Characterization of Ready-to-use Lipid Nanoparticle Delivery Kits for Efficient Intracellular Delivery of Nucleic Acids

Lipid Nanoparticles | Non-Viral Vectors
Nucleic Acid Delivery

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Introduction

Effective intracellular delivery of therapeutic molecules remains a major obstacle in drug development, particularly for gene and nucleic acid-based therapies. Biologics and nucleic acid-based drugs such as peptides, proteins, mRNA, plasmid DNA, siRNA, as well as small molecules often face critical delivery challenges, including poor cellular uptake, rapid degradation, and unfavourable pharmacokinetics. These limitations underscore the need for robust delivery systems that can improve solubility, stability, and bioavailability while minimizing cytotoxicity and immunogenicity.

Lipid nanoparticles (LNPs) have emerged as a leading non-viral delivery platform due to their high encapsulation efficiency, tuneable physicochemical properties, and established clinical relevance. However, designing and optimizing LNPs for different therapeutic cargos typically requires extensive in-house screening, specialized expertise, and time-consuming formulation development – barriers that limit accessibility and scalability, especially during early-stage research.

To streamline this process, we have developed a series of pre-formulated, ready-to-use LNP Delivery Kits specifically optimized for a range of cargos, including mRNA, peptides, proteins, and small molecules. These kits employ biocompatible, biodegradable lipid blends engineered for efficient cellular uptake and minimal cytotoxicity, offering a practical and reproducible solution for both academic and translational researchers.

Here we report on the characterization and performance of the mRNA LNP Delivery Kit using nano-flow cytometry (NanoFCM), Dynamic Light Scattering (DLS) and Nanoparticle Tracking Analysis (NTA). In addition, we tested delivery of plasmid DNA and Minicircle DNA encoding eGFP and Luciferase *in vitro* to show flexibility in transfecting a range of nucleic acid constructs.

Characterization of the LNP mRNA Delivery Kit

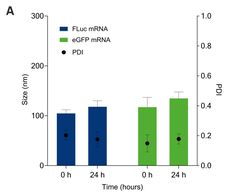

Figure 1 A. LNPs formulated with FLuc and eGFP mRNA are stable over at least 24h indicated by stable size and polydispersity index (measured with DLS).

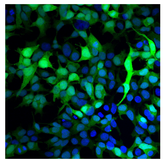

Figure 1 B. eGFP mRNA was successfully expressed in cell culture after transfection with LNPs (green signal). Nuclei were stained with Hoechst (blue signal).

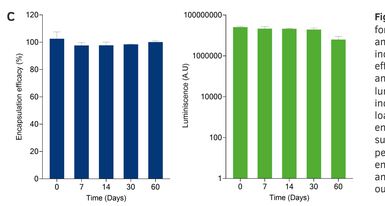

Figure 1 C. LNPs were formulated with FLuc mRNA and stored at 4 °C during indicated period. Encapsulation efficiency (EE%) was measured and cells were transfected and luminescence measured at the indicated time points. mRNA loaded particles showed high encapsulation efficiency and sustained functional performance over time, ensuring long-term stability and reliable gene expression outcomes.

Characterization of mRNA Loaded LNPs by Nano-Flow Cytometry (NanoFCM)

Figure 2. Flow NanoAnalyzer (by NanoFCM)

Concentration (particles/ml)	Median size (nm)	Mean size (nm)	St Dev
2.0E+11	135.2	134.8	34.1

Table 1. LNPs were formulated with mRNA and then measured unstained to examine particle size and concentration. The size distribution showed some degree of aggregation and polydispersity, consistent with a manual formulation process.

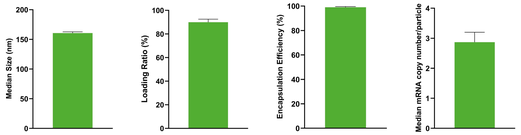

Figure 3. Utilizing SYTO 9 staining for mRNA detection to measure size, loading ratio, encapsulation efficiency and mRNA copy number per particle. LNPs loaded with stained mRNA had a median size of 160 nm. 90% of LNPs were loaded with mRNA. 99% of mRNA in the system was associated with an LNP, and loaded LNPs had a median copy number of 3 mRNA copies per particle.

Delivery of DNA Constructs with the mRNA LNP Delivery Kit

DNA construct	Size (bp)
pCMV-GFP	3487
mc.CMV-GFP	1788
pCMV-luc	6234
mc.CMV-luc	3645

Table 2. Constructs used for LNP delivery.

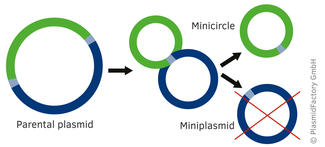

Figure 4. Overview of a Minicircle DNA construct.

