LipidLaunch™ C14-4 LNP Kit (Loadable)

Item No. 702860

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

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
401011	LipidLaunch™ C14-4 LNP (Loadable)	4 vials	-80°C
401013	LNP Dilution Buffer B1 (1X)	1 vial/20 ml	-20°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 LipidLaunch™ C14-4 LNP Kit (Loadable).

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. 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 C14-4, 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] C14-4 is an ionizable cationic aminolipid that has been used in combination with other lipids in the formation of LNPs for the delivery of cargos, such as mRNA, *in vivo*.[4,5] LNPs formulated with C14-4 effectively transfect T cells, a cell type that is notoriously difficult to transfect.[4] C14-4-containing LNPs encapsulating mRNA encoding chimeric antigen receptor (CAR) induce CAR expression in primary human T cells *ex vivo* to the same level as expression induced *via* electroporation, but with reduced cytotoxicity, and induce cytotoxicity to cancer cells *in vitro*. Cayman's LipidLaunch™ C14-4 LNP Kit (Loadable) uses the ionizable cationic aminolipid C14-4 to facilitate efficient payload delivery in research models with reduced toxicity compared to traditional methods.

About This Kit

Cayman's LipidLaunch™ C14-4 LNP Kit (Loadable) provides cargo-ready, empty C14-4 LNPs. These LNPs are capable of rapid encapsulation of nucleic acids, without the need for incubation steps, enabling subsequent delivery to target cells or other downstream research applications. LipidLaunch™ LNPs (Loadable) 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. LipidLaunch™ C14-4 LNPs (Loadable) enable users to encapsulate cargo in low reaction volumes offering greater flexibility in the scale of preparation while preserving costly nucleic acid cargo. The reagent has been optimized for transfection of primary and cultured T cells with mRNA.

LipidLaunch™ C14-4 LNP (Loadable) performance is dependent on a variety of factors, including cargo type and cell line, so optimization of these parameters is necessary. To aid in optimization, consider using Cayman's LipidLaunch™ LNP Screening Set (Loadable) (Item No. 502868).

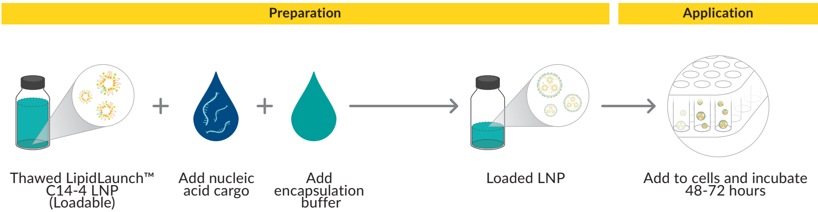

Figure 1. Protocol summary

PREPARATION

Reagent Preparation

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

   Each 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 kit provides a sufficient volume to transfect up to two 96-well plates. Once thawed, the loadable LNPs are should be kept on ice or at 4°C and used within the same day. Do not re-freeze.

2. **LNP Dilution Buffer B1 (1X) - (Item No. 401013)**

   This vial contains 20 ml LNP Dilution Buffer B1 (1X). This optional reagent is intended for optimizing the cargo:LNP loading ratio prior to encapsulation (see **Cargo Encapsulation**, page 10).

3. **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.
- If not using loaded LNPs immediately, store at 4°C for up to one day. Do not freeze.
- It is recommended to use loaded LNPs within one day. The stability of loaded LNPs may vary depending on the cargo type and handling prior to loading.
- Loaded LNPs can be diluted directly into culture medium or a neutral buffer of choice.
- If transfecting cells, optimal expression is typically observed using 2-4 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.

1. Cargo Encapsulation

- Prepare cargo in nuclease-free water. If using RNA, it is recommended to optimize by initially loading with 50-250 ng/µl.
- Loadable LNPs may be optionally diluted with LNP Dilution Buffer B1 (1X) prior to encapsulation.
- 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 40% of the combined volume of cargo and LNP.
- Table 1, on page 11, shows an example of recommended loading volumes. Scale accordingly.

