LipidLaunch™ LNP Screening Set (Loadable)

Item No. 502881

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Customer Service 800.364.9897
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1180 E. Ellsworth Rd · Ann Arbor, MI · USA

TABLE OF CONTENTS

GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -80°C kit. After opening the kit, store individual components as stated below.

Item Number	Item Name	Quantity/Size	Storage Temperature
400773	LipidLaunch™ ALC-0315 LNP (Loadable)	1 vial	-80°C
401011	LipidLaunch™ C14-4 LNP (Loadable)	1 vial	-80°C
401055	LipidLaunch™ MC3 LNP (Loadable)	1 vial	-80°C
400833	LipidLaunch™ (S)-C12-200 LNP (Loadable)	1 vial	-80°C
400648	LipidLaunch™ SM-102 LNP (Loadable)	1 vial	-80°C
400812	LNP Encapsulation Buffer Tablet	1 tablet	RT

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.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's LNP Screening Set (Loadable). This kit may not perform as described if any reagent or procedure is replaced or modified.

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

Kit components should be 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 source of nuclease-free water is recommended. Pure water - glass-distilled or deionized - may not be acceptable.
2. Adjustable pipettes; multichannel or repeating pipettor recommended

INTRODUCTION

Background

Lipid nanoparticles (LNPs) are a subset of lipid-based drug delivery (LBDD) systems that utilize ionizable cationic lipids, such as ALC-0315, C14-4, DLin-MC3-DMA (MC3), (S)-C12-200, and SM-102, for the delivery of nucleic acid payloads to cells.[1,2] LNPs are typically composed of four types of lipids: a cationic or ionizable cationic lipid, a helper phospholipid, a PEGylated lipid, and cholesterol. Release of LNP cargo into target cells is heavily influenced by the ionizable cationic lipid component, which undergoes protonation in the acidic environment of the endosomes, resulting in membrane disruption and release of cargo into the cell.[3] The ionizable cationic aminolipids in this kit have been used in combination with other lipids in the formation of LNPs for the delivery of various cargo *in vitro* and *in vivo* (see Table 1, on page 7). Cayman's LipidLaunch™ LNP Screening Set (Loadable) uses the ionizable cationic aminolipids to facilitate efficient payload delivery in research models with reduced toxicity compared to traditional methods.

Ionizable Cationic Lipid	pK_a	Cargo	Notable Features	References
ALC-0315	6.09	Small molecules, cyclic nucleotides, mRNA, siRNA, plasmid DNA	Accumulates in the liver, and, to a lesser extent, in the spleen in mice	4-12
C14-4	6.5	mRNA	Transfects T cells with reduced cytotoxicity compared with electroporation	12-15
MC3	6.44	Cyclic nucleotides, mRNA, siRNA, plasmid DNA	Accumulates in muscle, non-draining lymph nodes, and spleen in mice	11,12,16-18
(S)-C12-200	7.12	Cyclic nucleotides, mRNA, plasmid DNA	Accumulates in hepatocytes, hepatic endothelial cells, and hepatic Kupffer cells in mice	11,12,19
SM-102	6.68	Cyclic nucleotides, mRNA, siRNA, plasmid DNA	Accumulates in the liver and spleen in mice	11,12,20,21

Table 1. Summary of properties of LNPs containing ionizable cationic lipids

About This Kit

Cayman's LipidLaunch™ LNP Screening Set (Loadable) provides a versatile panel of five preformed, payload-ready LNP formulations featuring the following ionizable lipids: ALC-0315, C14-4, MC3, SM-102, and (S)-C12-200. This kit enables rapid screening of multiple LNP formulations in one convenient package. Each LNP included is *loadable*, allowing users to rapidly encapsulate their own nucleic acid using a simple mixing protocol. In addition, LipidLaunch™ Loadable LNPs demonstrate very low toxicity compared to traditional lipid-based transfection methods.

