In partnership with labcorp

Application Note

Biodistribution Assessment of Lipid Nanoparticle-Mediated mRNA Delivery Using *In Vivo* Imaging

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Key Features

- Cayman's LNP development services in partnership with Labcorp utilizes *in vivo* imaging technology to create a comprehensive LNP screening platform.
- With this approach, three LNP formulations were assessed *in vitro* and *in vivo* for potency of reporter gene expression and differential organ delivery.
- This comprehensive LNP formulation, characterization, and analysis service can be used to identify candidate LNP formulations with tissue-tropic distribution.

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Introduction

Lipid nanoparticles (LNPs) are an efficient platform for the *in vivo* delivery of nucleic acid therapies, representing a significant advancement over traditional transfection reagents that are largely limited to *in vitro* applications.

There is broad and current interest in developing approaches for organ-targeted nucleic acid delivery with LNPs. By leveraging both active (ligand-mediated) and passive (biophysical characteristic-mediated) targeting strategies, LNPs can be recognized and taken up by specific organs, tissues, and cells during *in vivo* delivery.

Here, a workflow for mRNA-LNP formulation and performance assessment is outlined. Three different LNP formulations, varying primarily in the ionizable cationic lipid used, encapsulating mRNA encoding firefly luciferase were generated by Cayman Chemical. Luciferase expression was tested in A549 lung epithelial cells and Huh7 hepatocytes *in vitro* and in naïve BALB/c mice using *in vivo* imaging through a partnership with Labcorp.

Experimental Design

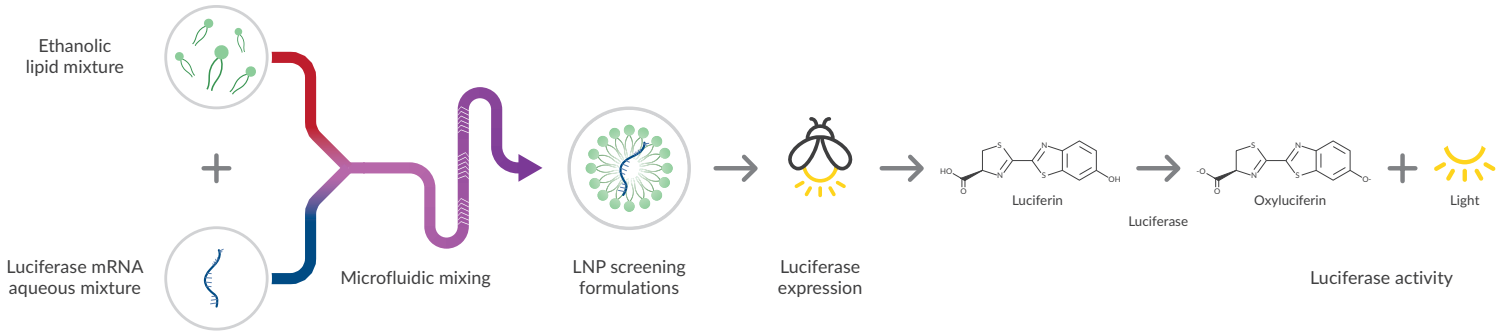
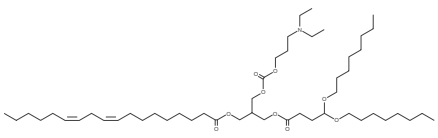
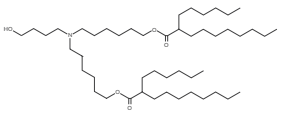
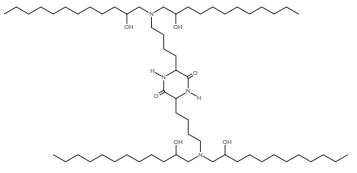


Figure 1. Schematic workflow of luciferase mRNA-LNP screening.

