LipidLaunch™ LNP-MC3 Uptake Kit (Near-Infrared Fluorescence)

Item No. 39066

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
34364	DLin-MC3-DMA	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
34954	1,1-Dioctadecyl-3,3,3,3-tetramethylindotricarbocyanine (iodide)	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. A device capable of measuring fluorescence with excitation and emission wavelengths of 750 and 780 nm, respectively
2. Absolute ethanol
3. 50 mM sodium acetate, pH 5.0
4. Nucleic acid payload
5. Commercial microfluidic device, off-the-shelf microfluidic device, extrusion kit, or sonicator
5. 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 DLin-MC3-DMA (MC3), for the delivery of nucleic acid (*e.g.* siRNA, mRNA, cyclic dinucleotides) payloads into 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 (theoretical pK_a for MC3 = 6.44), 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] MC3 becomes protonated and positively charged in the acidic environment of the endosomal compartment, promoting LNP endosomal escape and intracellular delivery.[6]

Cayman's LipidLaunch™ LNP-MC3 Uptake Kit (Near-Infrared Fluorescence) is intended to serve as a starting point for laboratories to explore the uptake of LNPs *in vivo* 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

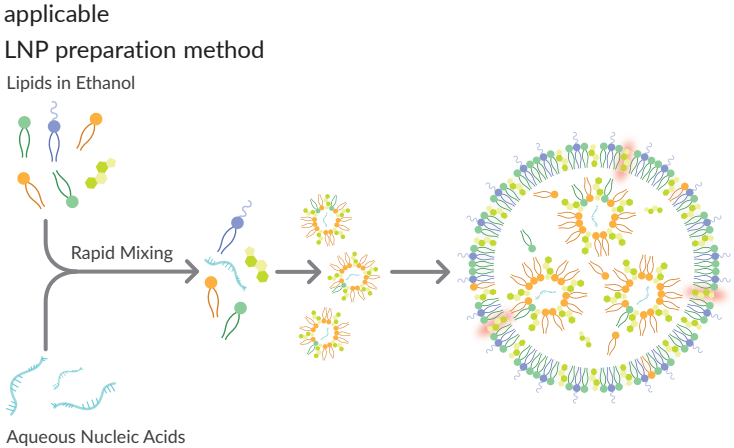

Figure 1. Schematic of nucleic acid-containing LNP formation with the LipidLaunch™ LNP-MC3 Uptake Kit (Near-Infrared Fluorescence)

Protocol

An example for preparing nucleic acid-containing LNPs with an ethanolic lipid mixture containing DLin-MC3-DMA (Item No. 34364), 1,2-distearoyl-*sn*-glycero-3-PC (1,2-DSPC; Item No. 15100), cholesterol (Item No. 9003100), DMG-PEG(2000) (Item No. 33945), and 3,3,3,3-tetramethylindotricarbocyanine (iodide) (DiR; Item No. 34954), at lipid molar ratios of 50:10:37.5:1.5:1, respectively, is shown below. mRNA-based vaccines using these lipids have been optimally formulated at this lipid molar ratio.[6] This example is shown with a lipid:nucleic acid (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: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.

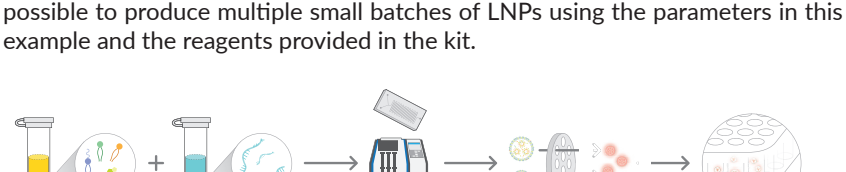

Figure 2. LipidLaunch™ LNP-MC3 Uptake Kit (Near-Infrared Fluorescence) workflow

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 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	3.25	32 µl
1,2-DSPC	25	790.2	10	0.80	32 µl
Cholesterol	5	386.7	37.5	1.47	293 µl
DMG-PEG(2000)	1	2,509.2	1.5	0.38	381 µl
DiR	1	1,013.4	1.0	0.10	103 µl
Absolute ethanol					159 µl
Total				6.00	1 ml