Conventionally loaded LNPs typically require specialized equipment to ensure consistent control of particle size, while also requiring large reaction volumes. Each loadable LNP in this kit enables users to encapsulate their payload in low reaction volumes offering greater flexibility in the scale of preparation while preserving costly nucleic acid cargo.

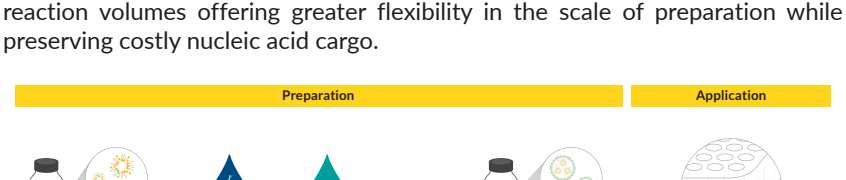

Figure 1. Protocol summary

Cayman also offers individual loadable LNP kits for each of these ionizable cationic lipids for further studies (see Table 2, below).

Item Number	Item Name
702750	LipidLaunch™ ALC-0315 LNP (Loadable)
702860	LipidLaunch™ C14-4 LNP (Loadable)
502868	LipidLaunch™ MC3 LNP (Loadable)
702780	LipidLaunch™ (S)-C12-200 LNP (Loadable)
702620	LipidLaunch™ SM-102 LNP (Loadable)
702760	LipidLaunch™ BODIPY SM-102 LNP Kit (Loadable)

Table 2. Additional loadable LNP kits available from Cayman

PREPARATION

Reagent Preparation

1. **LipidLaunch™ ALC-0315 LNP (Loadable) - (Item No. 401055)**

   This vial contains 200 µl of LipidLaunch™ ALC-0315 LNP (Loadable). After thawing, store on ice. Invert the vial or pipette up and down 2-3 times to fully mix. This vial of ALC-0315 LNPs provides sufficient volume to transfect a full 96-well plate. Once thawed, the loadable LNPs are stable for up to two weeks when stored at 4°C. Do not re-freeze.

2. **LipidLaunch™ C14-4 LNP (Loadable) - (Item No. 401011)**

   This vial contains 100 µl of LipidLaunch™ C14-4 LNP (Loadable). After thawing, store on ice. Invert the vial or pipette up and down 2-3 times to fully mix. This vial of C14-4 LNPs provides sufficient volume to transfect a full 96-well plate. Once thawed, the loadable LNPs should be kept on ice or stored at 4°C and used within the same day. Do not re-freeze.

3. **LipidLaunch™ MC3 LNP (Loadable) - (Item No. 401055)**

   This vial contains 150 µl of LipidLaunch™ MC3 LNP (Loadable). After thawing, store on ice. Invert the vial or pipette up and down 2-3 times to fully mix. This vial of MC3 LNPs provides sufficient volume to transfect a full 96-well plate. Once thawed, the loadable LNPs are stable for up to one week when stored at 4°C. If not using all at once, the loadable MC3 LNPs may be frozen and stored at -80°C for up to one month. Do not subject to more than one freeze-thaw cycle.

4. **LipidLaunch™ (S)-C12-200 LNP (Loadable) - (Item No. 400833)**

   Each vial contains 200 µl of LipidLaunch™ (S)-C12-200 LNP (Loadable). After thawing, store on ice. Invert the vial or pipette up and down 2-3 times to fully mix. This vial of (S)-C12-200 LNPs provides sufficient volume to transfect a full 96-well plate. Once thawed, the loadable LNPs are stable for up to two weeks when stored at 4°C. If not using all at once, the loadable (S)-C12-200 LNPs may be frozen and stored at -80°C for up to one month. Do not subject to more than one freeze-thaw cycle.

5. **LipidLaunch™ SM-102 LNP (Loadable) - (Item No. 400648)**

   Reconstitute one vial with 200 µl of nuclease-free water. Gently swirl the vial 2-3 times to fully resuspend. This vial of reconstituted SM-102 LNPs provides sufficient volume to transfect a full 96-well plate. Reconstituted SM-102 LNPs are stable for up to one week when stored at 4°C. Do not re-freeze.

