LipidLaunch™ ALC-0315 LNP Kit (Loadable)

Item No. 702750

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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
400773	LipidLaunch™ ALC-0315 LNP (Loadable)	2 vials	-80°C
400772	LNP Dilution Buffer B (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™ ALC-0315 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 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, 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] ALC-0315 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 small molecules, mRNA, siRNA, and plasmid DNA *in vitro* and *in vivo*.[4-9] In mice, ALC-0315-containing LNPs have been shown to accumulate in the liver and, to a lesser extent, in the spleen.[10] Cayman's LipidLaunch™ ALC-0315 LNP Kit (Loadable) uses the ionizable cationic aminolipid ALC-0315 to facilitate efficient payload delivery in research models with reduced toxicity compared to traditional methods.

About This Kit

Cayman's LipidLaunch™ ALC-0315 LNP Kit (Loadable) provides cargo-ready, empty ALC-0315 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™ ALC-0315 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.

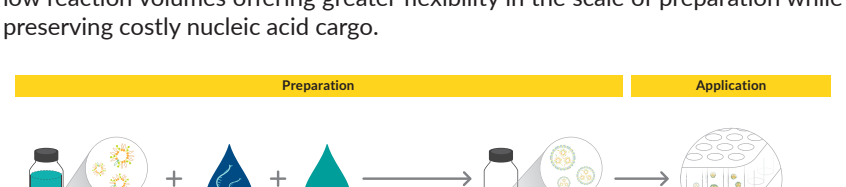

Figure 1. Protocol summary

PREPARATION

Reagent Preparation

1. **LipidLaunch™ ALC-0315 LNP (Loadable) - (Item No. 400773)**

   Each 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. Each vial of ALC-0315 LNPs provides a sufficient volume to transfect up to 50 wells using 24-well plates. Once thawed, the loadable LNPs are stable for up to two weeks when stored at 4°C. Do not re-freeze.

2. **LNP Dilution Buffer B (1X) - (Item No. 400772)**

   This vial contains 20 ml LNP Dilution Buffer B (1X). This optional reagent is intended for optimizing the cargo:LNP loading ratio prior to encapsulation (see **Cargo Encapsulation**, page 9).

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. Do not freeze.
- It is recommended to use loaded LNPs within one week. 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 1-3 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 10-100 ng/µl.
- Loadable LNPs may be optionally diluted with LNP Dilution Buffer B (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 one-third the combined volume of cargo and LNP.
- Table 1, below, 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 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.*[11]

Example Data

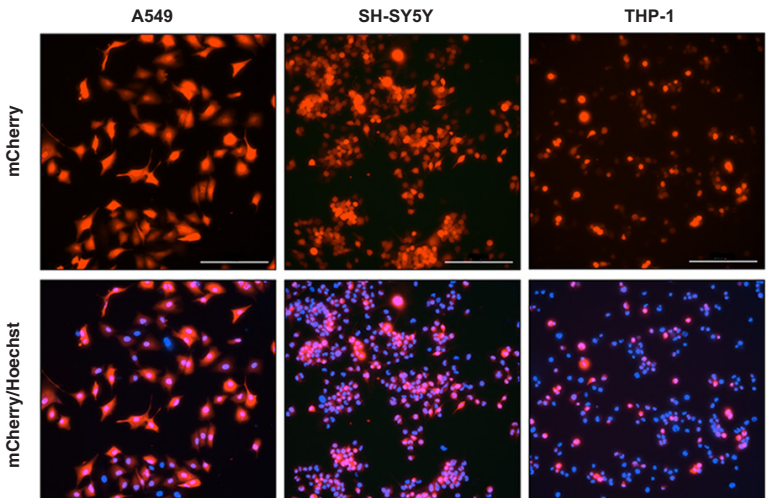

Figure 2. Typical transfection results with mCherry mRNA-loaded LipidLaunch™ ALC-0315 LNPs (Loadable). A549, SH-SY5Y, and THP-1 cells were transfected with loadable LNPs following encapsulation of mCherry mRNA. Fluorescence images were collected at 20X magnification 72 hours later (mCherry (upper), mCherry/Hoechst merge (lower); Bar size: 200 µm).

