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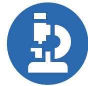 Laborgeräte & Service

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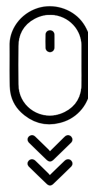
See the following pages for more information!



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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

LipidLaunch™ Loadable LNP SM-102 Kit

Item No. 702620

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
400648	LipidLaunch™ Loadable LNP SM-102	2 vials	-80°C
400649	LNP Dilution Buffer A (1X)	1 vial/20 ml	4°C
10009322	Cell-Based Assay 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™ Loadable LNP SM-102 Kit. 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

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 source of nuclease-free water is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
2. Adjustable pipettes; multichannel or repeating pipettor recommended.
3. Materials used for **Sample Preparation** (see page 8)
4. Materials and reagents needed for cell culture

INTRODUCTION

Background

Lipid nanoparticles (LNPs) are a subset of lipid-based drug delivery (LBDD) systems that utilize ionizable cationic lipids, such as SM-102, for the delivery of RNA payloads to cells.[1,2] They frequently 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 RNA. 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] SM-102 is an ionizable cationic amino lipid that has been used in combination with other lipids in the formation of LNPs.[4,5] Traditional lipid-based transfection methods commonly use fixed cationic lipids, resulting in high toxicity.[6] Cayman's LipidLaunch™ Loadable LNP SM-102 Kit's use of the cationic aminolipid SM-102 facilitates efficient RNA transfection with reduced toxicity compared to traditional methods.

Cayman's LipidLaunch™ Loadable LNP SM-102 Kit provides cargo-ready, empty, lyophilized SM-102 LNPs specially formulated for *in vitro* RNA transfection. Following resuspension, these LNPs are capable of rapid encapsulation of RNA, without the need for incubation steps, enabling subsequent delivery to target cells. 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 larger reaction volumes. LipidLaunch™ Loadable SM-102 LNPs enable users to encapsulate RNA cargo in reaction volumes as low as 50 µl, offering greater flexibility in the scale of preparation while preserving costly RNA cargo.

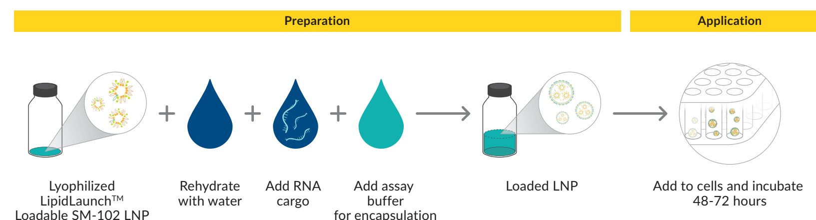

Figure 1. Protocol summary

PREPARATION

Sample Preparation

Dilute RNA cargo in nuclease-free water. The optimal loading concentration of cargo and the optimal final concentration of LNPs should be varied experimentally. Transfection efficiency will depend on both the cargo and cell type. It is recommended to optimize from an initial RNA concentration of 0.1 mg/ml.

Reagent Preparation

1. **LipidLaunch™ Loadable LNP SM-102 - (Item No. 400468)**

   Each vial of reconstituted SM-102 LNPs provides a sufficient volume to transfect up to 50 wells of a 24-well plate. Reconstitute one vial with 200 µl nuclease-free water. Gently swirl the vial 2-3 times to fully resuspend. Reconstituted LNPs are stable for up to one week when stored at 4°C.

2. **LNP Dilution Buffer A (1X) - (Item No. 400469)**

   This vial contains 20 ml of LNP Dilution Buffer A (1X). LNP Dilution Buffer A (1X) will be stable for at least 1 month when stored at 4°C. This optional reagent is intended for optimizing the cargo:LNP loading ratio prior to encapsulation (see **Cargo Encapsulation**, page 9).

3. **Cell-Based Assay Buffer Preparation**

   Dissolve the Cell-Based Assay Buffer Tablet (Item No. 10009322) in 100 ml of sterile-filtered nuclease-free water.

PROTOCOL

Once rehydrated, Loadable LNPs are briefly mixed with cargo (*i.e.* RNA) followed by a dilution step in Cell-Based Assay Buffer to promote encapsulation. Loaded LNPs are then ready for downstream delivery to target cells or other characterization. LNP loading can be performed at room temperature.

1. **Cargo Encapsulation**

   A. Add 1 volume of cargo to 4 volumes of reconstituted LNP. Gently pipette up and down to mix.

   - Optimal transfection is typically observed using 0.5-2 µl reconstituted LNP per final 100 µl media volume.
   - *Optional:* To test lower concentrations of lipids, dilute reconstituted LNPs with LNP Dilution Buffer A (1X) prior to mixing with cargo.
   - Optimal transfection is typically observed using 5-250 ng RNA per final 100 µl media volume.

   B. Dilute the cargo-LNP mix 4-fold with Cell-Based Assay Buffer to promote RNA encapsulation. Pipette up and down gently. For optimal results, loaded LNPs should be used within the same day. For short-term storage, loaded LNPs may be stored at 4°C for up to 7 days while still retaining transfection activity.

   NOTE: See Table 1, page 10, for recommended volumes needed for in vitro transfection.

2. *In Vitro* Transfection

For *in vitro* transfection, it is recommended to dilute the encapsulated cargo-LNP mix ten-fold into the relevant complete growth medium. The following example reaction is sufficient to transfect 20 wells of a 96-well plate, 4 wells of a 24-well plate, or 1 well of a 6-well plate. Scale volumes as needed.