6. **Encapsulation Buffer Preparation**

   Dissolve the LNP Encapsulation Buffer Tablet (Item No. 400812) in 10 ml of sterile-filtered nuclease-free water. The Encapsulation Buffer will be stable for six months when stored at 4°C.

PROTOCOL

Reagent Protocol

General Information

- LNP loading can be performed at room temperature.
- It is recommended to use loaded LNPs within the time frame specified in the **Reagent Preparation** section.
- Loaded LNPs can be diluted directly into culture medium or a neutral buffer of choice.
- If transfecting cells, optimal expression is typically observed using 0.5-2 µl of loaded LNPs per 100 µl of medium.
- Loaded LNPs may be sterile-filtered using 0.2 µm polyethersulfone (PES) syringe filters without affecting performance.
- If not using loaded LNPs immediately, store at 4°C. Do not freeze. Stability of loaded LNPs may vary depending on the cargo type and handling prior to loading.

1. **Cargo Encapsulation**

   - Prepare cargo in nuclease-free water. If using RNA, it is recommended to optimize by initially loading with 10-100 ng/µl.
   - LNPs are loaded by adding reagents in a specific order:

     1. Gently mix loadable LNPs with cargo.

     2. Add Encapsulation Buffer to complete loading.
   - Cargo and LNP volumes can be varied, however, **it is necessary that the volume of Encapsulation Buffer added is one-third the combined volume of cargo and LNP.**
   - Table 3, on page 12, shows an example of recommended loading volumes. Scale accordingly.

Procedure	Volume
Add Loadable LNP	20 µl
Add Cargo	4 µl
Pipette up and down to mix	
Add Encapsulation Buffer	8 µl
Pipette up and down to mix	

Table 3. Recommended initial loading conditions

2. Characterization

A variety of techniques are available to characterize LNPs prior to *in vitro* or *in vivo* use.

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-100
LNP morphology	Microscopy (cryo TEM, ESEM, AFM)
Translation or knockdown analyses	Cell-based reporter assays, Western blotting

Table 4. LNP attributes and corresponding assays. Adapted from Schoenmaker, L., *et al.*[22]