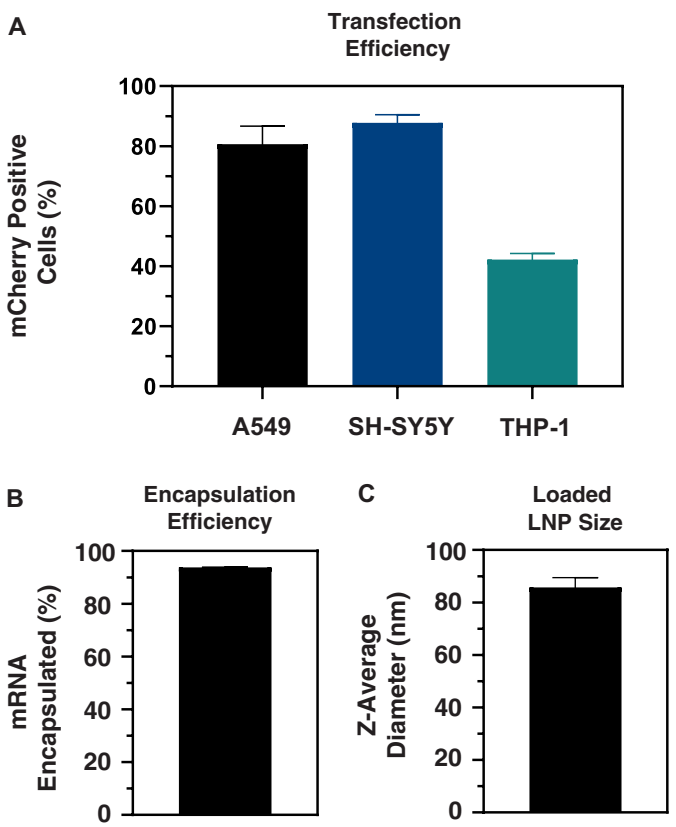

Figure 3. Characterization of mCherry mRNA-loaded LipidLaunch™ ALC-0315 LNPs (Loadable). (A) The transfection efficiency of the experiment shown in Fig. 2 was determined by scoring the percentage of mCherry-positive cells. (B) mRNA encapsulation efficiency was determined as previously described.[12] (C) The average loaded particle size was determined *via* DLS.

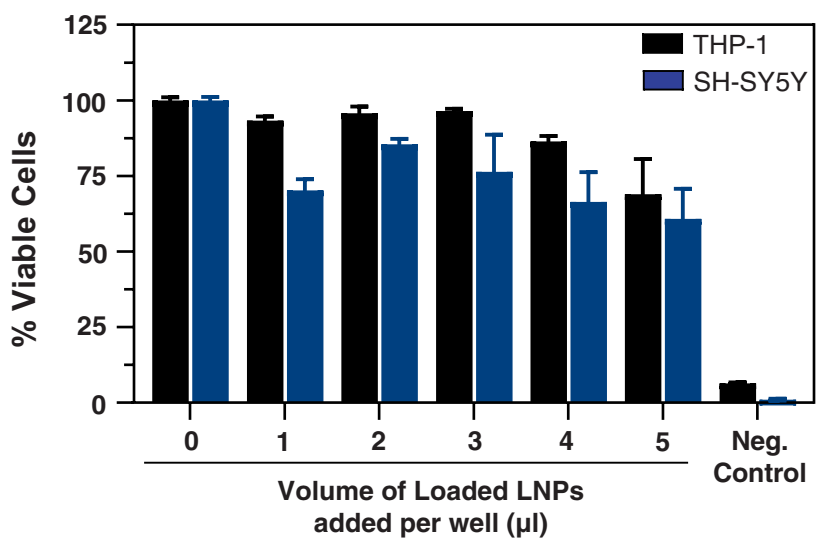

Figure 4. Viability of cells treated with LipidLaunch™ ALC-0315 LNPs (Loadable). THP-1 and SH-SY5Y cells were cultured in 96-well plates (100 µl medium/well) and incubated with mCherry mRNA-loaded (50 ng/well) LNPs without changing the media. Negative control wells were incubated with Triton-X 100. Cell viability was determined 72 hours later *via* the Resazurin Cell Viability Assay Kit (Item No. 702540).

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. Nat. Rev. Drug Discov.* **20(2)**, 101-124 (2021).
2. Viegas, C., Patrício, A.B., Prata, J.M., *et al. Pharmaceutics* **15**, 1593 (2023).
3. Han, X., Zhang, H., Butowska, K., *et al. Nat. Commun.* **12**, 7233 (2021).
4. Vogel, A.B., Kanevsky, I., Che, Y., *et al. Nature* **592(7853)**, 283-289 (2021).
5. Ferraresso, F., Strilchuk, A.W., Juang, L.J., *et al. Mol. Pharm.* **19(7)**, 2175-2182 (2022).
6. Chen, J., Ye, Z., Huang, C., *et al. Proc. Natl. Acad. Sci. USA* **119(34)**, e2207841119 (2022).
7. Yu, H., Sarkar, S., Shaw, Z.L., *et al. Small Sci.* **4(10)**, 2400145 (2024).
8. Ansell, S.M. and Du, X. *Acuitas Therapeutics, Inc.* US 10,166,298 B2 (2019).
9. Zhang, W., Pfeifle, A., Lansdell, C., *et al.* Tn mice. *Vaccines (Basel)* **11(10)**, 1580 (2023).
10. Lam, K., Leung, A., Martin, A., *et al. Adv. Mater.* **35(15)**, e2209624 (2023).
11. Schoenmaker, L., Witzigmann, D., Kulkarni, J.A., *et al. . Int. J. Pharm.* **601**, 120586 (2021).
12. Geall, A.J., Verma, A., Otten, G.R., *et al. PNAS* **109(36)**, 14604-14609 (2012).

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.