LNP Starter Kit
TAMARA Workflow
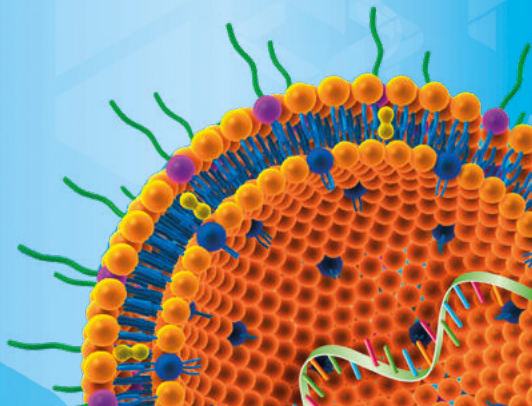
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General Information

Safety Data	**This material should be considered as 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.**
Precautions	**Please read these instructions carefully before beginning this assay.**

Storage and Stability	This kit will perform correctly if stored as specified and used before the expiration date as indicated on the label of the box.

Video of LNP Starter Kit with TAMARA Workflow

Scan me to get more info & watch the video «How to use our LNP Starter Kits»

Introduction

Background

Lipid NanoParticles (LNPs) belong to the family of lipid-based drug delivery systems that find lots of applications in the delivery of genetic material (e.g. siRNA, mRNA). LNPs are composed of different types of lipids, such as cationic or ionizable lipids, phospholipids, PEGylat ed lipids and cholesterol in different ratios.[1-3] Among them, the positively charged lipids represent the most important and abundant components of LNPs. This class of lipids is characterized by the presence of a positively charged head, which interacts with the negative charges of nucleic acids in order to favor their engulfment. Depending on whether it is a permanent or an inducible positive head, lipids are classified as Cationic or Ionizable.[1] One of the main advantages of ionizable lipids is that, having a pKa between 6.0 and 6.5, they are protonated at acidic pH, which is useful to efficiently encapsulate genetic material during LNP synthesis, but also to release the payload once inside the cell by fusing with the endosome membrane as soon as the pH decreases, thus providing an intracellular delivery.[4,5]

The process of LNP formation consists of the rapid mixing of a lipid-containing organic phase and a nucleic acids-containing aqueous phase, usually with an acidic pH. Once the collision occurs, the lipids immediately self-organize in one or more bilayers to minimize aqueous exposure. During this step the encapsu-

lation spontaneously occurs thanks to the interaction between nucleic acids' phosphate groups and cationic lipids (Fig. 1).[6]

Figure 1. *Schematic illustration of the process of Lipid NanoParticle formation with the LNP Starter Kit*
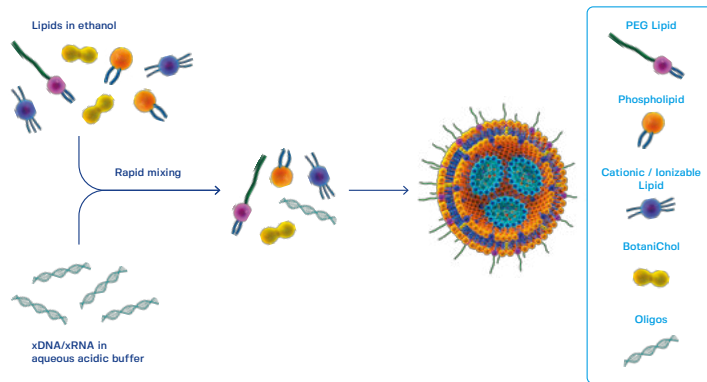
Background
(continued)

CordenPharma's LNP Starter Kit is an easy-to-use tool for laboratories to explore the feasibility of using LNPs for their individual applications. For this purpose, CordenPharma created a complete selection of lipid mix combinations to meet all requirements.

The optimization of the preparation conditions for the encapsulation of oligonucleotides must be determined by the final user. A list of adjustments that may facilitate this process is provided in the *General Rules and Adjustable Parameters for LNP Synthesis* section.
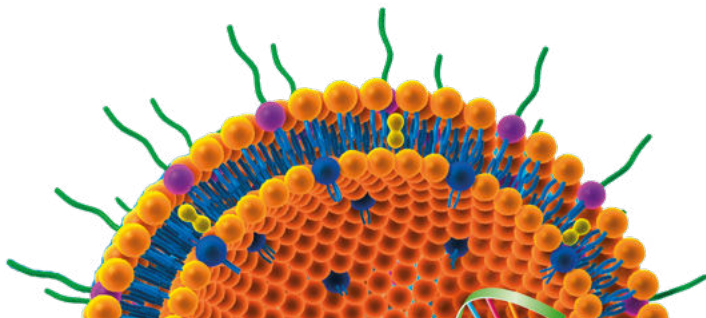
Experimental Protocol

