Echelon Biosciences Inc.

LNP Trailblazer Kit - Pre-mixed

K-6300M

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Description: Pre-packaged lipids for evaluating and optimizing delivery of RNA cargo via lipid nanoparticles. Aqueous buffer for preparation of RNA is also included.

Materials Provided

Catalog #	Description	Quantity
K-6301	RNA Dilution Buffer	5 mL
K-6302a-e	LNP Lipid Mix**	1 mL

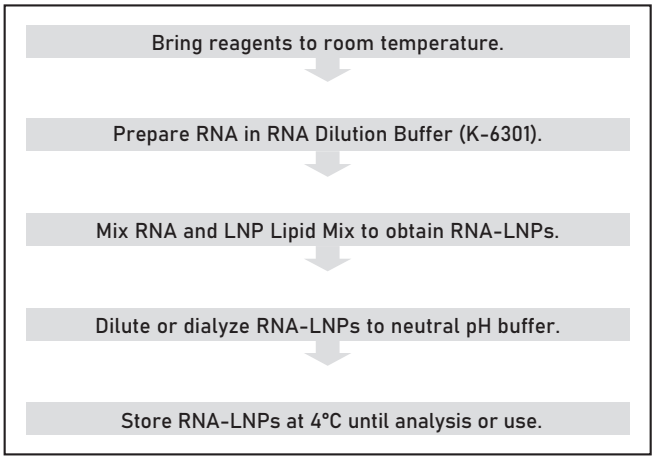
**refer to table below for the ionizable lipid in the specific LNP Lipid Mixes

K-6302a	ALC-0315
K-6302b	cKK-E12
K-6302c	SM-102
K-6302d	Lipid 5
K-6302e	Dlin-MC3-MCA

Additional Materials Provided by User

- mRNA for packaging and testing
- Electrophoresis reagents or plate reader with fluorescence capabilities
- RNA detection reagent
- Pipettes or benchtop microfluidic mixer

Quick Protocol



Storage

Upon receipt, individual lipids and the LNP lipid mix should be stored at -20°C. The RNA Dilution Buffer is stable at room temperature or 4°C. Under proper storage conditions, the kit components should remain stable for at least 6 months from date of receipt. Allow the reagents to warm to room temperature before use.

Precepitation of lipids in ethanol solution can be expected at temperatures below 4°C. Vortex and heat lipid solutions to 50°C as needed to solubilize lipids prior to use.

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Kit Design

The LNP Trailblazer Kits are designed to simplify testing of lipid nanoparticles for delivery of RNA cargo. K-6300M contains a pre-formulated mixture of lipids and low pH buffer for preparing RNA and the option to choose one or more ionizable lipids for testing.

Technical Notes

1. RNA stocks should be prepared at sufficiently high concentrations prior to dilution with the RNA Dilution Buffer. In our optimization experiments, RNA was stored at -80°C at 1 mg/mL in aqueous buffer and was stable for at least 30 days.
2. RNA-LNPs diluted or dialyzed to neutral buffers are stable for at least 30 days at 4°C. Longer term storage at -20°C is possible but some loss of activity may occur due to freeze-thaw effects on the lipids. Addition of cryoprotectants or lyophilization of RNA-LNPs for storage at -20 to -80°C should be evaluated by the user.
3. Successful delivery of RNA cargo may depend on cell type and the ionizable lipid being used. Results may also vary depending on the type of cell culture system, i.e. monolayers vs. 3D cultures. See Table 1 for additional details.
4. Use of serum free media for application of RNA-LNPs in cell culture is not required.

Table 1, Ionizable lipid transfection efficiency

	HEK293	NIH3T3
ALC-0315	++*	-
SM-102	++++	++
cKK-E12	+	-
Lipid 5	+++	+
Dlin-MC3-DMA	+++	+

Table 1 - Relative transfection efficiency of a GFP mRNA with different ionizable lipids in two common cell types.

*Efficacy with ALC-0315 varied between monolayer cells and portions of the cell culture that had some 3D characteristics.

Assay Protocol

Please read this entire section and assay notes before beginning.

This procedure is for a 5:1 RNA:lipid mix (v:v). In our optimization experiments, the mRNA used was diluted to 0.4 µM in RNA Dilution Buffer prior to mixing with lipids. LNPs may be prepared by hand mixing or by automated microfluidic devices. The procedure below describes small scale preparation using hand mixing which can be scaled as needed.