LNP Preparation

1. Cayman Chemical supplied all lipids and prepared, formulated, and characterized LNPs used in this study.
2. Lipid mixes were prepared in ethanol using the lipid mix molar ratios shown below:

LNP Formulations Screened

Ionizable Cationic Lipid	LP-01	ALC-0315	cKK-E12
			
Phospholipid	1,2-DSPC	1,2-DSPC	1,2-DOPE
Sterol lipid	Cholesterol	Cholesterol	Cholesterol
PEGylated lipid	DMG-PEG(2000)	ALC-0159	DMG-PEG(2000)
Molar lipid ratios (%)	45:9:44:2	46.3:9:4:42.2:1.6	50:10:38.5:1.5

3. The aqueous phase contained 200 µg/ml luciferase-mRNA in 50 mM sodium acetate, pH 4.5.
4. LNPs were formed by microfluidic mixing. The total flow rate was 10 ml/min with a flow rate ratio of 3:1 (mRNA:lipid).
5. LNPs were dialyzed against PBS, pH 7.4, overnight and stored at 4°C until use.
6. For *in vivo* studies, LNPs were concentrated using 30 MWCO Amicon concentrators and sterile filtered prior to injection.

Biophysical Characteristics

1. LNP size and polydispersity were determined by dynamic light scattering (DLS).
2. mRNA concentration and encapsulation efficiency were determined using a fluorescent RNA dye in the absence and presence of 0.5% Triton X-100.

In Vitro Studies

1. A549 lung epithelial and Huh7 hepatocytes were seeded for LNP treatment at 7,500 cells per well in 96-well plates.
2. LNPs were added to cells in media to a final luciferase-mRNA concentration of 125, 250, or 500 ng/ml.
3. Following treatment of the cells with LNPs for 24 hours, Promega's ONE-Glo™ assay kit was used to measure luciferase activity.

In Vivo Studies

1. Animal care and use was performed in accordance with applicable animal welfare regulations at an AAALAC International accredited animal program and IACUC approved. For all experiments, female BALB/c mice were administered LNPs intravenously at 0.75 mg/kg in DPBS. For control (no LNP) conditions, an equivalent volume of DPBS was administered.
2. At two hours post-injection, mice were anesthetized with isoflurane and imaged 10 minutes after intraperitoneal injection of 10 ml/kg D-luciferin (15 mg/ml).
3. At six hours post-injection, luciferin was again administered, and mice were euthanized and organs collected for *ex vivo* imaging. For *ex vivo* imaging, tissues were placed in 24 well plates containing a 300 µg/ml solution of D-luciferin.
4. Luciferase imaging was performed on an IVIS Spectrum (Perkin Elmer). Exposure times were adjusted to obtain at least several hundred counts per image and to avoid saturation of the CCD chip. Image analysis was performed using Living Image® 4.7.1 software (Perkin Elmer). Total flux (photons/second) were quantified from regions of interest at two- and six-hours post-injection.

Results & Discussion

LNP Preparation

LP-01 and ALC-0315 LNPs had an average diameter of less than 100 nm whereas cKK-E12 LNPs were larger, with an average diameter of approximately 150 nm (**Figure 2A**). All LNP formulations had low polydispersity index (PDI) below 0.2, indicating a narrow particle size distribution. LP-01 and ALC-0315 LNPs had high mRNA encapsulation efficiency, indicating minimal mRNA loss during preparation (**Figure 2B**). In contrast, cKK-E12 LNPs had less robust encapsulation efficiency and a lower concentration of encapsulated RNA, suggesting that further optimization of this formulation may be beneficial. All *in vitro* and *in vivo* experiments were conducted normalizing to encapsulated RNA concentration.

