Lipid Set (LNP Encapsulation)

Introductions

Lipid nanoparticles (LNPs) can be formed by rapidly mixing a lipid-containing ethanol phase with an acidic aqueous phase containing nucleic acid. Under acidic conditions, SM-102 becomes protonated and associates with negatively charged mRNA, facilitating nucleic acid encapsulation. After neutralization, the LNP surface approaches electrical neutrality. DSPC and cholesterol help maintain particle structure, whereas DMG-PEG2000 helps regulate particle formation, particle size, and colloidal stability. LNP encapsulation reduces direct exposure of mRNA to nucleases and supports cellular uptake and cytoplasmic delivery.

The kit provides four lipids, SM-102, DSPC, cholesterol, and DMG-PEG2000, together with 1 M Citrate Buffer (pH 3.2) for small-scale preparation of classical SM-102-based mRNA-LNPs. Prepare separate stock solutions of the four lipids in anhydrous ethanol, and then combine them at a molar ratio of 50:10:38.5:1.5 to prepare the Lipid Mix . Based on the reference condition of N/P = 6, one set theoretically supports the encapsulation of approximately 2 mg of mRNA.

Components and Storage

Components \ Size	1 Set	5 Set	10 Set
SM-102	30 mg	150 mg	300 mg
DSPC	10 mg	50 mg	100 mg
Cholesterol	20 mg	100 mg	200 mg
DMG-PEG2000	5 mg	25 mg	50 mg
1 M Citrate Buffer (pH 3.2)	10 mL	50 mL	100 mL

Shipping: blue ice **Storage: -20°C** **Shelf life: 2 years**

Protocol

The following procedure describes mRNA encapsulation using the classical formulation:

SM-102:DSPC:Cholesterol:DMG-PEG2000 = 50:10:38.5:1.5 (mol%)

Recommended starting conditions are N/P = 6, an aqueous-to-ethanol phase ratio of 3:1, and a total flow rate of 12 mL/min. Optimize the specific conditions according to the mRNA and mixing device.

1. Materials and Reagents Required but Not Provided

1) Anhydrous ethanol (molecular biology grade or equivalent), RNase-free water, and mRNA to be encapsulated; sterile 1× PBS (pH 7.4) or a validated Tris buffer; sterile sucrose solution if frozen storage is required.

2) RNase-free low-binding centrifuge tubes, syringes, and tubing; a 10-30 kDa MWCO dialysis device; and a 100 kDa MWCO centrifugal filter unit if concentration is required.

3) Nanoparticle mixer or compatible microfluidic device, magnetic stirrer, and refrigerated centrifuge. A DLS particle size analyzer and an assay for mRNA encapsulation efficiency are recommended.

2. Preparation of the Lipid-Anhydrous Ethanol Solution (Ethanol Phase)

After equilibrating each lipid to room temperature, prepare the individual stock solutions in anhydrous ethanol at the following concentrations: SM-102, 20 mg/mL; DSPC, 10 mg/mL; cholesterol, 20 mg/mL; and DMG-PEG2000, 5 mg/mL. Mix thoroughly and confirm that each solution is clear and free of visible precipitate. If dissolution is incomplete, incubate briefly at 37-50°C with intermittent mixing. Prepare 1 mL of Lipid Mix working solution at a total lipid concentration of 12.5 mM according to the table below:

Component	Stock Concentration (mg/mL)	Molecular Weight	Molar Percentage (%)	Final Concentration (mM)	1 mL Lipid Mix
SM-102	20	710.2	50	6.250	221.9 μL stock solution
DSPC	10	790.1	10	1.250	98.8 μL stock solution
Cholesterol	20	386.7	38.5	4.813	93.0 μL stock solution
DMG-PEG2000	5	2509.2	1.5	0.188	94.1 μL stock solution
Anhydrous ethanol					Bring to 1,000 μL (approximately 492.2 μL)

***Note: DMG-PEG2000 has an average molecular weight. Use the value specified for the product lot or by the supplier for calculations. Prepare the lipid stock solutions and Lipid Mix fresh. If short-term storage is required, seal, protect from light, aliquot, and store at -20°C. Before use, confirm complete re-dissolution and absence of precipitate.**

3. Preparation of the mRNA-Citrate Solution (Aqueous Phase)

1) The N/P ratio is the molar ratio of protonatable nitrogen atoms (N) in the ionizable lipid to phosphate groups (P) in the mRNA. An N/P ratio of 6 is recommended as the starting condition; optimization within the range of N/P = 4-8 may also be performed. Use the actual molecular weight of the mRNA sequence for the calculation whenever possible. If it is unknown, estimate using an average molecular weight of 330 g/mol per nucleotide residue. The aqueous-to-ethanol phase volume ratio is typically 3:1.