Protocol Overview

This protocol is intended to be an example for the preparation of oligonucleotide-containing LNPs with a lipid mixture containing:

Item Name	Molar ratio [%]	Quantity
ALC-0315	46.3	67 mg
DSPC	9.4	14 mg
BotaniChol®	42.7	31 mg
ALC-0159	1.6	7 mg

mRNA-based vaccines using these lipids have been optimally formulated at this molar ratio.[7] This example is shown with a lipid:oligonucleotide (w:w) ratio of 10:1 and an Aqueous:Organic Flow Rate Ratio (A/O FRR) of 3:1. The final user may scale volumes and adjust the reaction parameters as desired.

Note: **The amount of reagents provided in the kit is enough to produce a single large batch of LNP (50 mL) or multiple small batches.**

Materials Required (but not provided)	→ Absolute ethanol for lipids dissolution.
	→ Aqueous buffer for oligos dissolution (e.g., 50 mM sodium acetate, pH 5.0).
	→ Oligonucleotides.
	→ Dilution buffer (e.g., PBS, pH 7.4).
	→ Commercial microfluidic device, commercial TFF systems (for big volumes) or centrifugal filter units/dialysis devices (for small volumes).
	→ Equipments for the characterization of LNPs and for the quantification of the encapsulated oligos.

**Materials
Required**
(continued)

1. **Lipid Mix in Ethanol (Organic Phase)**
   Prepare individual stock solutions of all the lipids supplied by dissolving them in absolute ethanol. If necessary, it is possible to heat the EtOH up to 45 ºC to improve lipids solubility. Bring all stock solutions to room temperature prior to use and ensure they are totally dissolved. Transfer the appropriate volume of each lipid mixture component (considering the molar ratio selected for the LNP composition) to a single tube to prepare the organic phase. Mix lipids by vortexing or pipetting several times. On the LNP Starter Kit Card there is an example of organic phase composition.

2. **Oligos in Sodium Acetate Buffer pH 5 (Aqueous Phase)**
   Add 9.45 mg oligonucleotide to a separate tube and adjust the volume to 45 mL with 50 mM sodium acetate, pH 5.0.

See the LNP Starter Kit Card for the preparation of the Lipid mixture.

Performing the Protocol

Different techniques are suitable for the production of LNPs at laboratory-scale, such as the ethanol injection, the rapid mixing in T-junction and the microfluidic systems. All of them include the mixing of an aqueous phase in which nucleic acids are dissolved and a lipids-containing organic phase. A general manufacturing procedure for LNP production that involves a typical micro-mixing system consists of a flow control module and a mixing unit, which could be a microfluidic chip with the implemented designs of 2D or 3D structures that uses hydrodynamic flow focusing, NxGen mixing technology, T- or Y- channel, or TAMARA's SHM or baffle mixers.[8-9]

In all the mentioned techniques, the formation of LNPs initiates as a result of the rapid mixing of the two fluids. During this step the lipids in the ethanol phase begin to self-assemble (due to the hydrodynamic forces) to reduce water exposure. Therefore, if the xRNA/xDNA is present in the aqueous phase it will be spontaneously encapsulated inside the LNPs. During this process, the particle size can be controlled by adjusting the Flow Rate Ratio (FRR) and the Total Flow Rate (TFR).

Performing the Protocol
(continued)

Figure 2. Schematic illustration of Lipid NanoParticle Starter Kit workflow
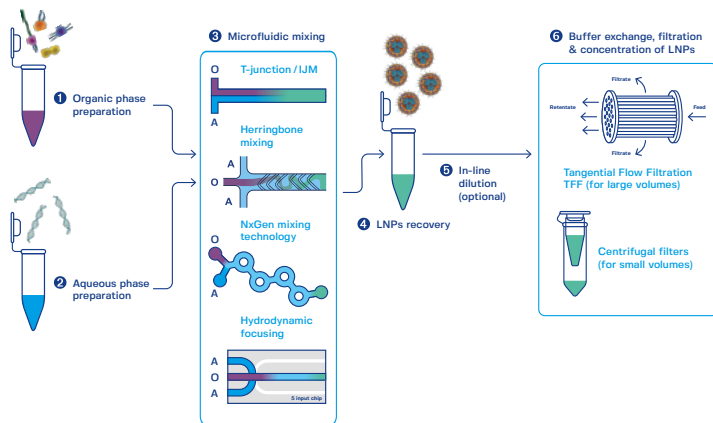
Performing the Protocol
(continued)

1. Perform the LNP formation following the instructions provided by the manufacturer of the selected instrument (See page 18).