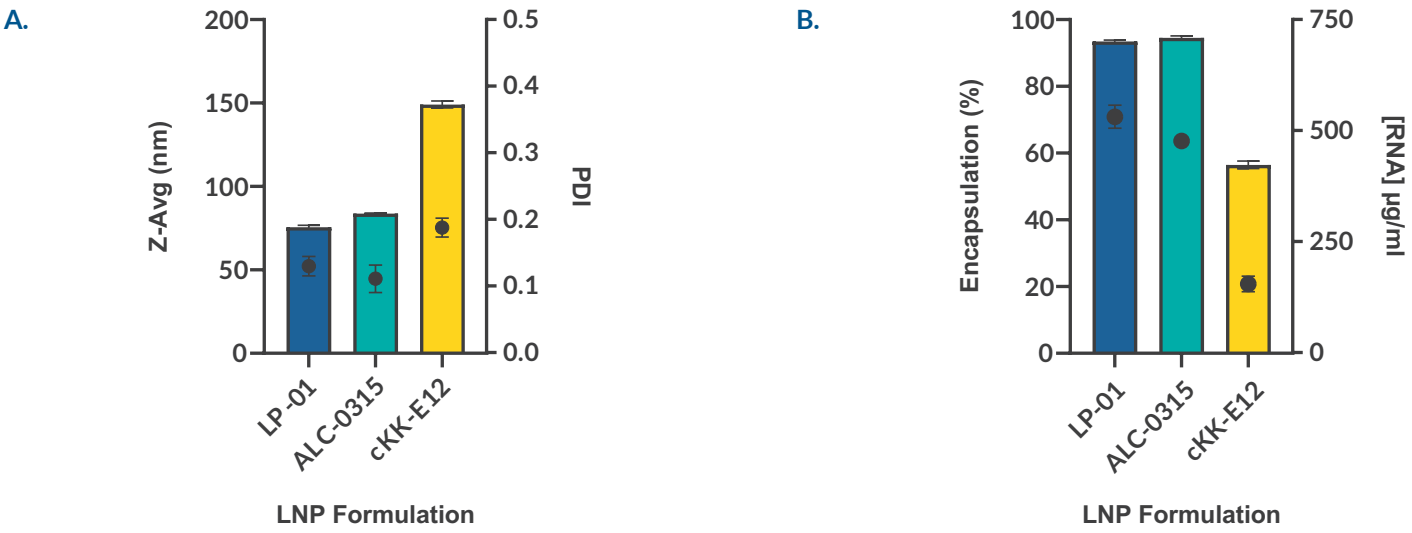


Figure 2. LNP characteristics. **A**) LNP average particle size (bars) and PDI (points) were measured using DLS. **B**) RNA encapsulation efficiency (bars) and concentration (points) were quantified using a fluorescent RNA dye in the absence and presence of 0.5% Triton X-100.

In vitro expression of mRNA-LNPs

Dose-response experiments were performed in A549 lung epithelial cells and Huh7 hepatocytes to evaluate expression differences in LNP formulations for luciferase mRNA delivery *in vitro*. Increasing luciferase expression was observed with increasing concentrations in both cell lines tested with all three LNP formulations. Luciferase activity was easily detected at the lowest concentration (125 ng/ml) used in this study. ALC-0315 LNPs were the most potent LNP formulation tested in A549 cells (**Figure 3**) and induced slightly greater luciferase activity at the highest concentration tested in Huh7 cells. All LNP formulations tested induced significant luciferase expression in both Huh7 and A549 cells with minimal differences at lower concentrations.

