

LNP Synthesis Protocol (Ethanol Injection)

1. Task

The task is to review a standard experimental protocol for synthesising lipid nanoparticles and then digitise the protocol into a script for the robot. Review the materials used, read the protocol, and develop a step-by-step script for the robot.

When finished, please email your document to robiochemistry@gmail.com

(Name Format Example: Group2_L2_Synthesis)

2. Materials

- **Lipids (each lipid is stored in a separate Eppendorf tube)**

SM-102 (ionizable lipid); DOPE (helper lipid); Cholesterol; DSPE-PEG2000

- **Payload (In an Eppendorf tube)**

Poly-A (mock RNA) (In an Eppendorf)

- **Buffers (In a Buffer Reservoir)**

Citrate buffer 50mM (pH=5.0); EtOH; PBS buffer x1 (In a buffer reservoir)

3. Procedure (Hands-On) To be used as a Reference

1. Turn on the heater and set to 55°C

2. To an empty 1.5ml Eppendorf, add 117 ul Ethanol

3. To the Eppendorf containing Ethanol, **add:**

- 18.1 ul SM-102 Ionizable lipid
- 7.6 ul DOPE Helper lipid
- 15.2 ul Cholesterol
- 19.4 ul DSPE-PEG 2000

4. Mix thoroughly

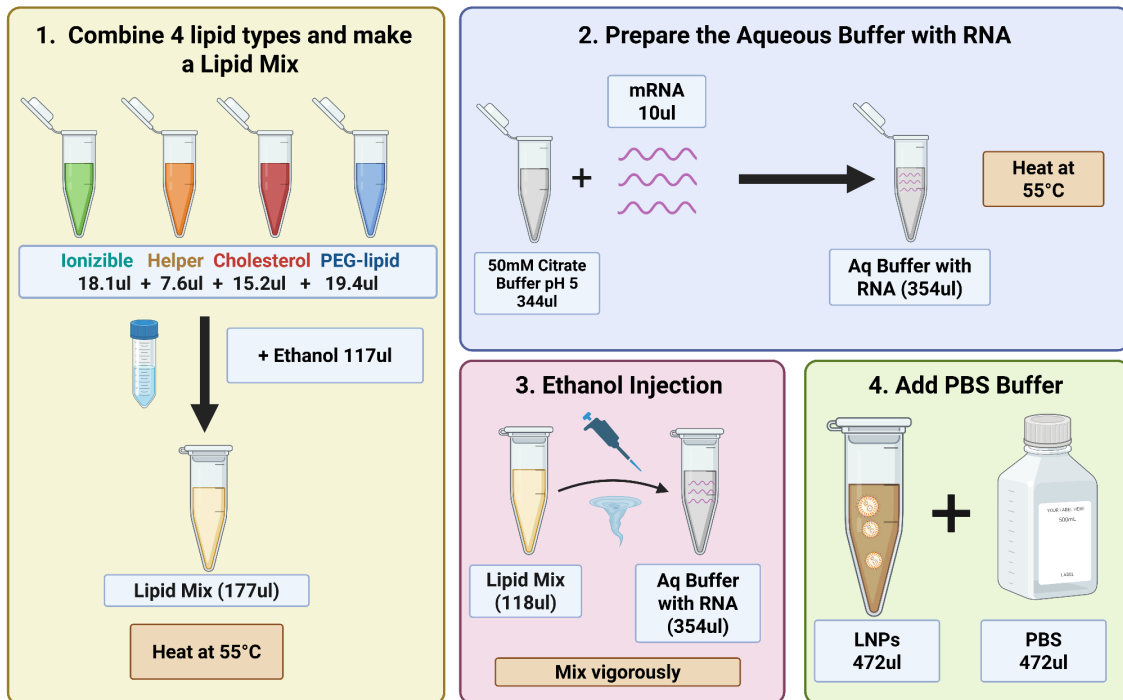
5. To another empty Eppendorf, add 344 µL of Citrate buffer 50 mM (pH=5) and then add 10 µL of PolyA (concentration 1 mg/mL, total payload weight 10 µg)

6. Close the cap and Incubate for 1 minute. The open the cap.

7. Aspirate **118 μ L** of the lipid mix and dispense into the **RNA-Aqueous buffer**. Mix vigorously for several cycles.

8. Add **472 μ L PBS x1 buffer** (makes the particles fuse and form faster) and mix.

9. Mix thoroughly and then close the cap



4. Procedure (Robot)

Example - First Commands

1. Group Start (Lipid Mix)
2. Turn on Bioshake 1
3. Get DiTis (1000ul)
4. Aspirate (117ulul) - Ethanol Reservoir
5. Dispense (117ul) - Bioshake 1 (Position 1)
6. Drop DiTis