Method

The stability of NanOZ LNP-mRNA (Luc) over a six-month period was investigated in this study. The physico-chemical characteristics (size, charge, mRNA encapsulation) of the particles and their transfection efficiency *in vitro* were evaluated. LNP-mRNA samples were stored either at room temperature (RT), 4°C or -80°C and were tested at several time points: from 1 week to 24 weeks. In addition, the effect of repeated freeze/thaw cycles on LNPs properties was also assessed. Transfections were performed with an mRNA dose of 1 µg of mRNA-loaded LNPs per well of 24-well plates. Classic transfection of mRNA Luc using RmesFect transfection reagent was included as reference. Luciferase expression was measured 24 hours post-transfection to assess transfection efficiency in HEK293.

Results

Size and Charge

Regardless of the storage conditions, NanOZ LNP-mRNA maintains a consistent size of around 100 nm, as illustrated in figure 1A. Over extended storage periods (up to 6 months) at -80°C, the NanOZ LNP-mRNA maintains a stable particle size of approximately 100 nm, demonstrating superior colloidal stability when stored under these conditions for prolonged durations as depicted in figure 1B. On the same way, the charge stays neutral to slightly positive and remains similar to the initial measurements taken right after production during the first two weeks across all three storage conditions and continues this way for up to six months when stored at -80°C (data not shown).

NOTE: the slight increase at -80°C is due to the first freeze/thaw cycle; size remains constant after this (refer to freeze/thaw paragraph)

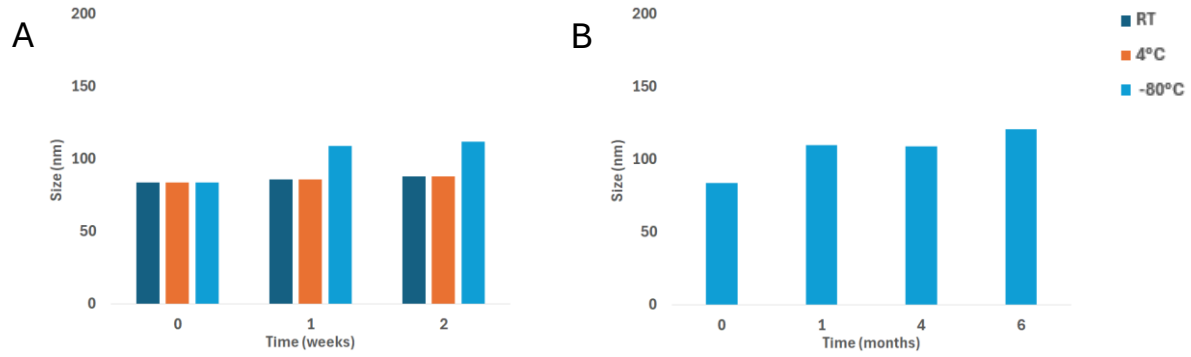

Figure 1: Size (nm) of NanOZ LNP-mRNA (Luc) stored in various conditions was measured by dynamic light scattering over a two week-period of storage (A) or over a six month-period storage (B).

Encapsulation efficiency

Similar to the physico-chemical characteristics, the encapsulation efficiency (EE) of NanOZ-LNPs stays stable during short-term storage at room temperature, +4°C, and -80°C as shown in figure 2A. The EE maintains its stability throughout long-term storage at -80°C for up to 4 months, but then shows a decline of roughly 20% at the 6-month mark (figure 2B).

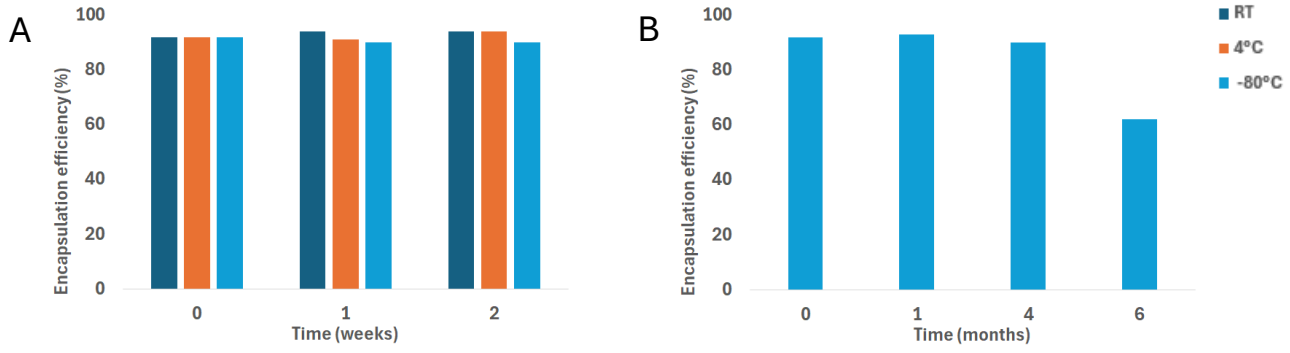

Figure 2: Percentage of Encapsulation efficiency of NanOZ LNP-mRNA (Luc) measured using the Ribogreen assay over a two week-period of storage (A) or over a six month-period storage (B).

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Biological efficacy

The biological efficacy remains constant over two weeks regardless of the storage method as shown in figure 3A. A slight decrease in luminescence was observed after six months, although this represented less than 10% compared to the first month of storage (figure 3B). Storage of particles at -80°C for 6 months results in particles that still yield conclusive results; however, this represents the acceptable storage limit.