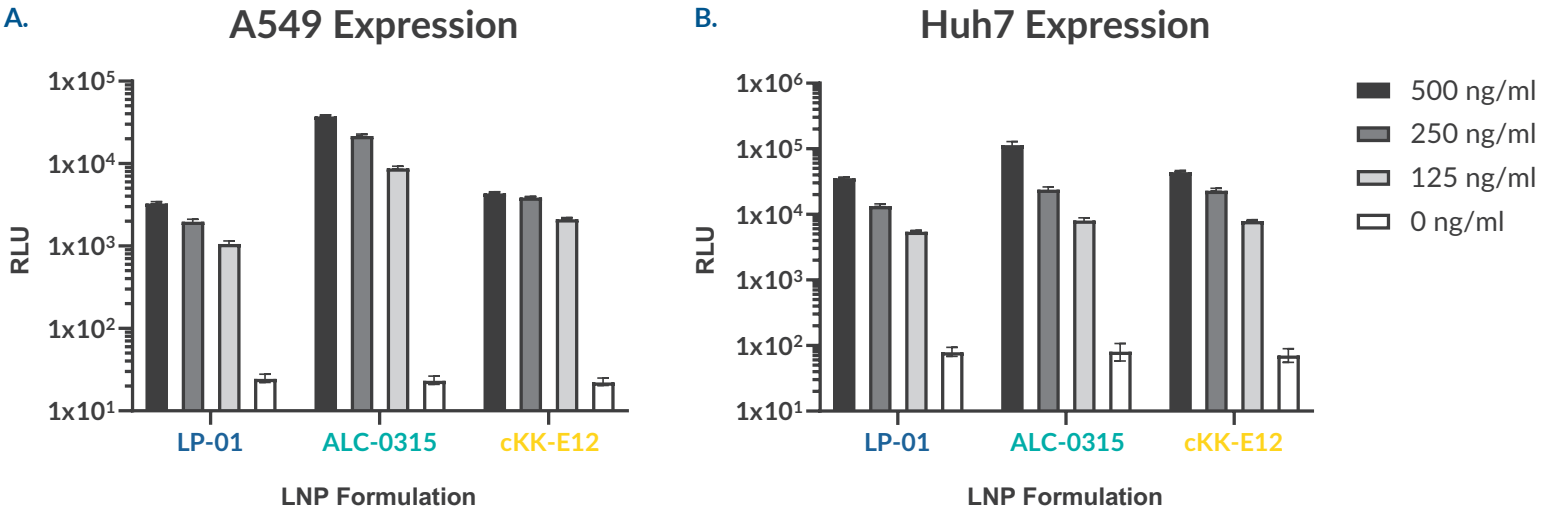


Figure 3. Luciferase activity in **A**) A549 lung epithelial cells and **B**) Huh7 hepatocytes.

In vivo expression of mRNA-LNPs

When LNPs were administered intravenously in mice, we found that ALC-0315 LNPs were the most potent formulation for luciferase expression two hours post-injection, consistent with our *in vitro* experiments (**Figure 4**). LP-01 and cKK-E12 LNPs also induced notable luciferase expression. Notably, a strong bioluminescent signal in the upper abdomen of the mice treated with all LNP formulations was observed.

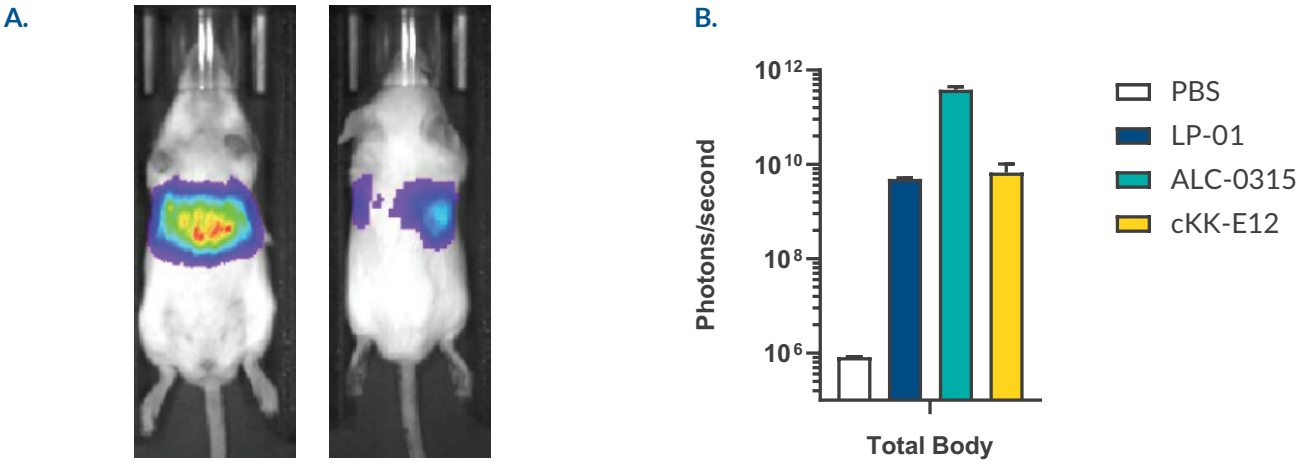


Figure 4. *In vivo* imaging of luciferase expression. **A**) Whole-body IVIS imaging showing luciferase activity in a representative mouse two hours post-injection of luciferase mRNA-LNP formulations. **B**) Total luminescent flux was quantified and plotted.

