User instruction: LNP-mRNA for In Vitro Applications

The Lipid nanoparticle (LNP) is a promising vehicle to deliver messenger RNA (mRNA), which has emerged as a new therapeutic agent. Compared to IVT mRNA delivered by commercial transfection reagents, LNP-encapsulated mRNA (LNP-mRNA) may demonstrate higher gene expression levels in vitro (Figure 1).

Storage and Handling

1. Storage conditions affect the long-term stability of LNP-mRNA formulations. Our products are supplemented with sucrose (a cryoprotectant) and are shipped on dry ice and should be stored at -80°C upon receiving.
2. Avoid freeze-thaw cycles. The melted LNP-mRNA can stay at room temperature for up to 1 hour.
3. Designate a special area for RNA work whenever possible.
4. Sanitize your bench surface and pipettes with RNase inactivating agents prior to work.
5. Always wear clean gloves and use RNase-free tips, tubes, and reagents when working with RNA.

Preparing Cells for Transfection

Typically, cells are plated for transfection **the day before**. As a general guideline, it is recommended that cells of interest reach a desired confluence of 40-80% at the time of transfection. Please refer to Table 1 below for recommended cell seeding density.

Table 1. Recommended Cell Seeding Density by Culture Vessel

Culture Vessel	Surface Area Per Well (cm^2)*	Adherent Cell Number Per Well**	Suspension Cell Number Per Well**
96-well plate	0.32	7.5×10^3 - 2.5×10^4	2.5×10^4
24-well plate	1.9	4.0×10^4 - 1.0×10^5	1.0×10^5
12-well plate	3.8	8.0×10^4 - 1.5×10^5	1.5×10^5
6-well plate/35 mm dish	9.5/9	1.5×10^5 - 4.0×10^5	4.0×10^5
60 mm dish/Flask 25 cm^2	21/25	2.0×10^5 - 8.5×10^5	1.6×10^6
100 mm dish/Flask 75 cm^2	55/75	1.0×10^6 - 4.0×10^6	4.0×10^6

Notes:

*The surface area sizes are provided based on Corning disposable culture vessels.

**Optimize the seeding density based on the size and growth rate of your cells of interest.

Thawing Procedure

1. Thaw LNP-mRNA in a refrigerator between 2°C and 8°C or on ice. The melted LNP-mRNA is colorless to slightly white.
2. Once the LNP-mRNA is melted, gently invert the vial several times. Do not shake or vortex the vial.
3. Stand the melted LNP-mRNA on ice and repeat step 2 each time prior to cell treatment to keep the consistency of the concentration of the LNP-mRNA added into each well.

Dilution Procedure

1. Normally, no dilution procedure is required considering our standard concentration of the LNP-mRNA products is 200 ug/ml. The recommended per well amount for the LNP-mRNA introduced into cells at 60-80% confluency, grown on a 12-well plate, is from 0.5 to 1 ug.
2. If a dilution procedure is required, dilute the LNP-mRNA with complete culture medium in a separate culture dish or sterile centrifuge tube.
3. Gently shake the dish or invert the tube several times to properly mix the dilution each time prior to cell treatment. Avoid shaking or vortexing. Use the restriction endonucleases shown in the PDF provided by VectorBuilder for each individual plasmid to confirm successful transformation and plasmid identity. Primers may also be used to confirm the construct.

Preparing Cells for Transfection

The following is an example protocol for transfecting 293T cells with EGFP IVT-mRNA or LNP-mRNA (Figure 1). The quantity of mRNA, LNP-mRNA, and the volume of transfection reagent used in the protocol indicate the per well amount for a 12-well plate. We strongly recommend performing optimization based on your specific experimental settings.

1. Seed cells in a 12-well plate at a density of 1.0×10^5 cells per well.
2. On the day of transfection, change the culture medium*.
3. Dilute 1 ug of EGFP IVT mRNA into 100 ul of transfection buffer.
4. Mix transfection reagent by vortexing or inverting for 5 sec.
5. Add 2 ul of transfection reagent into the RNA/transfection buffer mix by thorough pipetting.
6. Thaw and mix the LNP-mRNA (see Thawing and Dilution Procedure).
7. Add the transfection master mix or the appropriate amount of LNP-mRNA (see Dilution Procedure) to the cells in the growth medium **.
8. Gently rock the plate to disperse the transfection master mix or the LNP-mRNA.
9. Measure EGFP expression 6-72 h later***.

Notes:

*Please refer to the manual of the transfection reagent to find out whether it is compatible with serum and antibiotics.

**LNP-mRNA can be added directly to cells in the growth medium or mixed with the growth medium first before adding to the cells.

***The IVT mRNA in vitro expression can sometimes be detected as early as 6 h post-transfection, and the exogenous protein is transient. Of note, the time point of the peak protein expression varies by cell type and mRNA modification. In some cases, users may fail to detect strong protein expression 48-72 h after transfection. Hence, we recommend you closely monitor the gene expression at different time points in your own experimental settings.

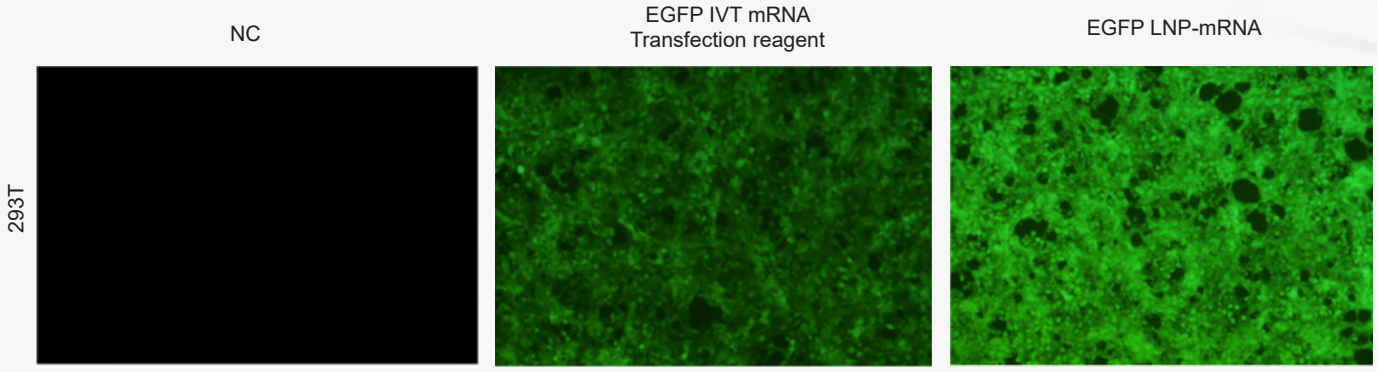

Figure 1. Comparison of the cellular expression levels of EGFP mRNA. 1 ug of EGFP mRNA was transfected into 293T cells at approximately 60% confluence, followed by fluorescent imaging 24 hours post-transfection. LNP-encapsulated mRNA (right) exhibited higher expression levels compared to EGFP mRNA delivered by a commercial transfection reagent (middle).