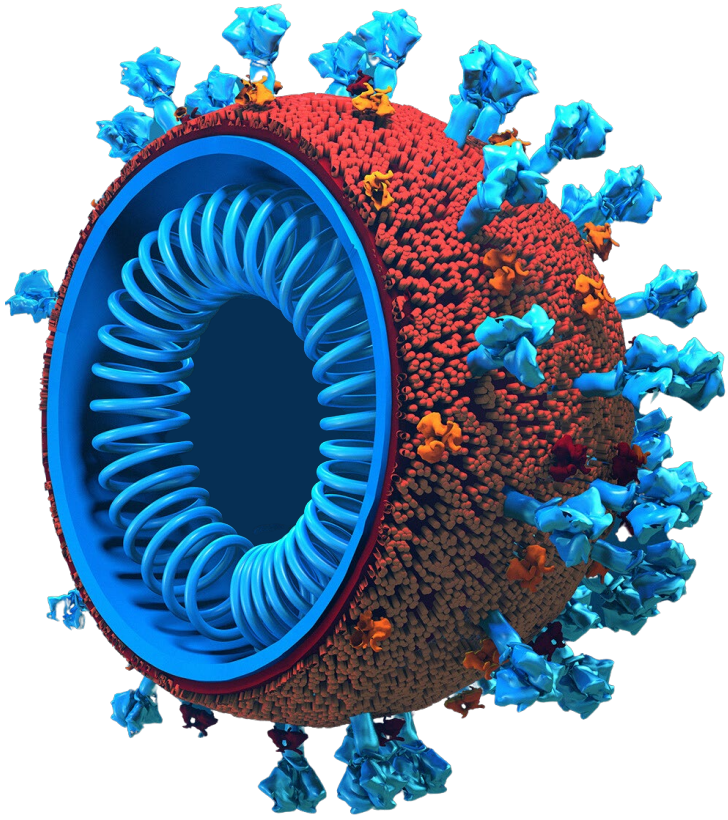
Microfluidics™

# Application Note

## Microfluidizer® Technology for Lipid Nanoparticle Production



### INTRODUCTION

Lipid Nanoparticles (LNPs) are promising non-viral delivery vehicles composed of a specific mixture of lipids including cationic/ionizable lipids, and other excipients.

A technology that has been in development for some time, the recent COVID-19 vaccines by companies such as Moderna and Pfizer have vaulted LNPs into the spotlight as critical delivery systems of nucleic acids, especially messenger RNA (mRNA). This technology not only protects the payload but also overcomes multiple physiological barriers. In addition to facilitating rapid vaccine development, LNPs are capable of carrying and delivering therapies that may be useful in a number of applications, including infectious disease, immuno-oncology, genetic disorders, and neurodegenerative conditions. Traditionally, the use of cationic lipids may cause concerns regarding toxicity, however, the specific cationic lipid in use in this study is 1,2-Dioleoyl-3-trimethylammonium propane (DOTAP), which is a widely used and tested lipid.

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### LNP MANUFACTURING METHODS

While there are a variety of ways to manufacture these particles, all processing technologies can be broken down as either top-down or bottom-up.

Bottom-up technologies, such as microfluidics mixing, create individual particles, whereas top-down methods reduce the size of pre-mixed lipid nanoparticle formulations, typically from the micron range to the sub-micron. The desired particle size for lipid nanoparticles is typically under 200 nm, while a tight particle size distribution with as small of a PDI as possible is also desired. One major drawback of the bottom-up method is the presence of a large amount of residual solvent, which not only leads to stability issues, but also requires additional downstream processes to remove the excessive solvent. Another challenge for this method is the lack of scalability since precipitation needs to be precisely controlled via flow rates and their ratios, which is hard to achieve on a larger scale.

### MICROFLUIDIZER® TECHNOLOGY

The Microfluidizer® processor is a top-down technology capable of producing these small, uniform nanoparticles by treating the particles with uniform high shear rates which are produced by the combination of the fixed geometry Interaction Chamber™ microchannels and constant high-pressure processing of up to 2,000 bar/30,000 psi.

The technology is linearly scalable up to thousands of liter batches and capable of cGMP manufacturing, plus, solvent may be completely avoided during the entire manufacturing process.

### CASE STUDY - WITH CAYMAN CHEMICAL COMPANY

The capability of the Microfluidizer® technology in producing LNPs is briefly demonstrated in the following case study which was collaborated with Cayman Chemical Company.

In this study, two Lipid Nanoparticle Exploration Kits, LNP-102 (which mimics Moderna's COVID-19 vaccine LNP formulation) and LNP-0135 (which mimics Pfizer's COVID-19 vaccine LNP formulation), available from Cayman Chemical, along with a cationic LNP formulation were processed through a lab-scale Microfluidizer® processor to produce various blank LNPs.

Note that no additional solvent, other than a small amount required to solubilize the ionizable lipids, was used when preparing the samples. All formulations were simply hydrated under mixing and then processed through the Microfluidizer® processor.

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## RESULTS

Table 1: Z Average size and PDI of the 3 formulations

| Formulation | Z-Average Size | PDI |
|---|---|---|
| LNP-102 | 112.4 nm | 0.183 |
| LNP-0135 | 106.6 nm | 0.241 |
| 3rd Formulation | 80.39 nm | 0.285 |

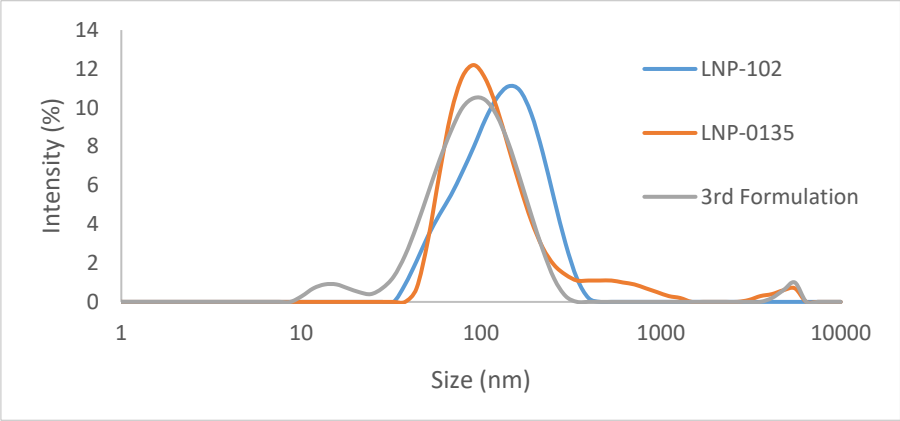


Figure 1: Particle Size Distribution of the 3 Formulations

As shown in the table and figure, the Microfluidizer® processor was able to produce LNPs with small and uniform particle size (sizes <120 nm and PDI <0.3) for all formulations.

These sizes and distributions are ideal for LNPs to deliver their payloads in an appropriate manner, while also being readily capable of being efficiently sterile filtered.