	Procedure	Reagent	96-well	24-well	6-well
Day before transfection	Seed cells	Adherent cells/ suspension cells	1-4 x 10^4	0.5-2 x 10^5	0.2-1 x 10^6
Day of transfection	Prepare LNP transfection media	Reconstituted LNP	0.5-2 µl	2.5-10 µl	10-40 µl
		LNP Dilution Buffer A	0-1.5 µl	0-7.5 µl	0-30 µl
		Vol. RNA	0.5 µl	2.5 µl	10 µl
		Vol. Assay Buffer	7.5 µl	37.5 µl	150 µl
		Vol. Growth medium	90 µl	450 µl	1,800 µl
	Transfect cells	Vol. of LNP transfection media per well	100 µl	500 µl	2,000 µl
48 to 72 hours post-transfection	Image/ analyze cells				

Table 1. Recommended volumes for *in vitro* transfection

3. 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
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.*[7]

4. Example data

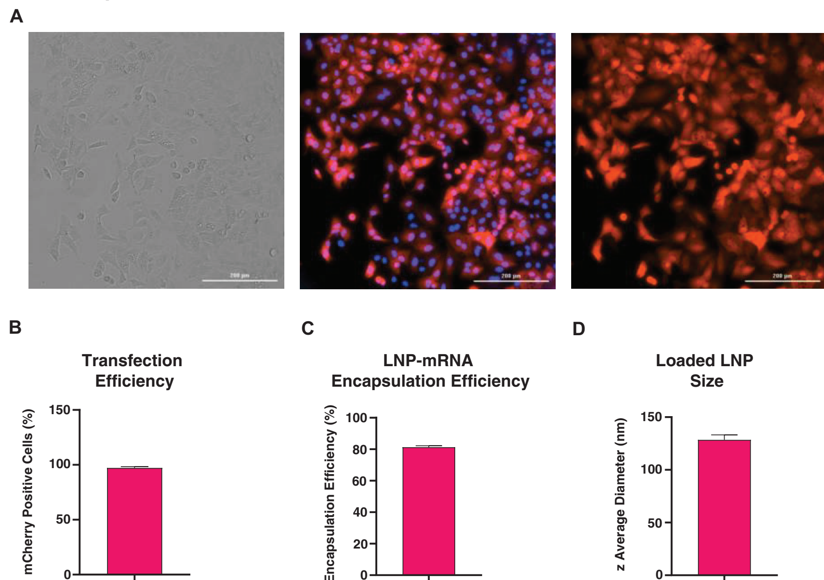

Figure 2. Characterization of mCherry mRNA-loaded LipidLaunch™ Loadable LNPs. A549 cells were transfected with Loadable LNPs following encapsulation of mCherry mRNA. Brightfield & fluorescence images were collected at 20X magnification 72 hours later (Panel A: Brightfield (left), mCherry/DAPI merge (center), and mCherry (right); Bar size: 200 µm), and transfection efficiency was determined by scoring the percentage of mCherry-positive cells (Panel B). mRNA encapsulation efficiency was determined as previously described (Panel C).[8] Average loaded particle size was determined *via* dynamic light scattering (Panel D).

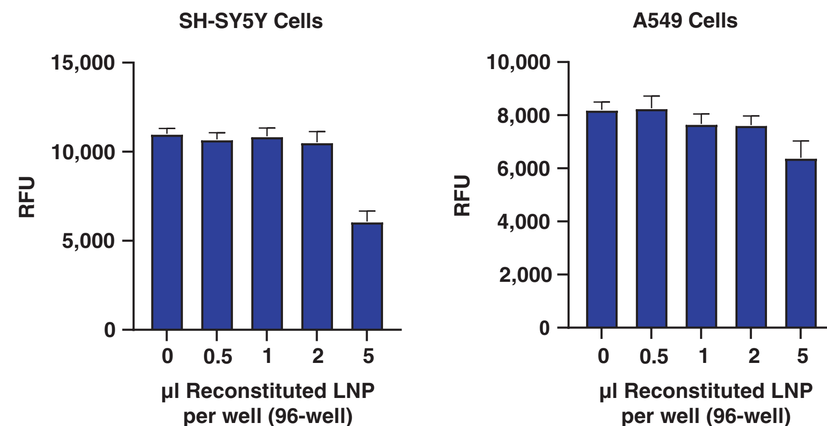

Figure 3. Cell viability of LipidLaunch™ Loadable LNP treated cells. SH-SY5Y and A549 cells were cultured in 96-well plates and treated with mCherry mRNA-loaded (50 ng/well) loadable LNPs without changing media. Cell viability was determined 72 hours later *via* the Resazurin Cell Viability Assay Kit (Item No. 702540).

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Cells dying after transfection	A. Expressed protein is cytotoxic B. Cargo is cytotoxic	A. Reduce LNP/cargo concentration B. Ensure a pure source of cargo is used
Low transfection efficiency	A. Sub-optimal LNP:cargo loading conditions B. RNA cargo is degraded	A. Adjust LNP/cargo loading concentration B. Use high quality source of RNA cargo; work in a nuclease-free environment

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. Sabnis, S., Kumarasinghe, E.S., Salerno, T., *et al. . Mol. Ther.* **26(6)**, 1509-1519 (2018).
5. Naidu, G.S., Yong, S.-B., Ramishetti, S., *et al. Adv. Sci. (Weinh).* **10(19)**, e2301929 (2023).
6. Schlich, M., Palomba, R., Costabile, G., *et al. Bioeng. Transl. Med.* **6(2)**, e10213 (2021).
7. Schoenmaker, L., Witzigmann, D., Kulkarni, J.A., *et al. . Int. J. Pharm.* **601**, 120586 (2021).
8. Geall, A.J., Verma, A., Otten, G.R., *et al. PNAS* **109(36)**, 14604-14609 (2012).

NOTES

Warranty and Limitation of Remedy

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