To assess organ tropism, organs were collected six hours after LNP administration for *ex vivo* imaging using IVIS. The liver consistently demonstrated the highest luciferase expression levels for all tested LNP formulations, indicating the liver is the primary site of LNP accumulation using these LNP formulations, as expected when administered intravenously (**Figure 5**). Across all tested LNP formulations, the organ tropism pattern followed a consistent ranking: liver > spleen > lung. Minimal luciferase activity was observed in the kidneys and brain for all LNP formulations.

Among all LNP formulations tested, ALC-0315 LNPs demonstrated the strongest induction of luciferase activity across all organs, suggesting superior overall expression capabilities. The potency of expression in all organs followed a consistent ranking: ALC-0315 > LP-01 > cKK-E12.

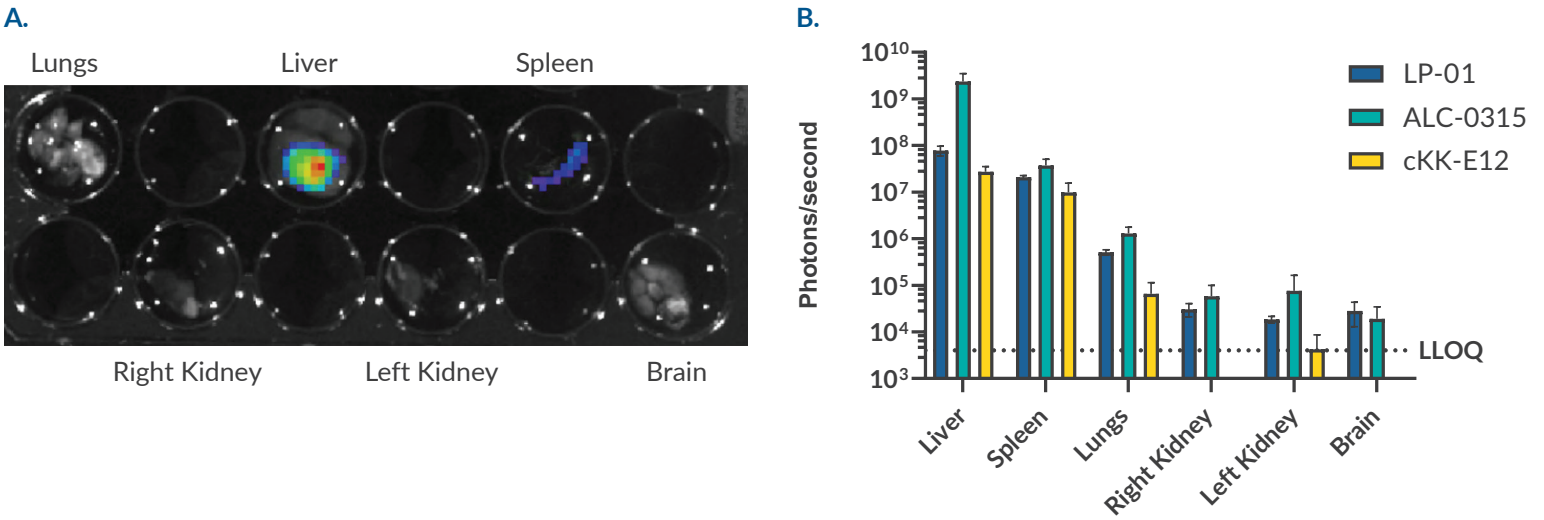


Figure 5. Organ tropism with mRNA-LNPs. **A**) IVIS imaging showing luciferase expression in representative mouse organs. **B**) Quantification of luciferase signals. Absent bars indicate signal below the lower limit of quantitation (LLOQ).

To evaluate the enrichment of the three LNP formulations in specific organs of targeting interest, we calculated the ratio of luciferase activity in the spleen, lung, brain, or kidney relative to the liver (**Figure 6**). ALC-0315-mediated activity was highly enriched in the liver, with very little relative luciferase activity in other organs, indicating excellent liver-targeting specificity. LP-01 particles induced luciferase expression in the broadest range of organs, whereas LNPs containing cKK-E12 showed an intermediate range of expression across the organs analyzed.