Parameter	SM-102 Formulation		
N/P Ratio	4	6	8
Theoretical Lipid Mix Volume Required to Encapsulate 1 mg mRNA (mL)	2.95	2.95	2.95
Flow Rate Ratio (Aqueous Phase:Ethanol Phase)	3:1	3:1	3:1
mRNA Preparation Concentration (μg/mL)	171.9	114.6	85.9

***Note: Use the actual molecular weight of the mRNA sequence whenever possible. If it is unknown, estimate using an average molecular weight of 330 g/mol per nucleotide residue. Equations: n(P) = mRNA mass ÷ average molecular weight per nucleotide residue; n(SM-102) = N/P × n(P); Lipid Mix volume = n(SM-102) ÷ 6.25 mM. One set theoretically supports approximately 2 mg of mRNA at N/P = 6; one set is insufficient for a 2 mg scale at N/P = 8.**

2) Dilute the mRNA to be encapsulated with RNase-free water to the target concentration shown in the table above. For example, at N/P = 6, dilute the mRNA to 114.6 μg/mL in a solution containing a final concentration of 50 mM Citrate Buffer. This constitutes the mRNA-citrate solution (aqueous phase). Mix the mRNA gently, add the buffer, and adjust to the required concentration. Avoid vigorous vortexing and repeated freeze-thaw cycles. Keep the aqueous phase on ice before loading. Before use, equilibrate it to the operating temperature required by the instrument and mix gently.

3) Prepare 50 mM Citrate Buffer as the collection solution for the sample exiting the microfluidic mixer. Dilute 1 M Citrate Buffer (pH 3.2) 20-fold to prepare 50 mM Citrate Buffer.

4. Preparation of LNPs by Microfluidic Mixing

Install the chip, tubing, and syringes according to the instrument instructions and remove all air bubbles. Load the Lipid Mix ethanol phase and the mRNA aqueous phase separately. Reference settings: aqueous-to-ethanol phase ratio = 3:1 and total flow rate = 12 mL/min, corresponding to 9 mL/min for the aqueous phase and 3 mL/min for the ethanol phase. Before mixing, add a volume of 50 mM Citrate Buffer (pH 3.2) equal to the total microfluidic effluent volume to the collection tube.

For example, if the combined ethanol and aqueous phase volume is 10 mL, prepare 10 mL of 50 mM Citrate Buffer in the collection tube. Direct the effluent into the collection tube containing the equal volume of Citrate Buffer, and gently invert to mix after completion. Incubate the collected LNP formulation at room temperature for 30 min to allow complete particle formation, and then proceed with buffer exchange as soon as possible.

***Note: At a 3:1 phase ratio, the ethanol volume fraction in the effluent is approximately 25%. After dilution with an equal volume of collection solution, it is approximately 12.5%. Residual ethanol and low pH may affect LNP stability; complete buffer exchange as soon as possible.**

5. Dialysis, Concentration, and Storage

1) Transfer the crude LNP preparation to a 10-30 kDa MWCO dialysis device and exchange against at least 500-1,000 sample volumes of neutral buffer at 4°C. Sterile 1× PBS (pH 7.4) or a validated 10-20 mM Tris buffer (pH 7.4) may be used. A reference dialysis time is 4 h to overnight; the required duration depends primarily on the dialysis volume, and complete buffer exchange should be ensured. Replace the dialysis buffer 2-3 times while maintaining gentle stirring.
2) If concentration is required, use a 100 kDa MWCO centrifugal filter unit and centrifuge at 3,000×g and 4°C according to the consumable manufacturer's instructions. Set the target mRNA concentration according to the intended application; 0.1-0.5 mg/mL may be used as a reference range for small-scale preparation. For short-term use, store at 2-8°C and complete the experiment as soon as possible. For frozen storage, adjust sucrose to 8-10% (w/v), aliquot into single-use portions, and store at -80°C. The actual storage period must be established using formulation-specific stability data.

***Note: Do not subject the prepared mRNA-LNPs to repeated freeze-thaw cycles. The labeled shelf life of the solid lipid kit is not the stability period of the prepared LNPs. Do not claim long-term stability of the prepared LNPs without supporting stability data.**

Note

1. RNase-free conditions must be maintained throughout the entire LNP preparation process.

2. This product is for research use only. It must not be used for human or animal diagnosis, prevention, or treatment.

3. mRNA encapsulation efficiency: It is recommended to use RiboGreen or an equivalent method to determine total RNA and free RNA. Take a small aliquot of LNPs and add ~1% Triton X-100 (disrupting agent) to release the mRNA inside the LNPs and obtain the total mRNA amount. The encapsulation efficiency can then be calculated from the difference in mRNA content before and after disruption: Encapsulation efficiency (%) = (quantification after disruption − quantification before disruption) / quantification after disruption

Troubleshooting

1. Particle size too large or PDI increased: Check whether the lipids are fully dissolved, whether the phase ratio and flow rates are correct, and whether there are bubbles or blockages in the chip. Also shorten the residence time of crude LNPs in the ethanol/acidic environment.

2. Low encapsulation efficiency: Verify mRNA integrity, the pH of the acidic aqueous phase, N/P calculation, stock solution concentrations, and equipment dead volume. Then perform small-scale optimization focusing on N/P ratio, mRNA concentration, and mixing conditions.

3. This manual provides reference starting conditions for a classical SM-102 system. Different mRNAs and different mixing devices may require re-optimization.

4. When used for siRNA, saRNA, circRNA, or other nucleic acids, the mRNA concentration and N/P table in this manual should not be applied directly. New calculations and verification are required.

5. For in vivo administration, the final dilution buffer, dosing concentration, dosing volume, and storage buffer should be determined by the user according to the animal ethics protocol and formulation compatibility data.