LipidLaunch™ LP-01 LNP Exploration Kit

Item No. 40098

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GENERAL INFORMATION

Materials Supplied

Item Number	Item Name	Quantity/Size	Storage Temperature
37278	LP-01	1 vial/25 mg	-20°C
15100	1,2-Distearoyl-*sn*-glycero-3-PC	1 vial/10 mg	-20°C
9003100	Cholesterol	1 vial/25 mg	-20°C
33945	DMG-PEG(2000)	1 vial/5 mg	-20°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the Materials Supplied section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Absolute ethanol
2. Aqueous acidic buffer, such as 50 mM sodium acetate, pH 4.0-5.0
3. Nucleic acid cargo
4. Commercial microfluidic device or pipettes for hand mixing
5. Neutral buffer, such as PBS, pH 7.4

INTRODUCTION

Background

Lipid nanoparticles (LNPs) are a subset of lipid-based drug delivery (LBDD) systems that utilize ionizable cationic lipids, such as LP-01, for the delivery of nucleic acid (*e.g.* siRNA, mRNA, cyclic dinucleotide) payloads to cells.[1] They consist of a lipid shell composed of structural phospholipids, cholesterol, and PEGylated lipids that surround an internal aqueous core, where the ionizable cationic lipids organize into inverted micelles around the encapsulated nucleic acids.[2] Ionizable cationic lipids are near-neutral at physiological pH and cationic in acidic environments (apparent pK_a for LP-01-containing particles is 6.1), a property that promotes the encapsulation of negatively charged nucleic acids during LNP preparation and facilitates intracellular delivery after cellular uptake.[1,3,4] LNPs are internalized into cells *via* endocytosis.[5] The ionizable cationic lipid becomes protonated and positively charged in the acidic environment of the endosomal compartment, promoting LNP endosomal escape and intracellular delivery.[6]

Cayman's LipidLaunch™ LP-01 LNP Exploration Kit is intended to serve as a starting point for laboratories to explore the feasibility of using LNPs containing LP-01, also known as CIN-16645, for their individual application without the need for specialized equipment. Optimal preparation conditions for the encapsulation of nucleic acids with LNPs must be determined by the end user. Adjustment of the following parameters may facilitate this process:

- Lipid molar ratio
- Lipid:nucleic acid (w:w) ratio
- Ionizable cationic lipid nitrogen:nucleotide phosphate (N:P) molar ratio
- Aqueous buffer: identity and ionic strength
- Particle size: extrusion size or microfluidic operating parameters, as applicable
- LNP preparation method

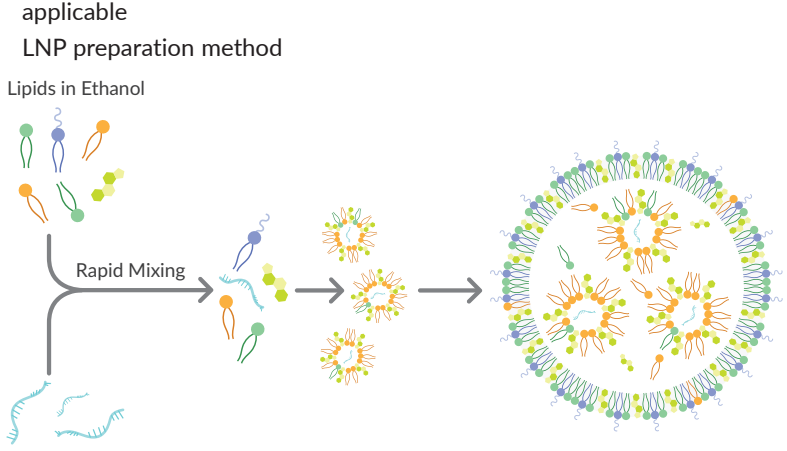

Figure 1. Schematic of nucleic acid-containing LNP formation with the LipidLaunch™ LP-01 LNP Exploration Kit

PROTOCOL PREPARATION

Protocol

An example for preparing nucleic acid-containing LNPs with an ethanolic lipid mixture containing LP-01 (Item No. 37278), 1,2-distearoyl-*sn*-glycero-3-PC (1,2-DSPC; Item No. 15100), cholesterol (Item No. 9003100), and DMG-PEG(2000) (Item No. 33945) at lipid molar ratios of 45:9:44:2, respectively, is shown below. mRNA-containing LNPs using these lipids have been optimally formulated at this lipid molar ratio.[6] This example is shown with a final lipid:nucleic acid (w:w) ratio of 22:1 and an aqueous:ethanol ratio of 3:1. The end user may scale volumes and adjust lipid molar and lipid:nucleic acid ratios as desired. It is possible to produce multiple small batches of LNPs using the parameters in this example and the reagents provided in the kit.