Here we utilize the mRNA LNP Delivery Kit to deliver additional types of nucleic acids into cells. We used plasmid DNA (pDNA) and Minicircle DNA constructs (mcDNA) to deliver reporter genes (GFP and Firefly Luciferase) into cells. Minicircle DNA is a supercoiled, circular DNA molecule that lacks bacterial backbone sequences, resulting in smaller size, lower cytotoxicity and more stable gene expression. The size, polydispersity and stability of the LNPs associated with pDNA or mcDNA GFP constructs were measured utilizing NTA and DLS (Figures 5 and 6). *In vitro* experiments showed the successful transfection of the different constructs (Table 2 and Figure 7).

NTA Analysis of DNA Loaded LNPs

Sample*	Particle Concentration (1/ml)	Diameter (nm)	D10 (nm)	D50 (nm)	D90 (nm)
Empty LNPs	1.7E+10 ± 2.4E+9	81 ± 4	50 ± 2	75 ± 2	121 ± 13
pDNA LNPs	6.3E+9 ± 8.2E+8	240 ± 8	141 ± 13	238 ± 1	342 ± 10
mcDNA LNPs	1.3E+10 ± 5.8E+08	96 ± 6	62 ± 2	89 ± 2	133 ± 5

Table 3. NTA Characterization of empty and DNA-loaded LNPs. D10, D50, D90 describe percentile -based distribution.

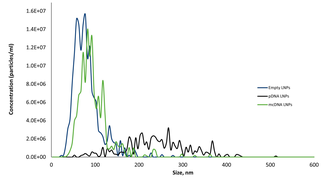

Figure 5. Particle concentration (particles/ml) vs. particle size (nm) for empty and DNA-loaded LNPs. pDNA LNPs showed a higher diameter and polydispersity, whereas empty and mcDNA filled LNPs showed comparable distribution.

DLS Analysis of DNA-loaded LNPs

Sample	Diameter (nm)	Normalized Intensity (kCnt/s)	PD Index	Particle Concentration (1/ml)
Empty LNPs	94 ± 2	4.99E+04 ± 5.15E+03	0.23 ± 0.03	5.6E+09 ± 1.2E+09
pDNA LNPs	304 ± 10	1.37E+05 ± 9.58E+03	0.39 ± 0.05	1.0E+08 ± 6.8E+06
mcDNA LNPs	113 ± 4	4.41E+04 ± 2.58E+03	0.21 ± 0.01	1.8E+09 ± 4.5E+08

Table 4. DLS/SLS characterization of empty and DNA-loaded LNPs

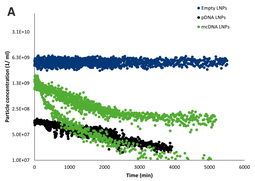

Figure 6A. Particle concentration over time for empty and DNA-loaded LNPs, measured by DLS/SLS. Empty LNPs showed constant concentration. LNPs filled with pDNA and mcDNA showed decreasing concentration indicating reduced stability over time.

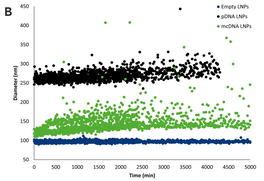

Figure 6B. Diameter over time for empty and DNA-loaded LNPs, measured by DLS/SLS. All LNPs showed stable diameters over time.

Expression Analysis of DNA-loaded LNPs

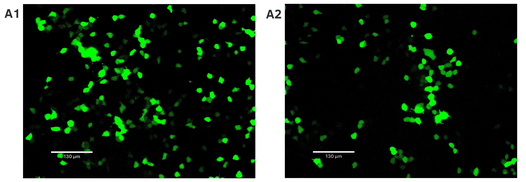

Figure 7 A. GFP expression in HEK293 T cells after LNP transfection with (1) mcDNA-GFP and (2) pDNA-GFP.

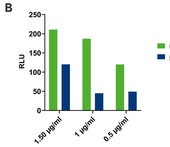

Figure 7 B. Quantification of Luciferase expression showed higher RLU signal with minicircle DNA constructs compared to plasmid DNA for three different DNA concentrations.

Overall, pDNA loaded LNPs showed larger diameters and a higher polydispersity index than mcDNA loaded LNPs. Both seem to become less stable after at least 4 hours of incubation. Functional experiments showed that mcDNA-luciferase constructs showed higher luciferase signals than pDNA suggesting better transfection and/or expression. Overall, the data indicated that for the mRNA LNP Delivery Kit mcDNA constructs are better suited than pDNA.

Conclusion

These results validate the potential of LNP Delivery Kits as reliable, off-the-shelf tools for nucleic acid delivery in preclinical applications. By eliminating the need for in-house lipid formulation and screening, these kits accelerate research workflows and advance the development of gene therapy and precision medicine strategies. mRNA LNP Delivery Kits are capable of delivering DNA constructs into cells, however, Minicircle DNA constructs perform better than plasmid DNA under the tested conditions.