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## Lipid Nanoparticle (LNP-MC3) Exploration Kit

Item No. 36970

[www.caymanchem.com](http://www.caymanchem.com)

Customer Service 800.364.9897

Technical Support 888.526.5351

1180 E. Ellsworth Rd • Ann Arbor, MI • USA

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## GENERAL INFORMATION

### Materials Supplied

Item Number	Item Name	Quantity/Size	Storage Temperature
37206	DLin-MC3-DMA (100 mg/ml)	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 this assay.

## 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. 50 mM sodium acetate, pH 5.0
3. Oligonucleotides
4. Commercial microfluidic device, off-the-shelf microfluidic device, extrusion kit, or sonicator
5. PBS, pH 7.4

## Background

Lipid nanoparticles (LNPs) are a subset of lipid-based drug delivery (LBDD) systems that utilize ionizable lipids, such as DLin-MC3-DMA (100 mg/ml) (MC3), for the delivery of oligonucleotide (e.g. siRNA, mRNA, cyclic dinucleotides) payloads into cells.<sup>1</sup> They consist of a lipid shell composed of structural phospholipids, cholesterol, and PEGylated lipids that surround an internal aqueous core, where the ionizable lipids organize into inverted micelles around the encapsulated oligonucleotides.<sup>2</sup> Ionizable lipids are near-neutral at physiological pH and cationic in acidic environments (theoretical  $pK_a$  for MC3 = 6.44), a property that promotes the encapsulation of negatively charged oligonucleotides during LNP preparation and facilitates intracellular delivery after cellular uptake.<sup>1,3,4</sup> LNPs are internalized into cells *via* endocytosis.<sup>5</sup> MC3 becomes protonated and positively charged in the acidic environment of the endosomal compartment, promoting LNP endosomal escape and intracellular delivery.<sup>6</sup>

Cayman's Lipid Nanoparticle (LNP-MC3) Exploration Kit is intended to serve as a starting point for laboratories to explore the feasibility of using LNPs for their individual application without the need for specialized equipment. Optimal preparation conditions for the encapsulation of oligonucleotides with LNPs must be determined by the end user. Adjustment of the following parameters may facilitate this process:

- Lipid molar ratio
- Lipid:oligonucleotide (w:w) ratio
- Ionizable 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

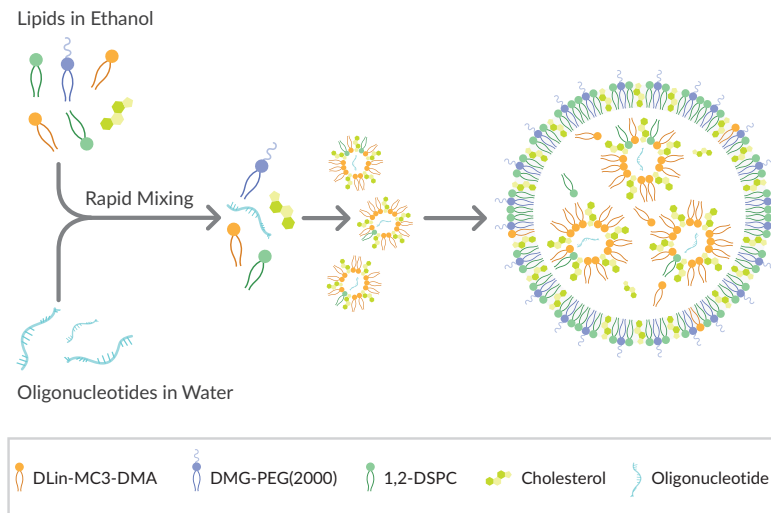


Figure 1. Schematic of Oligonucleotide-Containing LNP Formation with Lipid Nanoparticle (LNP-MC3) Exploration Kit

## Protocol

An example for preparing oligonucleotide-containing LNPs with an ethanolic lipid mixture containing DLin-MC3-DMA (100 mg/ml) (Item No. 37206), 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 50:10:38.5:1.5, respectively, is shown below. mRNA-based vaccines using these lipids have been optimally formulated at this lipid molar ratio.<sup>6</sup> This example is shown with a lipid:oligonucleotide (w:w) ratio of 10:1 and an ethanol:aqueous ratio of 1:3. The end user may scale volumes and adjust lipid molar and lipid:oligonucleotide 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