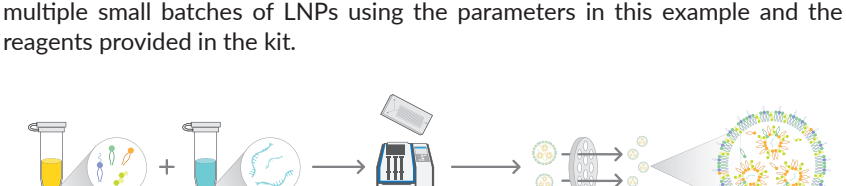

Figure 2. LipidLaunch™ LP-01 LNP Exploration Kit workflow

Reagent Preparation

1. **Ethanolic Lipid Mixture**

   Prepare individual lipid stock solutions of the three lipids supplied as crystalline solids in absolute ethanol. LP-01 is ready to use as supplied. Bring all stock solutions to room temperature prior to use and ensure they are well-dissolved. Heating to 37°C with intermittent vortexing may be required for dissolution. Transfer the appropriate volume of each lipid mixture component to a single tube as listed in the table below to prepare the ethanolic lipid mixture. Mix by pipetting several times. Individual lipid solutions and lipid mixes will be stable stored at -20°C for at least a week. Gentle heating may be required for re-solublization.

Lipid Mixture Component	Stock Solutions		Working Mixture		
	mg/ml	MW	Molar Ratio	mg	Required Volume
LP-01	100	852.3	45	4.55	45 µl
1,2-DSPC	25	790.2	9	0.84	34 µl
Cholesterol	5	386.7	44	2.02	403 µl
DMG-PEG(2000)	5	2,526	2	0.59	119 µl
Absolute ethanol					399 µl
Total				8.0	1 ml

Table 1. Preparation of ethanolic lipid mixture

2. **Aqueous Nucleic Acid Solution**

   Dilute mRNA (or other cargo) to a concentration of approximately 0.12 mg/ml in 50 mM sodium acetate, pH 4.0, prepared under RNase-free conditions. Prepare 3 volumes of aqueous solution for every 1 volume of lipid mix. Optimal cargo concentration and buffer should be determined experimentally for each nucleic acid cargo.

Performing the Protocol

Several methods are suitable for laboratory-scale, small-volume LNP production. Two of these methods are described briefly below. These methods are versatile and can be tailored for use with a range of basic-to-specialized equipment. The procedures are performed at room temperature unless otherwise indicated.

1. **Mixing**

   Commercial Microfluidic Device Mixing: Mix the ethanolic lipid mixture with the aqueous nucleic acid solution using a microfluidic device or chip with a staggered herringbone-, T-, or Y-channel design. Flow rate ratios (FRR) for the mixtures described here are 3:1 (aqueous:ethanolic), and total flow rates (TFR) can vary, usually between 10 and 25 ml/min.

   Hand Mixing: Hand mix the ethanolic lipid mixture with the aqueous nucleic acid solution *via* pipette by rapidly transferring the ethanolic lipid mixture into the aqueous nucleic acid solution. Volume ratios for the mixtures described here are 3:1 (aqueous:ethanolic). Mix by repeated pipetting for 15 seconds or vortexing briefly. Leave undisturbed for 10 minutes.

2. Final Preparation

LNPs are delicate structures and care should be taken to avoid shaking or pipetting LNP solutions too vigorously. Frequently, LNP solutions are immediately diluted into a neutral buffer (*e.g.* equal volume PBS, pH 7.4) to minimize potential damage to lipids in the low-pH environment. Subsequent preparation steps described below depend on the final application.

a. Dialyze LNPs in neutral buffer *(e.g.* PBS, pH 7.4) against 1,000 volumes of buffer using the appropriate molecular weight cut-off (MWCO) membrane overnight (generally, 30 kDa MWCO is appropriate).

b. If desired, LNP solutions may be concentrated by centrifugation using the appropriate MWCO filter.

c. LNP solutions can be filter-sterilized with a 0.22 µm filter if required for end use.

d. The LNP solution will be stable at 4°C for one week. If longer storage is required, the LNPs can be frozen at -80°C with the addition of a cryoprotectant such as 8-12% sucrose, but conditions for freezing should be optimized experimentally.

3. Characterization and Validation

A variety of techniques are available to characterize LNPs prior to *in vitro* or *in vivo* use. Contact Cayman Services for *in vitro* testing of your LNPs.

Attribute	Assay(s)
Particle size and distribution (PDI)	Dynamic light scattering (DLS)
Zeta potential	Laser doppler electrophoresis
Lipid quantification and integrity	RP-HPLC, SE-HPLC, IP-HPLC
Encapsulation efficiency	Fluorescent dyes (*e.g.* RiboGreen) with and without Triton-X 100
Apparent pK_a	TNS assay
LNP morphology	Microscopy (cryo TEM, ESEM, AFM)
Translation or knockdown analyses	Cell-based reporter assays, Western blotting

Table 2. LNP attributes and corresponding assays. Adapted from Schoenmaker, L., *et al.*[7]

Attribute	Typical Value
Particle size	50-150 nm
Polydispersity index (PDI)	<0.2
Encapsulation efficiency (%EE)	>85%

Table 3. Typical particle characteristics of LNPs made with the LipidLaunch™ LP-01 LNP Exploration Kit

References

1. Mitchell, M.J., Billingsley, M.M., Haley, R.M., *et al.* Engineering precision nanoparticles for drug delivery. *Nat. Rev. Drug Discov.* **20(2)**, 101-124 (2021).
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7. Schoenmaker, L., Witzigmann, D., Kulkarni, J.A., *et al.* mRNA-lipid nanoparticle COVID-19 vaccines: Structure and stability. *Int. J. Pharm.* **601**, 120586 (2021).

NOTES

Warranty and Limitation of Remedy

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