NanOZ LNP-DIY(SS1)
(Dried lipid mix as Do It Yourself (DIY) Lipid Nanoparticle (LNP))

Description

Ready-to-use dried lipid mix at the total lipid concentration of 25 mM when reconstituted in 1mL Ethanol, for **LNP-mRNA, LNP-DNA, LNP-saRNA or LNP-sgRNA** formulation.

LNPs represent the most effective and safe delivery systems for the translational success of nucleic acid drugs. LNPs are lipidic spherical vesicles formed by a combination of four main components: an ionizable cationic lipid, a helper phospholipid, cholesterol & a pegylated lipid, each having distinct functions (**Fig.1**). LNPs not only protect RNA from degradation, but also facilitate intracellular uptake and thus potentiate its efficacy. **LNP/RNA** systems self-assemble *via* electrostatic interactions between negatively charged RNA and ionizable cationic lipids.

NanOZ LNP-DIY(SS1) is designed for the development of LNP by customer at their ease by using different formulation methods at different N/P ratio. With our **NanOZ LNP-DIY(SS1)** system, customers can produce themselves a monodisperse LNP encapsulating mRNA/ DNS/saRNA/sgRNA within the size range of 50 nm to 200 nm through microfluidic/ impingement jets mixing (IJM)/T-junction mixing technology with an encapsulation efficiency of 80-96 % under optimized conditions.

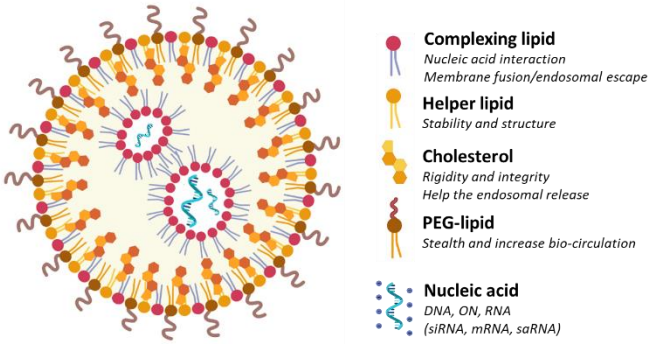

Fig.1. Schematic representation of LNPs-RNA

Kit contents

LDIY001: Dried 25 mM lipid mix having composition as described in table below:

Lipid mix components	Molecular weight	Molar ratio	mM	mg
SS1	1011.3	50	12.5	12.64
DSPC	790.17	10	2.5	1.98
Cholesterol	386.65	38.5	9.63	3.72
DMG-PEG 2000	2509.2	1.5	0.38	0.94
Total		**100**	**25**	**19.28**

Storage

NanOZ LNP-DIY(SS1)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.

Preparation of 25 mM ethanolic lipid mix stock

Bring the dry film of **NanOZ LNP-DIY(SS1)** at room temperature from -20°C. Add 1 mL of ethanol in it and vortex it until you see clear solution. 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 NanOZ LNP-DIY(SS1)

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 amount 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 5% 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.
   LNP-RNA formulation can be characterized and validated by various tests and quality parameters presented in below table. (**Note:** 25 mM 1 mL ethanolic solution of NanOZ LNP-DIY(SS1) can produce 4 mL of LNP-mRNA, LNP-DNA, LNP-saRNA or LNP-sgRNA formulation at 0.1 mg/mL RNA concentration)

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

Contact Us

Our company has developed a custom service platform for the LNPs (or polymeric nanoparticles (PNPs)) development using a highly performant microfluidic technology to potentiate nucleic acids/APIs activity.
Feel free to contact us for all complementary information and remember to visit our website to stay informed on the latest breakthrough technologies and updated on our complete product list. (www.ozbiosciences.com)

Technical questions: tech@ozbiosciences.com
For bulk inquiries: order@ozbiosciences.com
General information: contact@ozbiosciences.com

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Purchaser Notification

Limited License
The purchase of the LNP/mRNA grants the purchaser a non-transferable, non-exclusive license to use the included components. This reagent is intended for in-house research only by the buyer. Such use is limited to the transfection of nucleic acids as described in the product manual. In addition, research only use means that this formulation is excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences.
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Product Use Limitations
The LNP/mRNA is developed, designed, intended, and sold for research use only. It is not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the use of the component by following proper research laboratory practices.

OZ Biosciences SAS
163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09
FRANCE
Ph: +33 (0) 486 948 516
Fax: +33 (0)4 63 74 00 15
contact@ozbiosciences.com
order@ozbiosciences.com

OZ Biosciences INC
7975 Dunbrook Road,
Suite B,
San Diego, CA 92126,
USA
Ph: + 1-858-246-7840
Fax: + 1-855-631-0626
contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com