Preparation of MC3 Lipid Nanoparticles for RNA delivery

Materials Required

- DLin-MC3-DMA (Cat. No. 7946)
- DSPC (Cat. No. 7943)
- DMG-PEG 2000 (Cat. No. 7944)
- Cholesterol (Cat. No. 7945)
- Cargo RNA to be delivered
- Absolute ethanol
- Citrate buffer (10 mM, pH 4)
- PBS, pH 7.4 (Cat. No. 5564)
- Dialysis kit with a molecular weight cutoff (MWCO) of 3.5 kDa and capacity of 120 µL
- Optional: Vortex or Microfluidic device

A. Lipid Mix Solution Preparation:

The lipid mix solution consists of DLin-MC3-DMA (Cat. No. 7946), DSPC (Cat. No. 7943), Cholesterol (Cat. No. 7945), and DMG-PEG 2000 (Cat. No. 7944) at the molar ratio of 50/10/38.5/1.5, which has been optimally formulated for LNP applications.

1. Transfer 150 µL of DLin-MC3-DMA into a tared glass vial. Then, add 50 µL of pure ethanol to achieve a concentration of 75 mg/mL. Ensure the solution in the vial appears clear.
2. Weigh 10 mg of DSPC and transfer it to a tared glass vial. Next, add 1.0 mL of pure ethanol to dissolve DSPC, resulting in a concentration of 10 mg/mL. Verify that the solution in the vial is clear.
3. Weigh 10 mg of cholesterol and place it in a tared glass vial. Add 1.0 mL of pure ethanol to dissolve cholesterol, reaching a concentration of 10 mg/mL. Note that cholesterol may become insoluble at higher concentrations. Confirm that the solution in the vial is clear.
4. Weigh 10 mg of DMG-PEG 2000 and transfer it to a tared glass vial. Add 1.0 mL of pure ethanol to dissolve DMG-PEG 2000, achieving a concentration of 10 mg/mL. Ensure the solution in the vial appears clear.

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5. To prepare a complete lipid mix solution for formulating the LNPs, pipette 13.3 µL of the DLin-MC3-DMA solution from step 1, 24.6 µL of the DSPC solution from step 2, 46.4 µL of the cholesterol solution from step 3, and 11.7 µL of the DMG-PEG solution from step 4. Mix the solutions thoroughly to achieve a clear solution. This mixture contains 19 µg of total lipid per µL of ethanol.

B. Payload RNA Preparation:

The total lipids/RNA ratio should be 40/1 (wt/wt). Prepare a 1 mg/mL RNA stock solution with 10 mM citrate buffer (pH 4).

C. Mixing

There are three commonly used methods to achieve rapid mixing of the solutions from **A** and **B**: the pipette mixing method, the vortex mixing method, and the microfluidic mixing method (microfluidic mixing method is not shown here – please refer to recommended protocols from manufacturers and literature reports). All three mixing methods can be used for various applications.

Pipette Mixing Method:

1. Add 16.8 µL of the lipid mix solution (prepared in section A, step 5) to an RNase-free 1.5 mL tube.
2. Add 1.2 µL of ethanol to the above tube. Mix well.
3. In another RNase-free 1.5 mL Eppendorf tube, add 46 µL of citrate buffer (10 mM, pH 4), and then add 8 µL of the RNA stock (1.0 mg/mL). Mix well.
4. Pipette 54 µL of the RNA buffer solution from step 3 and quickly add it into the lipid mix ethanol solution from step 2. Pipette up and down rapidly for 20–30 seconds.
5. Incubate the resulting solution at room temperature for up to 15 minutes.
6. Dialyze the solution using a dialysis kit (MWCO 3500) against 1× PBS for at least 1 hour to remove the ethanol and acidic buffers.
7. After dialysis, transfer the solution to an RNase-free 1.5 mL tube and measure the volume. Add 1× PBS to reach a final volume of 800 µL.

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Vortex Mixing Method:

1. Add 21 µL of the lipid mix solution (prepared in section A, step 5) to an RNase-free 1.5 mL tube.
2. Add 9 µL of ethanol to the above tube. Mix well.
3. In another RNase-free 1.5 mL Eppendorf tube, add 80 µL of citrate buffer (10 mM, pH 4), and then add 10 µL of the RNA stock (1.0 mg/mL). Mix well.
4. Set the vortex mixer equipment to speed level '1'.
5. Vortex the mRNA buffer solution from step 3 at a moderate speed on the vortex mixer. Then, pipette 30 µL of the lipid ethanol mix solution from step 2 into the pipette. Quickly add it into the vortexing solution. Continue vortexing the resulting dispersion for another 20–30 seconds.
6. Incubate the resulting solution at room temperature for up to 15 minutes.
7. Dialyze the solution using a dialysis kit (MWCO 3500) 1× PBS for at least 1 hour to remove the ethanol and acidic buffers.
8. After dialysis, transfer the solution to an RNase-free 1.5 mL tube and measure the volume. Compensate the solution with 1× PBS to reach a final volume of 1,000 µL.

Reference

Wang *et al* (2020) Preparation of selective organ-targeting (SORT) lipid nanoparticles (LNPs) using multiple technical methods for tissue-specific mRNA delivery. Nat.Protoc. ***18*** 265. PMID: 36316378.

Support

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