Procedure	Volume
Add Loadable LNP	20 µl
Add Cargo	5 µl
Pipette up and down to mix	
Add Encapsulation Buffer	10 µl
Pipette up and down to mix	

Table 1. 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 2. LNP attributes and corresponding assays. Adapted from Schoenmaker, L., *et al.*[6]

Example Data

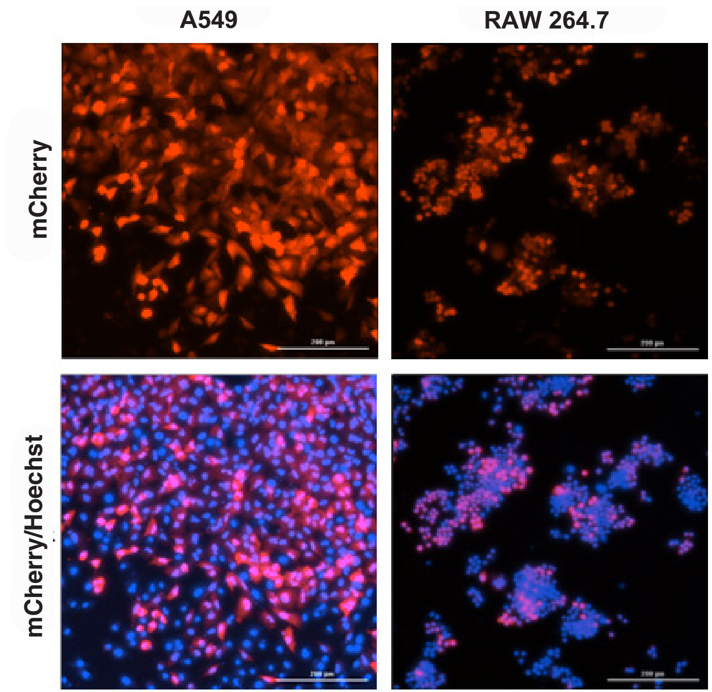

Figure 2. Typical transfection results with mCherry mRNA-loaded LipidLaunch™ C14-4 LNPs (Loadable). A549 and RAW 264.7 cells were transfected with 3-4 µl of C14-4 LNPs loaded with 100-250 ng mCherry mRNA (4-5 x 10^3 cells per well in 100-200 µl medium). Nuclei were stained with Hoechst 33258 (Item No. 16756). Fluorescence images were collected at 20X magnification 24 hours (A549) or 48 hours (RAW 264.7) post-transfection. Bar size: 200 µm.

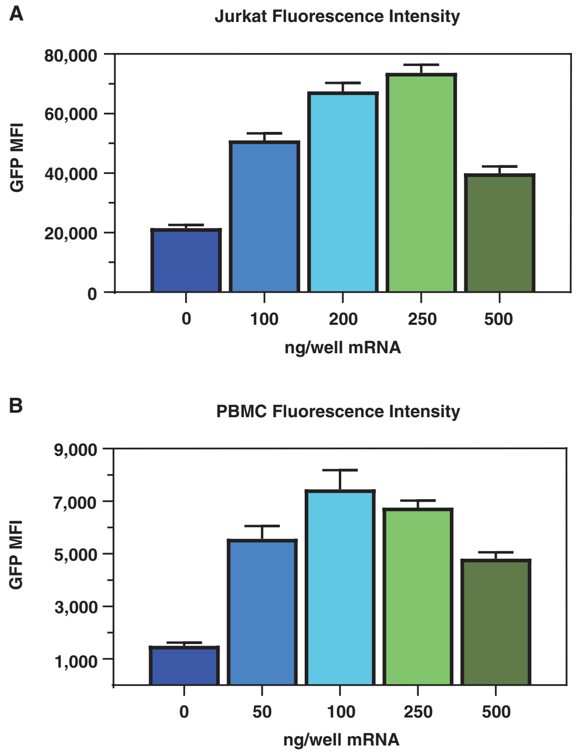

Figure 3. Typical transfection results with GFP mRNA-loaded LipidLaunch™ C14-4 LNPs (Loadable). (A) Jurkat cells (5 x 10^4 cells) and (B) activated human peripheral blood mononuclear cells (PBMCs; 1 x 10^5 cells) were transfected with 4 and 3 µl of C14-4 LNPs loaded with GFP mRNA (Item No. 39800), respectively, in 200 µl medium per well. After 48 hours, all transfected cells were stained with DAPI (Item No. 14285) and PBMCs with anti-CD3, then analyzed by flow cytometry. Mean GFP fluorescence for each of four mRNA concentrations is shown.