1. Bring LNP Lipid Mix to room temperature prior to use and vortex as needed to mix.
2. Thaw RNA stocks on ice.
3. Once thawed, bring the RNA to the desired concentration using RNA Dilution Buffer.
4. Calculate the amount of lipid required to achieve a 5:1 RNA:lipid ratio. Ex: for every 100 µL of RNA, 20 µL of lipid mix is required.
5. Add the lipid mix to the RNA solution and mix rapidly. The solution should become cloudy and opaque. We do not recommend mixing by vortex as this may shear free RNA.
6. Once the LNPs have formed, they should be diluted or dialyzed into a neutral pH buffer such as PBS (pH 7.2-7.4).

For small scale preparations a 20x dilution is sufficient to neutralize pH and dilute out the EtOH from the lipid mix. Ex: LNPs formed in a 5:1 RNA:lipid ratio with a 100 µg/mL concentration of RNA can be diluted 20x to 5 µg/mL RNA and then diluted further in buffer, vehicle, or media for testing in vitro or in vivo testing. Dialysis should be used if higher RNA concentrations are desired or if the volume of LNPs prepared is sufficiently large, i.e. >5-10 mL. All LNPs should be 0.2 micron filter sterilized before use if not prepared under sterile conditions.

Once LNPs are in a neutral pH buffer they are stable for at least one week at 4°C. In our experiments 5 µg/mL RNA LNPs were stable at 4°C up to 30 days. Stability may vary depending on individual RNAs and ionizable lipids and should be monitored by the user.

Analysis - Encapsulation Efficiency

Please read this entire section before beginning.

Quantitative – fluorescence-based quantification using reagents such as QuantiFluor®, Quant-it RiboGreen™, or similar products.

1. Prepare detection reagents as described by manufacturer in TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0).
2. Prepare a 1% v/v solution of Triton X-100 in TE buffer.
3. Add the 1% Triton X-100 solution to an aliquot of the LNPs to 0.2% v/v final concentration and incubate on ice for 10-15 minutes. Add an equivalent volume of TE alone to separate aliquots of the LNPs.
4. Add the prepared detection reagent to a 96-well plate (white or black, not clear) and add the required amount of LNP samples (+Triton or +TE). Mix briefly and incubate at room temperature for 5 minutes. Protect from light.
5. Read the plate according to the excitation/emission spectra for the detection reagent.
6. Encapsulation efficiency (EE) is calculated as follows:

   EE (%) = 100 * (T-U)/T

   where 'T' is the total RNA in the sample (+Triton) and 'U' is the free, unencapsulated RNA (+TE).

For absolute quantification of total encapsulated RNA, an RNA standard with known concentration should be run in parallel with the samples.

Qualitative – agarose gel-based assessment of encapsulated RNA.

1. Prepare a 1% agarose (w/v) TAE gel with sufficient lanes for all samples and a gel ladder. Add nucleic acid dye such as SYBR Safe at a 1:10,000 dilution.
2. Prepare a 1% v/v solution of Triton X-100 in TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0).
3. Add the 1% Triton X-100 solution to aliquot of the LNPs to 0.2% v/v final concentration and incubate on ice for 10-15 minutes. Add an equivalent volume of TE alone to separate aliquots of the LNPs.
4. Add gel loading buffer to LNP samples (+Triton or +TE) and load all samples and the gel ladder to the prepared gel.
5. Run the gel for approximately 45 minutes at 100V and image under UV light.

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Successful LNP preparation and encapsulation should result in little to no visible bands for the lanes of the gel with +TE (untreated) samples as the RNA is protected from the dye in the gel by the lipids. Conversely, +Triton (treated) samples should have readily apparent bands on the gel due to release of the RNA from the LNPs due to Triton treatment.

References

1. R. Tenchov, R. Bird, A. E. Curtze, Q. Zhou (2021) "Lipid Nanoparticles—From Liposomes to mRNA Vaccine Delivery, a Landscape of Research Diversity and Advancement" ACS Nano
2. Dong et al. (2014) Lipopeptide nanoparticles for potent and selective siRNA delivery in rodents and nonhuman primates. PNAS Mar 18;111(11):3955-60
3. Sabnis S, Kumarasinghe ES, Salerno T, et al. A Novel Amino Lipid Series for mRNA Delivery: Improved Endosomal Escape and Sustained Pharmacology and Safety in Non-human Primates. Mol Ther. 2018;26(6):1509-1519
4. Jayaraman, M., Ansell, S.M., Mui, B.L., et al. (2012) "Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing in vivo." Angew. Chem. Int. Ed. Engl. 51(34), 8529-8533. DOI: 10.1002/anie. 201203263.

Related Products

Catalog #	Products
N-1020	ALC-0315
N-1012	cKK-E12
N-1282	Dlin-MC3-DMA
N-1438	4A3-SC8
N-1102	SM-102
N-1005	Lipid 5
N-1006	Lipid 6
N-1380	DOTAP
N-1919	9A1P9
N-1220	c12-200

Please visit our website at www.echelon-inc.com for more LNP and lipid products.

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