NanOZmix-Do it Yourself (DIY) Protocol

Description

Ready-to-use dried lipid mix at the total lipid concentration of 25 mM when reconstituted in 1mL ethanol, for LNP-mRNA formulation.

Protocol

Preparation of 25 mM ethanolic lipid mix stock

Bring the dry film of **NanOZmix-DIY** at room temperature from -20°C. Add 1 mL of ethanol in it and vortex it until you see clear solution.

Troubleshot: If you see sign of precipitation, try to sonicate or heat the ethanolic solution at 60°C for 1-2 min and vortex again.

General protocol for LNP-RNA formulation using **NanOZmix-DIY** (Recommended N/P ratio 6)

1. Bring the 25 mM ethanolic lipid mix at room temperature

2. The ethanolic mix can be used as it is or diluted further in ethanol as per customers requirement for different amounts of RNA encapsulation as well as exploring different N/P ratio

3. Prepare the RNA stock solution of desired concentration either in 50 mM citrate or acetate buffer of pH 4.0

4. Mix the RNA stock solution with ethanolic lipid mix at 3:1 v/v ratio using microfluidic/impingement jets mixing (IJM)/T-junction mixing technology at desired flow rate from 1-12 mL/min

5. Once the LNPs are prepared they must be diluted or dialyzed with your buffer of choice (1X PBS buffer, Tris buffer or HEPES buffer). Please note that 10 % sucrose w/v can be added as cryoprotectant

6. Filter the final LNP-RNA formulation through sterile PES 0.2 µM filters

7. LNP-RNA formulations can be concentrated by centrifugation using suitable concentrating centrifugal filters if required

8. Final formulations can be stored at 4°C for 2 weeks and for longer storage at -80°C.

Note: 25 mM in 1 mL ethanolic solution of NanOZmix-DIY can produce 4 mL of LNP-mRNA formulation at 0.1 mg/mL mRNA concentration **at N/P 6**.

LNP-RNA formulation can be characterized and validated by various tests and quality parameters presented in below table (table 1).

Test and quality parameters	Assays
Particle size and distribution	Dynamic light scattering (DLS)
Charge/zeta potential	Electrophoretic light scattering (ELS) and electroacoustic determination
Encapsulation efficiency	Fluorescent RiboGreen assay or equivalent.
LNP morphology	Microscopy (TEM, Cryo-TEM)
Translation or knockdown efficiency	Cell based reporter assay, Western blot, qPCR
Lipid quantification or stability	HPLC, LCMS

Table 1: Recommended tests after formulation to ensure the characterization of the LNPs

Use, handling and storage

For Research Use Only. Not for use in humans. Not for use in diagnostic or therapeutic purposes.

NanOZmix-DIY must be stored at -20°C as a dried film and at -80°C as ethanolic solution. Dried lipidic film can be stored for 6 months whereas ethanolic solution must be used within one month.

We recommend to minimize freeze-thaw cycles to preserve LNPs integrity.

Related Products

Ref	Description
#LDIY102	NanOZ LNP-DIY(SM102)
# LDIY105	NanOZ LNP-DIY(FP105)
# LDIY105S	NanOZ LNP-DIY(FP105S)
# LDIY001	NanOZ LNP-DIY(SS1)

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