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Frequently Asked Questions (FAQ) - LNP Starter Kit

1. How many experiments can be performed using one kit? How is the kit used upon receipt?

The number of experiments depends on the scope of the study. Each kit contains enough lipids to produce up to **15 mL of ethanol (EtOH) organic phase**, supporting formulation optimization, formulation development, and process scale-up studies.

For example, if a total volume of **2 mL formulation** is prepared at a Flow Rate Ratio (FRR) of 3:1, 0.5 mL of the EtOH organic phase is required. This **2 mL formulation is** sufficient for physicochemical and in-vitro studies, while the remaining **14.5 mL** can be stored for further studies.

The amount of RNA encapsulated depends on multiple factors, including RNA size, FRR, and input RNA in the aqueous phase. Generally, the kit can encapsulate up to 20 mg of RNA and is suitable for producing multiple small batches.

2. How are the starter kits shipped?

- Shipments for Switzerland are done with a cool pack and all destinations except Switzerland are shipped with 10-12 kg of dry ice, with both options dispatched from the CordenPharma Switzerland site.
- The valid USA/Canada shipments are done with a cool pack from the CordenPharma Colorado site.
- The valid Japan shipments are done by our distributor Pyxchemi using dry ice with insulation packaging. <u>Email our Japan contact</u> for more information.

3. What is the lead time for an LNP Starter Kit?

If the kit is in stock, it can be **shipped and delivered within 2 weeks.** The main storage location is in Switzerland, but stock is available in the US/EU and Japan for most items.



4. What are the storage conditions for the starter kits?

Before use, the lipids are dissolved in ethanol. Each kit includes a **one-page instruction card** explaining how to prepare the total lipid mixture.

Prior to use: it is recommended to store the pre-dissolved components as it is written on the card.

Once dissolved we do not recommend storage to avoid degradation.

5. What is the physical form of the lipids?

Ionizable/Cationic Lipids: Solid form at -20°C (some may be highly viscous oils).

Helper Lipids, Sterols and Stabilizers Usually solid, some in powder form.

6. Does the kit require any additional equipment to make LNPs?

To encapsulate nucleic acids into LNPs, benchtop equipment is recommended, including:

- T-junction mixing
- Impingement jet mixing
- Microfluidics

Purification can be achieved through:

- Dialysis
- Amicon centrifugal filtration
- Tangential flow filtration (TFF) for large-scale production

The kits include **a booklet** detailing the required materials, storage conditions, and additional instructions.



7. Which LNP starter kit is best suited for in vivo liver delivery (hepatocytes)?

For commercially validated in-vivo liver delivery/vaccine application formulations: Kits #1 and #10 have the same composition and ratios found in the commercial Covid vaccines and therefore are recommended as a starting point for intramuscular injection-based vaccine applications. (Kit #26, the LNP formulation components include a Certest ionizable that has been tested and compared to the Moderna and Pfizer vaccines)

For early-stage studies: Various kits can be tested to assess the impact of lipid component variations on cell culture studies. Adjusting the molar ratio can also optimize performance.

8. Which kit provides the best transfection for CAR-T cells?

Currently, no data indicates that any of our kits specifically target T-cells

Factors influencing RNA delivery to T-cells:

- Biology and LNP formulation process
- Room temperature buffers
- ApoE supplementation for enhanced LNP internalization
- Activator presence pre- and post-transfection
- CD25 expression should exceed 80% before LNP addition

For well-established cell culture protocols, **Kits #12 and #14** are recommended, as they contain Beta-sitosterol and DOPE providing a synergistic effect on transfection, or **#15** and **#23** are recommended, as they include gold standard ionizable lipids with Beta-sitosterol to increase the transfection efficiency.

9. What is the output per kit (mRNA-LNP yield/concentration)?

LNP yield depends on various formulation and process parameters, such as:

- Formulation process control
- Amicon filters used for reconcentration
- Buffer composition, strength, and concentration



• RNA integrity maintenance

These factors significantly impact the final LNP concentration and encapsulation efficiency.

10. Is there a kit for gene editing purposes?

Kits #38 and #39 are recommended, as they contain a well-known ionizable lipid, LP-01, for that purpose.

11. What is the reason for having so many kits?

Because of the unlimited variables that can be tested for screening LNP formulations, the kits provide a quick and easy way to compare some of the most relevant ingredient substitutions for your specific application. Since the components are pre-weighed, the intention is to save time and effort in order to identify which specific formulation changes have the biggest impact on your application.