2. Regardless of what was the applied mixing technique for the preparation of LNPs, by following these steps users could apply an in-line dilution to reduce the amount of EtOH in the final product thus stabilizing the LNPs.

3. Then, the final steps of the LNP preparation will be the buffer exchange, the filtration and concentration of the LNPs to remove the residual EtOH and replace the acidic buffer with a more physiological one (e.g., PBS, pH 7.4). Based on the final volume obtained, users could choose between the Tangential Flow Filtration (TFF) systems (recommended for larger volumes) or the centrifugal filter units/dialysis devices (more suitable for small volumes).

4. It is highly recommended to filter-sterilize the LNP suspension with a 0.22 μm filter.

5. Store the LNP suspension at 4 °C up to 1 week.

LNP Characterization

After preparation, the evaluation of the size, charge, and homogeneity of nanoparticles is fundamental, as well as the estimation of the encapsulation efficiency. For this purpose, there are many available techniques for the characterization of the LNPs.[7]

The most commonly used are listed in the table on the next page:

LNP Characterization
(continued)

Analytical panel for LNP characterization[7]

Assay	Attribute
Dynamic Light Scattering (DLS)	→ Particle size → Polydispersity Index → Surface charge (Zeta Potential) → Particle concentration
HPLC and mass spectrometry	Lipid identification, quantification and integrity
Ribogreen/Picogreen Assay and RNA/DNA-seq (RNA/DNA identification)	Encapsulation Efficiency (EE%)
RNA-Seq and qPCR	RNA identification and quantification
Cryo TEM	LNP morphology
Cell-based reporter assays, Western blotting	Transfection and translation efficacy

General Rules & Adjustable Parameters for LNP Synthesis

→ Increasing the amount of PEG-lipids leads to a decrease in the size of LNPs. (Less than 2% PEG lipids were used in the currently approved covid-19 vaccines.)

→ The optimal pKa range for Ionizable Lipids is 6.2–6.5.

→ The pH of the aqueous buffer is usually acidic (e.g. pH 4).

→ Organic buffer is usually EtOH.

→ By changing the Aqueous: Organic Flow Rate Ratio (A/O FRR) it's possible to modulate the size of LNPs:

  → Increasing the A/O FRR results in smaller LNPs.
  → Decreasing the A/O FRR results in bigger LNPs.

→ Also the Total Flow Rate (TFR) may impact LNP dimension. Faster TFR results in smaller LNPs.

→ N/P ratio is usually from 3 to 8. Usually lower values are recommended for short sequence oligos (e.g. siRNA); while higher ratios are recommended for mRNA.[7]

→ After LNP preparation a 1:1 dilution in a neutral buffer (pH ~ 7.4) is recommended, prior to filtration and purification, to reduce the concentration of ethanol in the final product, which affects LNP stability.

RNA-LNP formulation protocol using TAMARA

Background

Controlled and reproducible LNP manufacturing is essential to achieve optimal formulation quality and performance. Microfluidic technology provides an ideal solution for LNP formulation at the R&D scale, enabling precise particle size control, high reproducibility, and minimal reagent consumption.

The **TAMARA** system from **Inside Therapeutics** is a plug-and-play microfluidic platform covering all R&D stages, from screening to in vivo studies. TAMARA is fully compatible with **CordenPharma's LNP Starter Kits**, creating a synergistic workflow for reproducible and scalable formulation. TAMARA offers precise TFR and FRR control with minimal dead volume and high material efficiency. Its reusable chip includes staggered herringbone (SHM) and baffle mixers with automated cleaning for multiple runs.

**Full protocol in video:
(https://bit.ly/TAMUnbox)**

Performing the microfluidic formulation

1. **System setup:** Turn on the TAMARA formulation system. Ensure that the microfluidic chip and reservoirs are clean and dry before starting. For first-time use, perform a pressure test (see user guide, p.23). Use the top-left-corner menu of the TAMARA interface to set the chip design, solvent, and temperature.

2. **Microfluidic parameters:** Click «New Run» to set the microfluidic parameters.
   → Example: FRR = 3, TFR = 5 mL/min, Total Volume = 1 mL

3. **Load reservoirs:** Carefully pipette the solutions into their designated reservoirs. To minimize bubbles, dispense the liquid gently at the center and bottom of each reservoir. Always load the aqueous phase first, followed by the organic phase.
   → Example: Aqueous phase (RNA in buffer) = 750 μL, Organic phase (lipid mix in ethanol) = 250 μL

4. **Install chip & Collection tube:** Place the gasket and chip, align properly, close the lid, and insert a collection tube.