Example Data

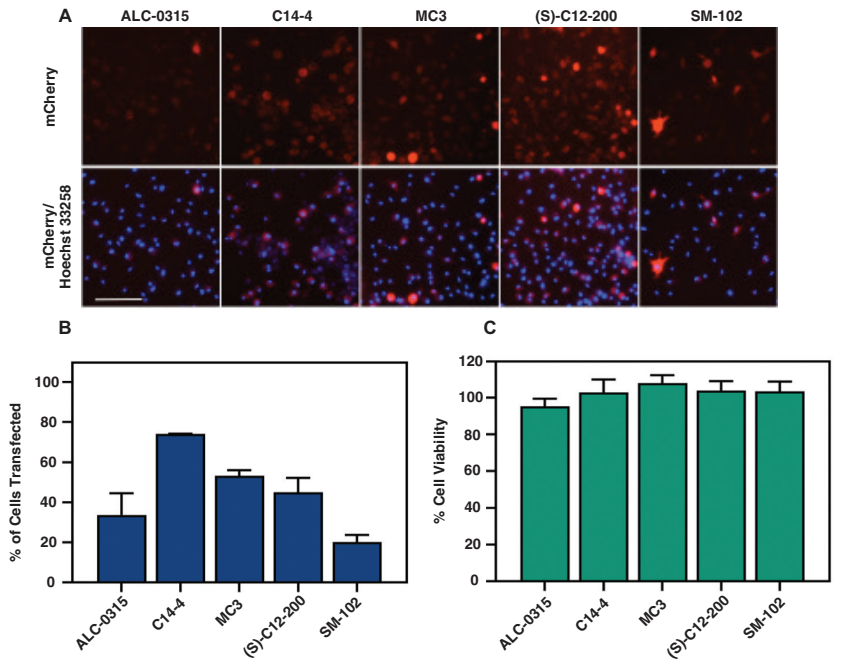

Figure 2. Transfection of murine primary cells with LipidLaunch™ Loadable LNPs. (A) Bone marrow-derived macrophages and dendritic cells harvested from 4-month-old BALB/c mice were transfected with LipidLaunch™ ALC-0315, C14-4, MC4, (S)-C12-200, or SM-102 LNPs loaded with mCherry mRNA (4 µl LNP, 50 ng mRNA per 100 µl medium). Nuclei were stained with Hoechst 33258 (Item No. 16756) and images were taken 48 hours post-transfection. **(B)** Transfection efficiency was determined by scoring the percentage of mCherry-positive cells. **(C)** The viability of cells was determined *via* Cayman's Resazurin Cell Viability Assay Kit (Item No. 702540). Scale bar = 100 µm.

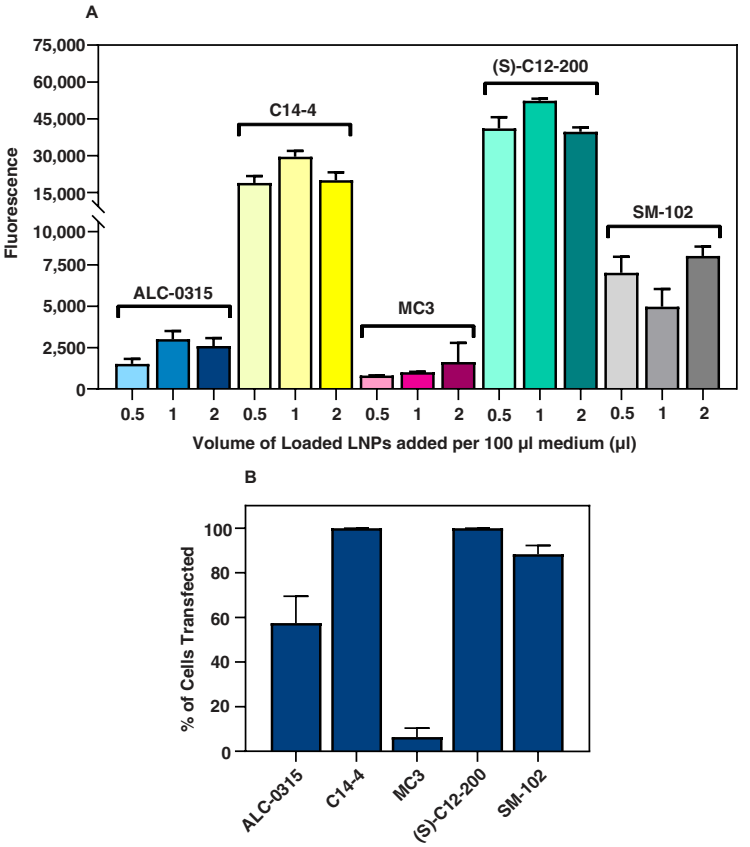

Figure 3. Typical transfection results with mCherry mRNA-loaded LNPs in A549 cells. (A) A549 cells were transfected with loadable LNPs following encapsulation of mCherry mRNA (100 ng/µl mRNA). Fluorescence was determined 48 hours later. **(B)** Transfection efficiency was determined by scoring the percentage of mCherry positive cells.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Poor encapsulation efficiency	A. RNA/cargo is degraded B. Reagents added in incorrect order C. RNA was prepared incorrectly D. Suboptimal cargo:LNP ratio	A. Use fresh RNA/cargo B. Ensure cargo and LNP are mixed prior to adding the Encapsulation Buffer C. Dissolve RNA in nuclease-free water D. Optimize cargo concentration and cargo:LNP ratio

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NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.