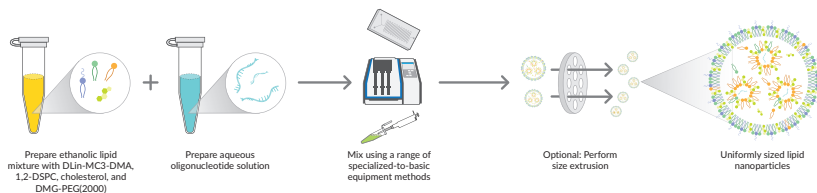


Figure 2. Lipid Nanoparticle (LNP-MC3) Exploration Kit Workflow

## Reagent Preparation

### 1. Ethanolic Lipid Mixture

Prepare individual lipid stock solutions of the three lipids supplied as crystalline solids in absolute ethanol. DLin-MC3-DMA (100 mg/ml) is ready to use as supplied. Bring all stock solutions to room temperature prior to use and ensure they are well-dissolved. 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.

Lipid Mixture Component	Stock Solutions		Working Mixture		
	mg/ml	MW	Molar Ratio	mg	Required Volume
DLin-MC3-DMA	100	642.1	50	3.21	32 $\mu$ l
1,2-DSPC	25	790.2	10	0.79	32 $\mu$ l
Cholesterol	5	386.7	38.5	1.48	296 $\mu$ l
DMG-PEG(2000)	1	2,526	1.5	0.38	378 $\mu$ l
Absolute ethanol					262 $\mu$ l
Total				5.86	1 ml

Table 1. Preparation of ethanolic lipid mixture

### 2. Aqueous Oligonucleotide Solution

Add 0.62 mg oligonucleotide to a separate tube and adjust the volume to 3.0 ml with 50 mM sodium acetate, pH 5.0.

## Performing the Protocol

Several methods are suitable for laboratory-scale, small-volume LNP production. These are described briefly below, and they may be adapted for use with a range of specialized-to-basic 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 acidic oligonucleotide solution using a microfluidic device or chip with a staggered herringbone-, T-, or Y-channel design. The inlet flow rates can be controlled with syringe pumps and should be optimized by the end user.

**Off-the-Shelf Microfluidic Device Mixing:** Mix the ethanolic lipid mixture with the aqueous acidic oligonucleotide solution using an off-the-shelf microfluidic mixing device. These mixers can be assembled with common and inexpensive materials. Two inlets, composed of individual syringes containing the ethanolic lipid mixture and the aqueous acidic oligonucleotide solution, can be connected to opposite ends of a T- or Y-connector (2 mm I.D.) with appropriate tubing (1.5 mm I.D.) and fittings. A single outlet fitted with the appropriate tubing will direct the LNPs into a collection tube. The inlet flow rates can be controlled with syringe pumps and should be optimized by the end user.

**Solvent-injection Mixing:** Rapidly inject the ethanolic lipid mixture into the aqueous acidic oligonucleotide solution using a syringe with the needle placed in the center of the solution. Stir at 400 rpm for 30 minutes above the 1,2-DSPC transition temperature ( $T_m = 55^\circ\text{C}$ ).

**Hand Mixing:** Hand mix the ethanolic lipid mixture with the aqueous acidic oligonucleotide solution *via* pipette by rapidly transferring the ethanolic lipid mixture into the aqueous acidic oligonucleotide solution. Mix by repeated pipetting for 15 seconds. Leave undisturbed for 10 minutes.

### 2. Final Preparation

- Perform size extrusion if necessary. Size extrusion is typically required following mixing techniques that produce large and heterogenous LNPs (e.g. off-the-shelf microfluidic device mixing, solvent-injection, and hand mixing) to yield a narrower LNP size distribution.
- 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) tubing overnight.
- If desired, LNP solutions may be concentrated by centrifugation using the appropriate MWCO filter.
- Filter-sterilize LNP solutions with a 0.22  $\mu\text{m}$  filter and store at 4°C until use. The LNP solutions will be stable at 4°C for one week. If longer storage is required, the LNPs can be lyophilized and stored at -80°C.

### 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	Dynamic light scattering (DLS)
Zeta potential	Laser doppler electrophoresis
Lipid quantification and integrity	RP-HPLC, SE-HPLC, IP-HPLC
Encapsulation efficiency	Fluorescent dyes (RiboGreen); UV spectroscopy with Triton-X
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.*<sup>6</sup>

## RESOURCES

### 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).
2. Guevara, M.L., Persano, F., and Persano, S. Advances in lipid nanoparticles for mRNA-based cancer immunotherapy. *Front. Chem.* **8**, 589959 (2020).
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4. Jayaraman, M., Ansell, S.M., Mui, B.L., *et al.* Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing *in vivo*. *Angew. Chem. Int. Ed. Engl.* **51(34)**, 8529-8533 (2012).
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### Warranty and Limitation of Remedy

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