**Performing
the microfluidic
formulation**
(continued)

5. **Run the experiment:** Launch the experiment and collect the LNP sample in the collection tube.

6. **Clean the system:** Place a 50 mL waste tube.
   → Between runs using the same formulation: Wash once with water and ethanol.
   → Between runs using different formulations: Sequentially wash with water + ethanol, water + water, ethanol + ethanol.

Example formulation data

LNPs were formulated on the **TAMARA** microfluidic platform using the SHM configuration with **CordenPharma's LNP Starter Kits — ALC-0315 (Kit #1), SM-102 (Kit #10), and LP-01 (Kit #38) —** to encapsulate eGFP siRNA at an N/P ratio of 6. Each 1 mL formulation was produced at TFRs of 5 or 10 mL/min (FRR = 3), then purified by dialysis and analyzed by DLS and Ribogreen assay.

At a TFR of 5 mL/min, LNPs displayed uniform sizes between 71–79 nm with PDIs below 0.20. Increasing the TFR to 10 mL/min resulted in smaller particles (65–73 nm) while maintaining similarly narrow distributions (PDI ≤ 0.20) (Fig. 3a). Encapsulation efficiency remained consistently high, exceeding 80% across all formulations after dialysis (Fig. 3b).

Example formulation data
(continued)

ALC-0315
(Acuitas,
Kit #1)

SM-102
(Moderna,
Kit #10)

LP-01
(Novartis,
Kit #38)
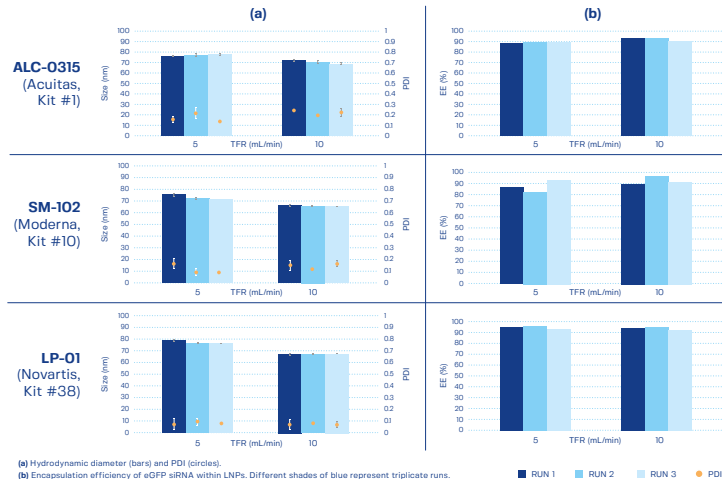
(a) Hydrodynamic diameter (bars) and PDI (circles).
(b) Encapsulation efficiency of eGFP siRNA within LNPs. Different shades of blue represent triplicate runs.

■ RUN 1　■ RUN 2　■ RUN 3　● PDI

Figure 3. *Physicochemical characterization of RNA-LNPs prepared at different total flow rates (TFR = 5 mL/min vs 10 mL/min).*

Practical considerations for LNP synthesis using TAMARA

→ **Sample volume:** TAMARA allows controlled nanoparticle synthesis from small screening volumes (~0.2 mL) up to larger-scale batches (~30 mL) suitable for in vivo studies.

→ **Mixer choice:**
  → **Staggered Herringbone Mixer (SHM):** Channel ridges create chaotic advection—stretching and folding fluid layers for rapid laminar mixing, typically yielding smaller nanoparticles at a given FRR and TFR.
  → **Baffle Mixer:** Channel obstacles induce vortices and recirculation at higher flow rates, enabling inertial mixing and producing larger nanoparticles.

→ **Chip reusability:** Reusability depends on the formulation, particularly lipid type and concentration. For standard LNP formulations, chips can typically be reused for up to nine runs (three triplicates) if properly cleaned. However, only the first use is guaranteed on our side for optimal performance.

Practical considerations for LNP synthesis using TAMARA (continued)

→ **Key parameters for LNPs:** FRR and TFR influence particle size, with TFR having the larger impact. Higher TFR or FRR accelerates ethanol dilution, promoting nucleation over growth and producing smaller particles. Total volume has little effect; minor batch variations (±5–10 nm size, ±0.02–0.05 PDI) are typical.

→ **Lipid concentrations:** Typical total lipid concentrations range from 1–10 mg/mL, though the system can accommodate concentrations up to ~100 mg/mL.

More detailed protocol available on Inside Therapeutics' website: **insidetx.com**

Full protocol in video:
(https://bit.ly/TAMUnbox)

Full protocol in PDF:
(https://bit.ly/4qaQHNY)

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