“Coronavirus Vaccine”, the contents of each of which are incorporated herein by reference for purposes described herein.)

[5a] In one aspect, the present disclosure provides a formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that comprises one or more mRNAs; ii) lipids that include:((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of 8:1:1.5:3 to 9:1:2:3.5; b) sucrose at a concentration of about 10% w/v; trehalose at a concentration of about 10% w/v; or sucrose at a concentration of about 5% w/v, and trehalose at a concentration of about 5% w/v; and c) Tris buffer, wherein i) no sodium chloride salt is added to the Tris buffer, or ii) the Tris buffer comprises about 6 mg/ml sodium chloride, and is at a concentration of about 10 mM. In another aspect, the present disclosure provides a method of preparing a formulation of as described herein, the method comprising steps of: a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises: i) a payload that comprises one or more mRNAs; ii) lipids that include:((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of 8:1:1.5:3 to 9:1:2:3.5; and b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises: i) Tris buffer, wherein (i) no sodium chloride salt is added to the Tris buffer, or (ii) the Tris buffer comprises about 6 mg/ml sodium chloride, and is at a concentration of about 10 mM; and ii) sucrose at a concentration of about 10% w/v; trehalose at a concentration of about 10% w/v; or sucrose at a concentration of about 5% w/v, and trehalose at a concentration of about 5% w/v. In yet another aspect, the present disclosure provides a dosage form of a formulation, wherein the formulation comprises: a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml; ii) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml; iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml; iv) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml; v) cholesterol at a concentration of about 3.1 mg/ml; b) sucrose at a concentration of about 10% w/v; trehalose at a concentration of about 10% w/v; or sucrose at a concentration of about 5% w/v, and trehalose at a concentration of about 5% w/v; c) Tris buffer, wherein i) no sodium chloride salt is added to the Tris buffer, or ii) the Tris buffer comprises about 6 mg/ml sodium chloride, and is at a concentration of about 10 mM; wherein the dosage form is diluted from the formulation.

[5b] In a further aspect, the present disclosure provides a formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of 8:1:1.5:3 to 9:1:2:3.5; and b) sucrose at a concentration of about 10% w/v; trehalose at a concentration of about 10% w/v; or sucrose at a concentration of about 5% w/v, and trehalose at a concentration of about 5% w/v; and c) His buffer, wherein the His buffer is at a concentration of about 10 mM and no sodium chloride salt is added; or HEPES buffer, wherein the HEPES buffer is at a concentration of about 10 mM and no sodium chloride salt is added. In another aspect, the present disclosure provides a method of preparing a formulation as described herein, the method comprising steps of: a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises: i) a payload that comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of 8:1:1.5:3 to 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises: i) His buffer, wherein the His buffer is at a concentration of about 10 mM and no sodium chloride salt is added; or HEPES buffer, wherein the HEPES buffer is at a concentration of about 10 mM and no sodium chloride salt is added; and ii) sucrose at a concentration of about 10% w/v; trehalose at a concentration of about 10% w/v; or sucrose at a concentration of about 5% w/v, and trehalose at a concentration of about 5% w/v. In another aspect, the present disclosure provides a dosage form of a formulation, wherein the formulation comprises: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) mRNA at a concentration of about 0.5 mg/ml; ii) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml; iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml; iv) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml; v) cholesterol at a concentration of about 3.1 mg/ml; b) sucrose at a concentration of about 10% w/v; trehalose at a concentration of about 10% w/v; or sucrose at a concentration of about 5% w/v, and trehalose at a concentration of about 5% w/; and d) His buffer, wherein the His buffer at a concentration of about 10 mM and no sodium chloride salt is added; or HEPES buffer, wherein the HEPES buffer is at a concentration of about 10 mM and no sodium chloride salt is added; wherein the dosage form is diluted from the formulation.

[5c] In another aspect, the present disclosure provides a formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of 8:1:1.5:3 to 9:1:2:3.5; and b) sucrose at a concentration of about 10% w/v; and c) PBS buffer, wherein i) no sodium chloride salt is added to the PBS buffer, or ii) the PBS buffer comprises about 6 mg/ml sodium chloride.

