User Instructions: LNP-mRNA for in vivo applications

Messenger RNA (mRNA) has emerged as a new therapeutic agent to prevent and treat various diseases. In order to function properly in vivo, mRNA requires safe, effective, and stable delivery systems that protect the nucleic acid from degradation and allow for cellular uptake and mRNA release. To overcome the extracellular and intracellular barriers, lipid nanoparticle (LNP)-mRNA formulations have been developed. In this system, mRNA molecules are encapsulated in the interior core of LNPs and associate with the lipids through electrostatic interactions. Proper safety precautions and handling techniques are crucial for in vivo administration of LNP-mRNA formulations.

Storage and Handling

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. Avoid freeze-thaw cycles.

Thawing and Dilution Procedure

1. Thaw LNP-mRNA in a refrigerator between 2°C and 8°C or on ice.
2. Allow LNP-mRNA to come to room temperature before using in vivo. Vials can be stored at room temperature for up to 1 hour before mixing.
3. Once the LNP-mRNA has reached room temperature, gently invert the vial for several times. Do not shake the vial.

Note: The melted LNP-mRNA is colorless to slightly white.

4. Use phosphate buffered saline (PBS) to dilute the LNP-mRNA if necessary.

Administration Routes

Administration routes can greatly influence biodistribution, expression, and therapeutic outcomes of LNP-mRNA formulations. It has been reported that both intravenous (i.v.) and local administration routes, such as intramuscular (i.m.) and subcutaneous (s.c.) injection, can produce robust immune responses at well-tolerated doses in human trials. The intended therapeutic outcome should be taken into consideration when choosing the administration route. For example, i.v. administration of LNP-mRNA formulations can supplement proteins that are missing in hematological disorders, or produce antibodies to neutralize pathogens present in circulation.

Protocol for Mouse Intramuscular Injection (i.m.)

Materials

0.5 ml syringe with 22-25 gauge 1/2-inch needle

Procedure

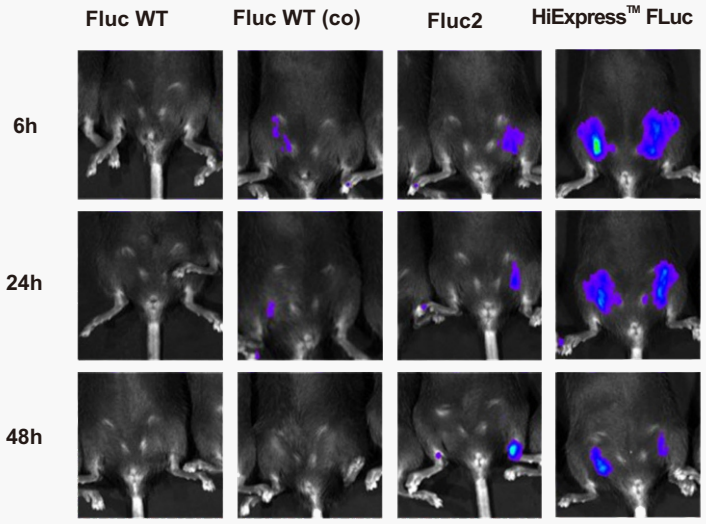
1. Restrain the mouse by grasping the skin along its back with your non-dominant hand.
2. Insert the needle into the thigh muscle of the hind limb and direct the needle away from the femur avoiding the sciatic nerve.

Note: Disinfecting the injection site with 70% ethanol is not necessary before injection. Use a new needle and syringe for each animal.

3. Pull back the plunger to aspirate the syringe. Any blood indicates improper needle placement, and the needle must be repositioned.
4. Slowly and steadily administer the LNP-mRNA formulation.

Note: Ideally, the injection volume should be less than 100 ul per site for mouse thigh muscle injections.

Intramuscular Injection of LNP-mRNA Validation

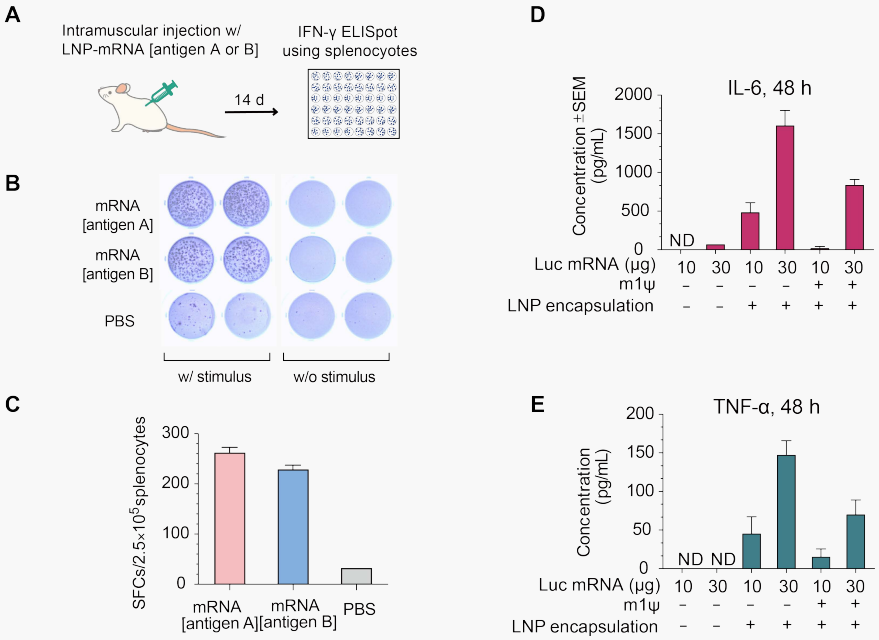


HiExpress™ Firefly Luciferase IVT mRNA expression in vivo. Luciferase activity was measured in adult C57BL/6 mice injected intramuscularly with 30 ug of LNP encapsulated mRNA at 6 h, 24 h, and 48 h post injection. FLuc WT indicates wild-type firefly luciferase. FLuc WT (co) indicates codon-optimized wild-type firefly luciferase. FLuc2 indicates Luc2 firefly luciferase.

Safety Concerns

Lipid components of LNP-mRNA formulations may activate host immune responses. Hence, the immunogenicity is an important safety concern, although eliciting cellular and humoral immunity may be advantageous for vaccination. Cationic and ionizable lipids have also been reported to stimulate the secretion of pro-inflammatory cytokines, raising the safety concern of unwanted inflammation. Therefore, LNP-mRNA formulations should be screened for their potential side effects including inflammation and organ damage. Additionally, production of antibodies against certain lipids could result in faster systemic clearance of subsequently administered LNP-mRNA decreasing the bioavailability of the drug encapsulated in the LNP. Due to all these safety concerns, studies should be conducted to detect the LNP-mRNA biodistribution and immunogenicity in your application.

Intramuscular Injection of LNP-mRNA Validation



Screening of immunogenicity upon LNP-mRNA administration. (A) Balb/C mice at the 8-week age were intramuscularly injected with 30 ug of LNP-encapsulated mRNA coding for viral antigen A, viral antigen B, or control PBS. 14 days post intramuscular injection, IFN-γELISpot assay was performed on splenocytes derived from Balb/C mice. (B and C) The results of spot forming cells (SFCs) indicated LNP-mRNA injection stimulated IFN-γ secretion in splenocytes. (C and E) Two pro-inflammatory cytokines, IL-6 and TNF-α, were quantified in the serum at 48 h post-injection. Error bars represent standard errors.