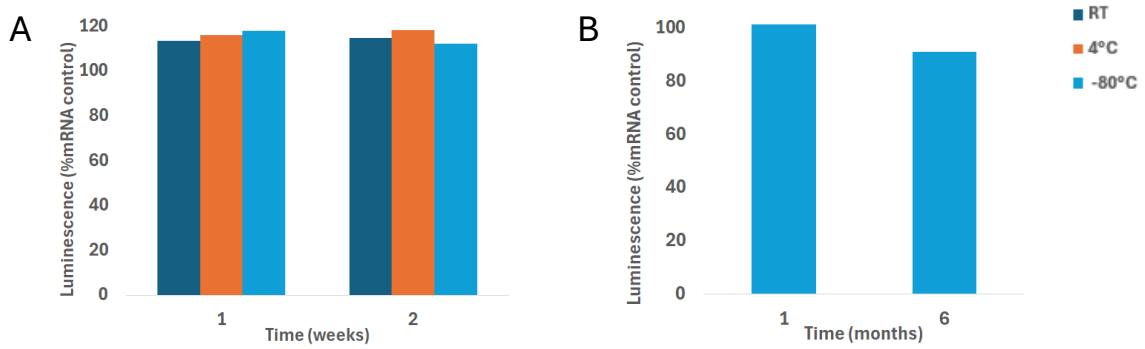

Figure 3: Luciferase activity 24h post transfection in HEK-293 cells after one and two weeks of storage (A) and at one and six months (B), normalized to FLuc mRNA transfected with RmesFect.

Freeze-thaw cycles

The stability of the particles after several freeze/thaw cycles at -80°C was also examined in our study. Particle size and mRNA encapsulation evolution upon repeated freeze/thaw cycles are presented in figure '. Overall, particle size is increased successively with each freeze/thaw cycle by approximately 25 nm, with about 200 nm being reached after the 5th cycle, which represents our upper limit for quality control. Conversely, encapsulation efficiency gradually decreases as the number of cycles increases. No change was observed after the first cycle, followed by a 10% loss being observed with each subsequent cycle. These data suggest that repeated freezing and thawing should be avoided by users to obtain optimal results.

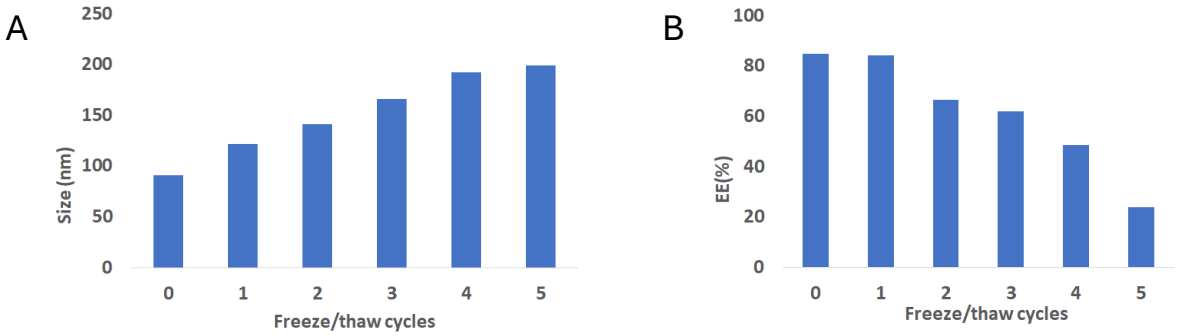

Figure 4: Physico-chemical properties of NanOZ LNP-mRNA (GFP) after several freeze/thaw cycles. A Size (nm) of the NanOZ-GFP measured by dynamic light scattering. B Encapsulation efficiency (%) of the NanoZ-GFP measured using the RiboGreen assay.

⚠ Storage at -20°C

Samples stored at -20°C exhibit particle size instability over time with a size increase of more than 300 nm in 2 weeks, suggesting potential aggregation or structural changes that make them completely inactive (figure 5).

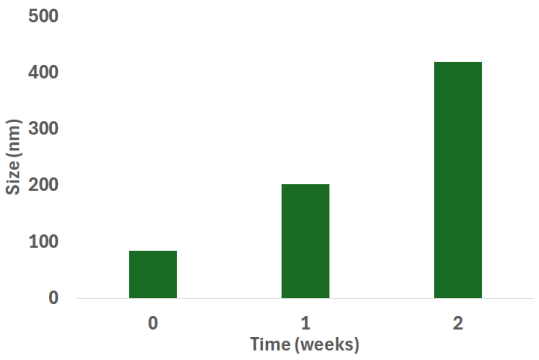

Figure 5: Size (nm) of NanOZ LNP-mRNA (Luc) measured by dynamic light scattering after two weeks of storage at -20°C.

Conclusion

Considerable stability is demonstrated by NanOZ-LNPs when stored at -80°C and used within 6 months.

It should be noted that NanOZ can also be kept at RT or +4°C for 2 weeks.

In summary, the following storage guidelines are recommended for optimal results:

• Long-term storage (several months): -80°C should be used

• Short-term storage (for a few days): +4°C should be used

• Freeze–thaw cycles: Should be minimized as much as possible; up to 3 freeze-thaw cycles are considered acceptable. If more cycles are required by your experiment, sample aliquoting is recommended, or the sample should be kept at +4°C."

⚠ Avoid storage at -20°C

For more information, please contact us at ***tech@ozbiosciences.com.***