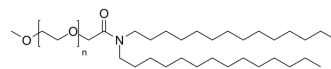


ALC-0159

Cat. No.:	HY-138300
CAS No.:	1849616-42-7
Molecular Formula:	$(C_2H_4O)_n C_{31}H_{63}NO_2$
Target:	Liposome
Pathway:	Metabolic Enzyme/Protease
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 6 months -20°C 1 month



SOLVENT & SOLUBILITY

In Vitro	DMSO : 100 mg/mL (Need ultrasonic) Ethanol : ≥ 50 mg/mL * "≥" means soluble, but saturation unknown.
In Vivo	<ol style="list-style-type: none"> Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline Solubility: ≥ 2.5 mg/mL (Infinity mM); Clear solution Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.5 mg/mL (Infinity mM); Clear solution Add each solvent one by one: 10% DMSO >> 90% corn oil Solubility: ≥ 2.5 mg/mL (Infinity mM); Clear solution

BIOLOGICAL ACTIVITY

Description	ALC-0159, a polyethylene glycol (PEG) lipid conjugate, could be used as vaccine excipient ^[1] .
In Vitro	<p>Preparation of Lipid Nanoparticles</p> <p>Here we provide lipid molar ratios for LNPs in FDA-approved BNT162b2 (a COVID-19 mRNA vaccine). The molar ratio of lipids in this formulation is ALC-0315 : DSPC : Cholesterol : ALC-0159 = 46.3 : 9.4 : 42.7 : 1.6, and RNA to lipid weight ratio is 0.05 (wt/wt)^[1].</p> <p>A. Lipid Mixture Preparation</p> <ol style="list-style-type: none"> Dissolve lipids in ethanol and prepare 10 mg/m stock solutions. The lipid stock solutions can be stored at -20°C for later use. <p>Note 1: The ionizable lipid is usually a liquid. Due to the viscosity, it should always be weighed rather than relying on the autopipette volume.</p> <p>Note 2: Cholesterol in solution should be kept warm (>37°C) to maintain fluidity. Transfer the cholesterol solution promptly to avoid cooling.</p>

2. Prepare the lipid mixture solution as described. For each mL of lipid mixture add the following: 560 μ L of 10mg/mL ALC-0315 (HY-138170), 261 μ L of 10mg/mL Cholesterol (HY-N0322), 117 μ L of 10mg/mL DSPC (HY-W040193), and 62 μ L of ALC-0159 (HY-138300). Mix the solutions thoroughly to achieve a clear solution. This mixture contains 10 mg of total lipid.

Note 3: The choice of lipids and ratios may be changed as desired and this will affect the LNP properties (size, polydispersity, and efficacy) and the amount of mRNA required.

B. mRNA Preparation

1. Prepare a 166.7 μ g/mL mRNA solution with 100 mM pH 5 sodium acetate buffer.

Note 4: The lipid:mRNA weight ratio influences the encapsulation efficiency. Other weight ratios may be prepared as alternative formulations and should be adjusted accordingly by user.

C. Mixing

There are three commonly used methods to achieve rapid mixing of the solutions: the pipette mixing method, the vortex mixing method, and the microfluidic mixing method. All these mixing methods can be used for various applications.

It is important to note that pipette mixing method and vortex mixing method may yield more heterogeneous LNPs with lower encapsulation efficiencies and is prone to variability. Microfluidic devices enable rapid mixing in a highly controllable, reproducible manner that achieves homogeneous LNPs and high encapsulation efficiency. Within these devices, the ethanolic lipid mixture and aqueous solution are rapidly combined in individual streams. LNPs are formed as the two streams mix and are then collected into a single collection tube.

1. Pipette Mixing Method:

1.1. Pipette 3 mL of the mRNA solution and quickly add it into 1 mL of the lipid mixture solution (A 1:3 ratio of ethanolic lipid mixture to aqueous buffer is generally used.) Pipette up and down rapidly for 20–30 seconds.

1.2. Incubate the resulting solution at room temperature for up to 15 minutes.

1.3. After mixing, the LNPs were dialyzed against PBS (pH 7.4) for 2 h, sterile filtered using 0.2 μ m filters, and stored at 4°C.

2. Vortex Mixing Method:

1.1. Vortex 3 mL of mRNA solution at a moderate speed on the vortex mixer. Then, Quickly add 1 mL of the lipid mixture solution into the vortexing solution (A 1:3 ratio of ethanolic lipid mixture to aqueous buffer is generally used.). Continue vortexing the resulting dispersion for another 20–30 seconds.

1.2. Incubate the resulting solution at room temperature for up to 15 minutes.

1.3. After mixing, the LNPs were dialyzed against PBS (pH 7.4) for 2 h, sterile filtered using 0.2 μ m filters, and stored at 4°C.

3. Microfluidic Mixing Method:

1.1 The 3 mL of mRNA buffer solution and 1 mL of the lipid mixture solution were mixed at a total flow rate of 12 mL/min in a microfluidic device (A 1:3 ratio of ethanolic lipid mixture to aqueous buffer is generally used.).

Note 5: Parameters such as the flow rate ratio and total flow rate can be altered to fine-tune LNPs.

1.2. After mixing, the LNPs were dialyzed against PBS (pH 7.4) for 2 h, sterile filtered using 0.2 μ m filters, and stored at 4°C.

Reference

1. Curr Issues Mol Biol. 2022 Oct 19;44(10):5013-5027.

2. Curr Protoc. 2023;3(9):e898.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- ACS Nano. 2023 Aug 29.
- PLoS One. 2023 Aug 29;18(8):e0289510.
- Research Square Preprint. 2023 Sep 12.
- bioRxiv. 2023 Jun 10.

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REFERENCES

[1]. S Moein Moghimi, et al. Allergic Reactions and Anaphylaxis to LNP-Based COVID-19 Vaccines. Mol Ther. 2021 Mar 3;29(3):898-900.

Caution: Product has not been fully validated for medical applications. For research use only.

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