In yet another aspect, the present disclosure provides a method of preparing a formulation as described herein, the method comprising steps of: a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises: i) a payload that comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of 8:1:1.5:3 to 9:1:2:3.5; and b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises: i) PBS buffer, wherein i) no sodium chloride salt is added to the PBS buffer, or ii) the PBS buffer comprises about 6 mg/ml sodium chloride; and ii) sucrose at a concentration of about 10% w/v. In yet another aspect, the present disclosure provides a dosage form of a formulation, wherein the formulation comprises: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) mRNA at a concentration of about 0.5 mg/ml; ii) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml; iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml; iv) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml; v) cholesterol at a concentration of about 3.1 mg/ml; b) sucrose at a concentration of about 10% w/v; and c) PBS buffer, wherein i) no sodium chloride salt is added to the PBS buffer, or ii) the PBS buffer comprises about 6 mg/ml sodium chloride; wherein the dosage form is dilution from the formulation. In yet another aspect, the present disclosure provides a method of preparing a formulation as described herein, the method comprising steps of: a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises: i) a payload that comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of 8:1:1.5:3 to 9:1:2:3.5; and b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises: i) PBS buffer, wherein the PBS buffer comprises about 6 mg/ml sodium chloride; and ii) sucrose at a concentration of about 10% w/v.

[5d] In another aspect, the present disclosure provides a dosage form of a formulation, wherein the formulation comprises: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) mRNA at a concentration of about 0.5 mg/ml; ii) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml; iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml; iv) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml; v) cholesterol at a concentration of about 3.1 mg/ml; b) sucrose at a concentration of about 10% w/v; and c) PBS buffer, wherein the PBS buffer comprises about 6 mg/ml sodium chloride; wherein the dosage form is diluted into the formulation. In yet another aspect, the present disclosure provides a method of preparing a formulation as described hereine, the method comprising steps of: a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises: i) a payload that comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of 8:1:1.5:3 to 9:1:2:3.5; and b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises: i) PBS buffer, wherein the PBS buffer comprises about 6 mg/ml sodium chloride; and ii) sucrose at a concentration of about 10% w/v wherein the first buffer system comprises sucrose at a concentration of about 10% w/v.

[5e] In another aspect, the present disclosure provides a formulation as described herein or a dosage form as described herein for use in delivering a nucleic acid into a cell in a subject. In another aspect, the present disclosure provides a formulation as described herein or a dosage form as described herein for use in inducing an immune response in a subject. In another aspect, the present disclosure provides a dose of the formulation as described herein, which dose includes 1 µg to 100 µg of mRNA. In another aspect, the present disclosure provides a dose of the formulation as described herein, which dose includes less than 60 µg of mRNA.

[6] In one example, a formulation provided herein comprises: (a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; (b) sucrose at a concentration of about 10% w/v in the formulation; and (c) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

[7] In some examples, a formulation provided herein is a frozen formulation comprising: (a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; (b) sucrose at a concentration of about 10% w/v in the formulation; and (c) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

[8] In some examples, a formulation provided herein is a dry formulation comprising: (a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; (b) sucrose at a concentration of about 10% w/v in the formulation before drying; and (c) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation before drying.

Claims

We claim:

1. A formulation comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of 8:1:1.5:3 to 9:1:2:3.5;

b) sucrose at a concentration of about 10% w/v;
trehalose at a concentration of about 10% w/v; or
sucrose at a concentration of about 5% w/v, and trehalose at a concentration of about 5% w/v; and

c) Tris buffer, wherein i) no sodium chloride salt is added to the Tris buffer, or ii) the Tris buffer comprises about 6 mg/ml sodium chloride, and is at a concentration of about 10 mM.

2. The formulation of claim 1, wherein the formulation is a frozen formulation.

3. A method of preparing a formulation of claim 1, the method comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of 8:1:1.5:3 to 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) Tris buffer, wherein (i) no sodium chloride salt is added to the Tris buffer, or (ii) the Tris buffer comprises about 6 mg/ml sodium chloride, and is at a concentration of about 10 mM; and

ii) sucrose at a concentration of about 10% w/v;
trehalose at a concentration of about 10% w/v; or
sucrose at a concentration of about 5% w/v, and trehalose at a concentration of about 5% w/v.

4. A dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) sucrose at a concentration of about 10% w/v;
trehalose at a concentration of about 10% w/v; or
sucrose at a concentration of about 5% w/v, and trehalose at a concentration of about 5% w/v;

c) Tris buffer, wherein i) no sodium chloride salt is added to the Tris buffer, or ii) the Tris buffer comprises about 6 mg/ml sodium chloride, and is at a concentration of about 10 mM;

wherein the dosage form is diluted from the formulation.

5. The formulation of claim 1, comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of 8:1:1.5:3 to 9:1:2:3.5; and

b) sucrose at a concentration of about 10% w/v; and

c) Tris buffer, wherein the Tris buffer is at a concentration of about 10 mM and no sodium chloride salt is added.

6. The method of claim 3, the method comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of 8:1:1.5:3 to 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) Tris buffer, wherein the Tris buffer is at a concentration of about 10 mM and no sodium chloride salt is added; and

ii) sucrose at a concentration of about 10% w/v.