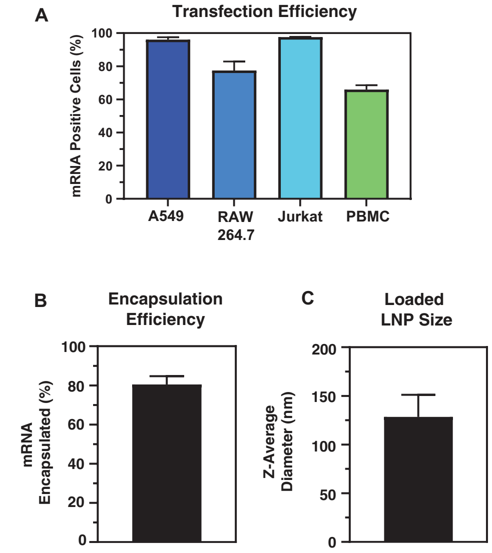

Figure 4. Characterization of mRNA-loaded LipidLaunch™ C14-4 LNPs (Loadable). (A) The transfection efficiency of the experiments shown in Figures 2 and 3 were determined by scoring the percentage of mRNA-positive cells. (B) mCherry mRNA encapsulation efficiencies was quantified. (C) The average mCherry mRNA-loaded particle size was determined *via* DLS.

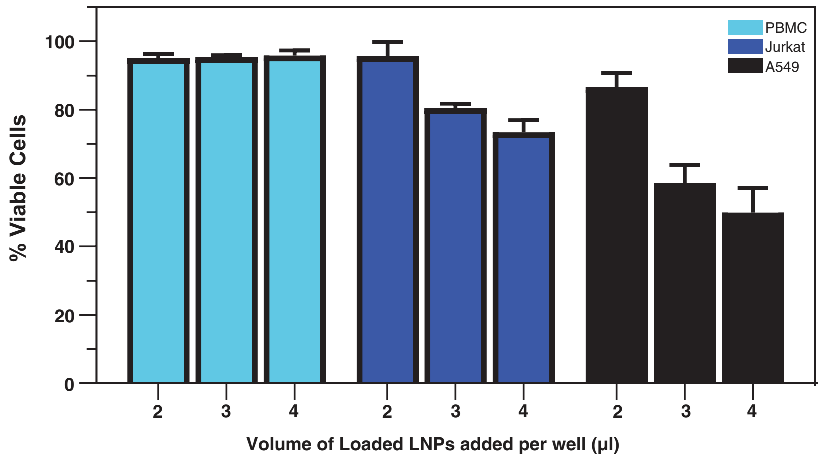

Figure 5. Viability of cells treated with mRNA-loaded LipidLaunch™ C14-4 LNPs (Loadable). Cell viability was determined at 48 hours (PBMCs and Jurkat) and at 24 hours (A549) post-transfection. Viability was normalized to the untreated (mRNA only) control, which was set at 100%.

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

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. Viegas, C., Patrício, A.B., Prata, J.M., *et al.* Solid lipid nanoparticles vs. nanostructured lipid carriers: A comparative review. *Pharmaceutics* **15**, 1593 (2023).
3. Han, X., Zhang, H., Butowska, K., *et al.* An ionizable lipid toolbox for RNA delivery. *Nat. Commun.* **12**, 7233 (2021).
4. Billingsley, M.M., Singh, N., Ravikumar, P., *et al.* Ionizable lipid nanoparticle-mediated mRNA delivery for human car T cell engineering. *Nano Lett.* **20(3)**, 1578-1589 (2020).
5. Patel, S.K., Billingsley, M.M., Mukalele, A.J., *et al.* Bile acid-containing lipid nanoparticles enhance extrahepatic mRNA delivery. *Theranostics* **14(1)**, 1-16 (2024).
6. 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

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.