (ORF) that comprises a nucleotide sequence having at least

80% identity to the nucleotide sequence of SEQ ID NO: 28 and encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 29.

21. The composition of claim 20, wherein the lipid nanoparticle comprises a PEG-modified lipid, a non-cationic lipid, a sterol, an ionizable cationic lipid, or any combination thereof.

22. The composition of claim 21, wherein the lipid nanoparticle comprises 0.5-15 mol% PEG-modified lipid; 5-25 mol% non-cationic lipid; 25-55 mol% sterol; and 20-60 mol% ionizable cationic lipid.

23. The composition of claim 22, wherein the PEG-modified lipid is 1,2 dimyristoyl-sn-glycerol, methoxypolyethyleneglycol (PEG2000 DMG), the non-cationic lipid is 1,2 distearoyl-sn-glycero-3-phosphocholine (DSPC), the sterol is cholesterol; and the ionizable cationic lipid has the structure of Compound 1:



(Compound 1).

24. A messenger ribonucleic acid (mRNA) comprising an open reading frame (ORF) that comprises a nucleotide sequence having at least 80% identity to the nucleotide sequence of any one of SEQ ID NOs 3, 7, 10, 13, 16, 19, 22, 25, 31, 48, 50, 52, 54, 56, 61, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, or 106.

25. The mRNA of claim 24, wherein the ORF comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 98%, or at least 100% identity to the nucleotide sequence of any one of SEQ ID NOs.: 3, 7, 10, 13, 16, 19, 22, 25, 31, 48, 50, 52, 54, 56, 61, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, or 106.

26. The mRNA of claim 24, wherein the ORF encodes a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs.: 5, 8, 11, 14, 17, 20, 23, 26, 32, 33, 34, 35, 47, 49, 59, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, or 85.

27. The mRNA of claim 24, wherein the ORF encodes a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 98%

identity of any one of SEQ ID NOs.: 5, 8, 11, 14, 17, 20, 23, 26, 32, 33, 34, 35, 47, 49, 59, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, or 85.

28. The mRNA of claim 24, wherein the RNA comprises a 5′ untranslated region (UTR) and a 3′ UTR.

29. The mRNA of claim 28, wherein the 5′ UTR comprises the nucleotide sequence of SEQ ID NO: 2 or SEQ ID NO: 36.

30 The mRNA of claim 28, wherein the 3′ UTR comprises the nucleotide sequence of SEQ ID NO: 4 or SEQ ID NO: 37.

31. The mRNA of claim 24, wherein the mRNA comprises a chemical modification.

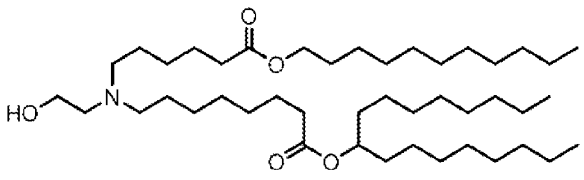
32. The mRNA of claim 31, wherein the mRNA is chemically with 1-methyl-pseudouridine, such that each U in the sequence is a 1-methyl-pseudouridine.

33. A composition comprising a lipid nanoparticle and a messenger RNA (mRNA), wherein the mRNA is an mRNA of any of claims 24-33.

34. The composition of claim 33, wherein the lipid nanoparticle comprises a PEG-modified lipid, a non-cationic lipid, a sterol, an ionizable cationic lipid, or any combination thereof.

35. The composition of claim 34, wherein the lipid nanoparticle comprises 0.5-15 mol% PEG-modified lipid; 5-25 mol% non-cationic lipid; 25-55 mol% sterol; and 20-60 mol% ionizable cationic lipid.

36. The composition of claim 35, wherein the PEG-modified lipid is 1,2 dimyristoyl-sn-glycerol, methoxypolyethyleneglycol (PEG2000 DMG), the non-cationic lipid is 1,2 distearoyl-sn-glycero-3-phosphocholine (DSPC), the sterol is cholesterol; and the ionizable cationic lipid has the structure of Compound 1:



(Compound 1).

37. A method comprising administering to a subject a messenger ribonucleic acid (mRNA) comprising an open reading frame (ORF) that encodes a SARS-CoV-2 spike (S) protein antigen having a double proline stabilizing mutation in an amount effective to induce a neutralizing antibody response against SARS-CoV-2 in the subject.

38. The method of claim 37, wherein the double proline stabilizing mutation is at positions corresponding to K986 and V987 of a wild-type SARS-CoV-2 S protein.

39. The method of any one of claims 37-38, wherein the mRNA is administered in an amount effective to induce a T cell immune response, optionally a $CD4^+$ and/or a $CD8^+$ T cell immune response against SARS-CoV-2 in the subject.

40. The method of any one of claims 37-39, wherein the subject is immunocompromised.

41. The method of any one of claims 37-39, wherein the subject has a pulmonary disease.

42. The method of any one of claims 37-39, wherein the subject is 65 years of age or older.

43. The method of any one of claims 37-39, comprising administering to the subject at least two doses of the composition.

44. The method of any one of claims 37-43, wherein detectable levels of the SARS-CoV-2 S protein are produced in serum of the subject at 1-72 hours post administration of the RNA or composition comprising the RNA.

45. The method of any one of claims 37-43, wherein a neutralizing antibody titer of at least 100 NU/ml, at least 500 NU/ml, or at least 1000 NU/ml is produced in the serum of the subject at 1-72 hours post administration of the RNA.

46. An immunizing composition comprising:

(a) a first ribonucleic acid (RNA) comprising an open reading frame (ORF) that encodes a coronavirus antigen capable of inducing an immune response, such as a neutralizing antibody response, to a SARS-CoV-2; and

(b) a second ribonucleic acid (RNA) comprising an open reading frame (ORF) that encodes a coronavirus antigen capable of inducing an immune response, such as a neutralizing antibody response, to a SARS-CoV-2, wherein the ORF of the first RNA is different from the ORF of the second RNA.