7. The dosage form of claim 4, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) sucrose at a concentration of about 10% w/v; and

c) Tris buffer, wherein the Tris buffer is at a concentration of about 10 mM and no sodium chloride salt is added;

wherein the dosage form is diluted from the formulation.

8. A formulation comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of 8:1:1.5:3 to 9:1:2:3.5; and

b) sucrose at a concentration of about 10% w/v;

trehalose at a concentration of about 10% w/v; or

sucrose at a concentration of about 5% w/v, and trehalose at a concentration of about 5% w/v; and

c) His buffer, wherein the His buffer is at a concentration of about 10 mM and no sodium chloride salt is added; or

HEPES buffer, wherein the HEPES buffer is at a concentration of about 10 mM and no sodium chloride salt is added.

9. A method of preparing a formulation of claim 8, the method comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of 8:1:1.5:3 to 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) His buffer, wherein the His buffer is at a concentration of about 10 mM and no sodium chloride salt is added; or
HEPES buffer, wherein the HEPES buffer is at a concentration of about 10 mM and no sodium chloride salt is added; and

ii) sucrose at a concentration of about 10% w/v;
trehalose at a concentration of about 10% w/v; or
sucrose at a concentration of about 5% w/v, and trehalose at a concentration of about 5% w/v.

10. A dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) sucrose at a concentration of about 10% w/v;
trehalose at a concentration of about 10% w/v; or
sucrose at a concentration of about 5% w/v, and trehalose at a concentration of about 5% w/; and

c) His buffer, wherein the His buffer is at a concentration of about 10 mM and no sodium chloride salt is added; or

HEPES buffer, wherein the HEPES buffer is at a concentration of about 10 mM and no sodium chloride salt is added;

wherein the dosage form is diluted from the formulation.

11. A formulation comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of 8:1:1.5:3 to 9:1:2:3.5; and

b) sucrose at a concentration of about 10% w/v; and

c) PBS buffer, wherein i) no sodium chloride salt is added to the PBS buffer, or ii) the PBS buffer comprises about 6 mg/ml sodium chloride.

12. A method of preparing a formulation of claim 11, the method comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of 8:1:1.5:3 to 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) PBS buffer, wherein i) no sodium chloride salt is added to the PBS buffer, or ii) the PBS buffer comprises about 6 mg/ml sodium chloride; and

ii) sucrose at a concentration of about 10% w/v.

13. A dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) sucrose at a concentration of about 10% w/v; and

c) PBS buffer, wherein i) no sodium chloride salt is added to the PBS buffer, or ii) the PBS buffer comprises about 6 mg/ml sodium chloride;

wherein the dosage form is dilution from the formulation.

14. The formulation of claim 11, comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of 8:1:1.5:3 to 9:1:2:3.5; and

b) sucrose at a concentration of about 10% w/v;

c) PBS buffer, wherein the PBS buffer comprises about 6 mg/ml sodium chloride.

15. A method of preparing a formulation of claim 14, the method comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of 8:1:1.5:3 to 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) PBS buffer, wherein the PBS buffer comprises about 6 mg/ml sodium chloride; and

ii) sucrose at a concentration of about 10% w/v.

16. A dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) sucrose at a concentration of about 10% w/v; and

c) PBS buffer, wherein the PBS buffer comprises about 6 mg/ml sodium chloride;

wherein the dosage form is diluted into the formulation.

17. A method of preparing a formulation of claim 14, the method comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of 8:1:1.5:3 to 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) PBS buffer, wherein the PBS buffer comprises about 6 mg/ml sodium chloride; and

ii) sucrose at a concentration of about 10% w/v

wherein the first buffer system comprises sucrose at a concentration of about 10% w/v.

18. A formulation of any one of claims 1, 2, 5, 8, 11, or 14 or a dosage form according to any one of claims 4, 7, 10, 13 or 16 for use in delivering a nucleic acid into a cell in a subject.

19. A formulation of any one of claims 1, 2, 5, 8, 11, or 14 or a dosage form according to any one of claims 4, 7, 10, 13 or 16 for use in inducing an immune response in a subject.

20. The formulation or dosage form for use of claim 19, wherein the immune response is against a viral antigen, or epitope thereof, encoded by the mRNA.

21. The formulation or dosage form for use of claim 20, wherein the viral antigen is an antigen of a coronavirus.

22. The formulation or dosage form for use of claim 21, wherein the coronavirus is a SARS-CoV-2 virus.

23. The formulation or dosage form for use of claim 21 or claim 22, wherein the antigen comprises an S protein.

24. The formulation of any one of claims 1-2, 5, 8, 11, or 14, the method of any one of claims 3, 6, 9, 12, 15, or 17, the dosage form of any one of claims 4, 7, 10, 13 or 16, or the formulation or dosage form for use of any one of claims 18 to 23, wherein the one or more mRNAs encodes one or more polypeptides.