Table 1. Preparation of ethanolic lipid mixture

2. Aqueous Nucleic Acid Solution

In a separate tube, bring nucleic acid payload to a concentration of 50-200 µg/ml in 3 ml of 50 mM sodium acetate, pH 5.0. Optimal nucleic acid:lipid ratio should be determined empirically for each payload.

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 nucleic acid 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 nucleic acid solution using an off-the-shelf microfludic 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 nucleic acid 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 nucleic acid 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°C).

   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. Mix by repeated pipetting for 15 seconds. Leave undisturbed for 10 minutes.

2. **Final Preparation**

   a. 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.

   b. 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.

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

   d. Filter-sterilize LNP solutions with a 0.22 µm 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, stabilizing reagents such as sucrose may be added for storage at -20°C. Stability testing should be conducted for each LNP formulation method to optimize storage conditions.

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	RNA-compatible nucleic acid dye with and without detergent, such as 0.5% Triton X-100; UV spectroscopy
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.*[6]

4. Uptake Analysis

DiR incorporated into the LNPs will fluoresce at excitation/emission wavelengths of 750/780 nm, respectively. For *in vitro* studies, dilute prepared LNPs in medium containing serum at approximately 1:100 to 1:500, then add to cells. After 24-48 hours, exchange medium for PBS, and image or capture total fluorescence using a plate reader. Formulated nanoparticles can also be used *in vivo* to determine organ targeting with near-infrared-compatible *in vivo* imaging systems.

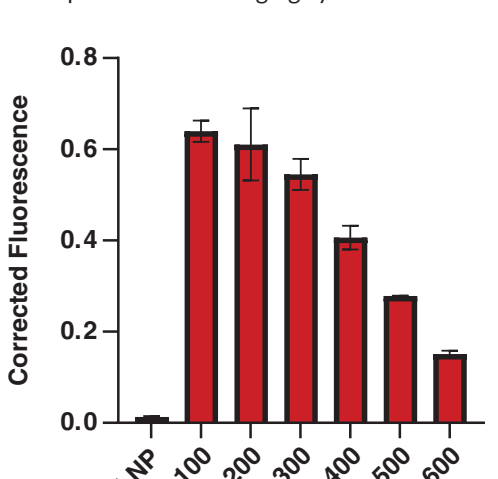

Figure 3. LNP-MC3 uptake in hepatocytes. LNP-MC3 was formulated in a Nunchuck device from Unchained Labs using a flow rate ratio of 3 and a total flow rate of 15 ml/min. After dialysis against PBS, LNPs were diluted in cell culture media and added to Huh7 hepatocytes for 24 hours. Hoescht 33342 (Item No. 15547) and DiR fluorescence were measured using a Tecan Infinite M Plex microplate reader. Hoescht 33342 was used to calculate the corrected fluorescence of LNP-MC3 uptake.

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).
3. Buschmann, M.D., Carrasco, M.J., Alishetty, S., *et al.* Nanomaterial delivery systems for mRNA vaccines. *Vaccines (Basel)* **9(1)**, 65 (2021).
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).
5. Degors, I.M.S., Wang, C., Rehman, Z.U., *et al.* Carriers break barriers in drug delivery: Endocytosis and endosomal escape of gene delivery vectors. *Acc. Chem. Res.* **52(7)**, 1750-1760 (2019).
6. Carrasco, M.J., Alishetta, S., Alameh, M.-G., *et al.* Ionization and structural properties of mRNA lipid nanoparticles influence expression in intramuscular and intravascular administration. *Commun. Biol.* **4(1)**, 956 (2021).
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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