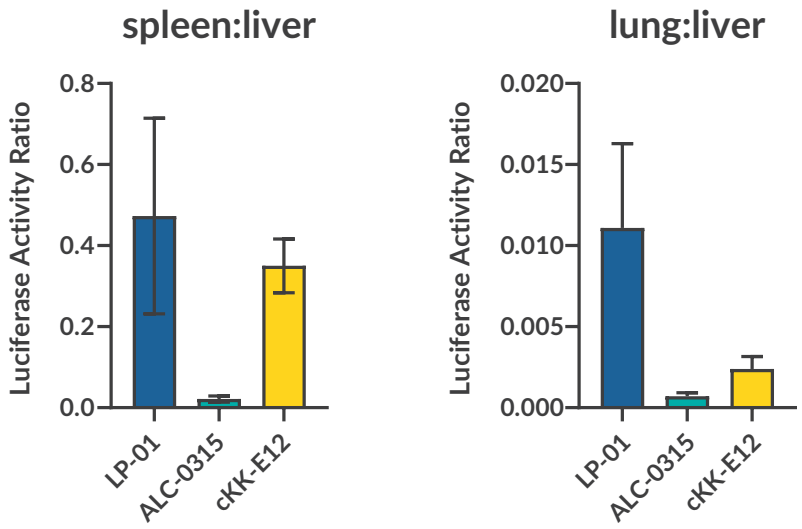


Figure 6. Specificity of organ tropism with mRNA-LNPs. The liver was selected for normalization as it is the primary site of LNP accumulation following intravenous administration.

It was noted that cKK-E12 induced nearly equivalent luciferase activity to LP-01 in the spleen, but substantially less in the lung, suggesting preferential spleen tropism with this LNP. Direct comparison of the spleen to lung activity ratios highlighted this enrichment of cKK-E12 LNP-mediated activity in the spleen (**Figure 7**).

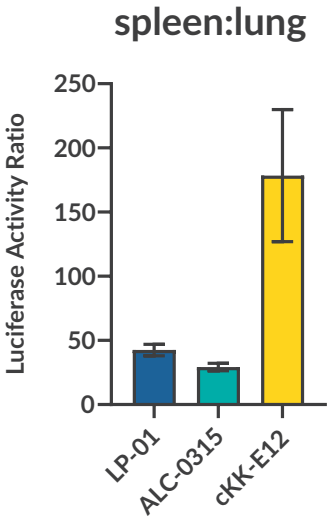


Figure 7. Enrichment of mRNA-LNP-mediated luciferase activity in spleen as compared with lung.

Conclusions

Expression of an LNP's encapsulated cargo in the tissue of interest is the first critical check of the utility of that LNP for the chosen application. With this workflow, a variety of mRNA-LNPs can be formulated and tested for their *in vitro* and *in vivo* expression efficiency.

Herein, we used luciferase-encoding mRNA to investigate cell and organ tropism following mRNA-LNP administration, but other reporter mRNAs can be used, such as green fluorescent protein (GFP) or mCherry.

Microfluidic mixing of LNP formulations enables simple and efficient screening of various LNP formulations, altering parameters such as lipid selection, cargo selection, and mixing parameters with ease. Leveraging *in vitro* studies and *in vivo* imaging with iterative LNP design helps identify and optimize candidate LNP formulations with targeted tropism or superior expression efficiency.

Appendix

Materials

LP-01	Cayman Chemical Item No. 37278
ALC-0315	Cayman Chemical Item No. 34337
cKK-E12	Cayman Chemical Item No. 36700
1,2-DSPC	Cayman Chemical Item No. 15100
1,2-DOPE	Cayman Chemical Item No. 15091
Cholesterol	Cayman Chemical Item No. 9003100
DMG-PEG(2000)	Cayman Chemical Item No. 33945
ALC-0159	Cayman Chemical Item No. 34336
Firefly luciferase mRNA	Cayman Chemical Item No. 39801
LipidLaunch™ LNP Formulation Buffer	Cayman Chemical Item No. 400813
RNA quantification	Fluorescent RNA dye ± 0.5% Triton X-100
Cell lines	A549 lung epithelial cells and Huh7 hepatocytes
Cell media	ATCC recommended

Equipment

LNP preparation	Sunshine – Unchained Labs
Particle analysis	Stunner – Unchained Labs
IVIS Spectrum	Perkin Elmer