25. The formulation, method, dosage form or the formulation or dosage form for use of claim 24, wherein the one or more polypeptides comprise an epitope for inducing an immune response against an antigen in a subject.

26. The formulation of any one of claims 1-2, 5, 8, 11, or 14, wherein the one or more mRNAs comprise an epitope for inducing an immune response against an antigen in a subject.

27. The formulation of any one of claims 1-2, 5, 8, 11, 14, or 26, or the method of any one of claims 3, 6, 9, 12, 15, or 17, the dosage form of any one of claims 4, 7, 10, 13 or 16, or the formulation or dosage form for use of any one of claims 18 to 23, wherein the one or more RNAs comprise self-amplifying RNA molecules.

28. The formulation of any one of claims 1-2, 5, 8, 11, 14, 26 or 27, or the method of any one of claims 3, 6, 9, 12, 15, 17 or 27, the dosage form of any one of claims 4, 7, 10, 13, 16 or 27, or the formulation or dosage form for use of any one of claims 18 to 23 or 27, wherein the one or more RNAs comprise modified RNA molecules, or non-modified RNA molecules.

29. The formulation of any one of claims 1-2, 5, 8, 11, 14 or 26-28, or the method of any one of claims 3, 6, 9, 12, 15, 17, 27 or 28, the dosage form of any one of claims 4, 7, 10, 13, 16, 27 or 28, or the formulation or dosage form for use of any one of claims 18 to 23, 27 or 28, wherein the one or more RNAs comprise non-modified uridine RNA molecules.

30. The formulation of any one of claims 1-2, 5, 8, 11, 14, or 26-29, or the method of any one of claims 3, 6, 9, 12, 15, 17, 27 to 29, the dosage form of any one of claims 4, 7, 10, 13, 16, 27 to 29, or the formulation or dosage form for use of any one of claims 18 to 23, 27 to 29, wherein the one or more RNAs comprise nucleoside modified RNA molecules.

31. The formulation, method, dosage form or the formulation or dosage form for use of claim 24, wherein at least one of the one or more polypeptides is derived from a SARS-CoV-2 S Protein of SEQ ID NO: 1 or 7.

32. The formulation, method, dosage form or the formulation or dosage form for use of claim 24, wherein at least one of the one or more polypeptides comprises at least 85% sequence identity to the SARS-CoV-2 S Protein of SEQ ID NO: 1 or 7.

33. The formulation, method, dosage form or the formulation or dosage form for use of claim 24, wherein at least one of the one or more polypeptides is or comprises a full length SARS-CoV-2 S Protein of SEQ ID NO: 1 or 7.

34. The formulation, method, dosage form or the formulation or dosage form for use of claim 24, wherein at least one of the one or more polypeptides comprises at least 85% sequence identity to the Receptor Binding Domain (RBD) of SARS-CoV-2 S Protein of SEQ ID NO: 1 or 7.

35. The formulation, method, dosage form or the formulation or dosage form for use of claim 24, wherein at least one of the one or more polypeptides comprises a Receptor Binding Domain (RBD) of SARS-CoV-2 S Protein of SEQ ID NO: 1 or 7.

36. The formulation of any one of claims 1-2, 5, 8, 11, or 14, or the method of any one of claims 3, 6, 9, 12, 15, or 17, the dosage form of any one of claims 4, 7, 10, 13 or 16, or the formulation or dosage form for use of any one of claims 18 to 23, wherein the one or more mRNAs are associated or encapsulated in the LNP.

37. The formulation of any one of claims 5, 8, 11, or 14, wherein the formulation is a frozen formulation.

38. A dose of the formulation of any one of claims 1-2, 5, 8, 11, 14, 24-36, or 37, which dose includes 1 μg to 100 μg of mRNA.

39. A dose of the formulation of claims 1-2, 5, 8, 11, 14, 24-36, or 37, which dose includes less than 60 µg of mRNA.

40. The method of any one of claims 3, 6, 9, 12, 15, 17, 24, 25 or 27-36, wherein the formulation includes 1 µg to 100 µg of mRNA per dose.

41. The method of any one of claims 3, 6, 9, 12, 15, 17, 24, 25 or 27-36, wherein the formulation includes less than 60 µg of mRNA per dose.

42. The dosage form of any one of claims 4, 7, 10, 13, 16, 24, 25 or 27-36, or the dosage form for use of any one of claims 18-23, wherein 1 µg to 100 µg of mRNA is to be administered.

43. The dosage form of any one of claims 4, 7, 10, 13, 16, 24, 25 or 27-36, or the dosage form for use of any one of claims 18-23, wherein less than 60 µg of mRNA is to be administered.