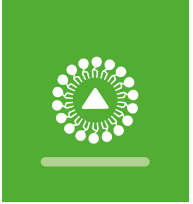


LNP mRNA Delivery Kit

Lipid nanoparticles for promoting effective intracellular transfection of mRNA.

Cat. No.:	PR6104
Contents:	1 test kit
Storage box	Before formulating: -20°C After formulating: 2–8°C
Version:	01

For research use only! Not for use in humans!

PROGEN

Table of Contents

About the Nanoparticles

Overview

PROGEN's Delivery Nanoparticles are a biocompatible, biodegradable, and cell-friendly technology designed to enhance intracellular delivery of biomolecules, paving the way towards clinical translation.

PROGEN's mRNA Delivery Nanoparticles demonstrate exceptional efficacy, ensuring safe mRNA delivery while maintaining cell integrity, standing out from viral and cationic-based transfection methods. They can accommodate a wide range of mRNA sizes (900–4,500 nucleotides), expanding research possibilities. Formulations outside this range could be feasible, but their performance has not been thoroughly assessed and cannot be guaranteed without further testing.

PROGEN's nanoparticles are easily internalized by cells and can penetrate more complex structures, such as 3D cell cultures and organoids. Additionally, they can be adapted to various routes of administration for evaluation in animal models, maximizing targeted biodistribution and enhancing their therapeutic effect. Contact PROGEN for specific recommendations for *in vivo* experiments.

Components

4x **mRNA Prep** vials for reconstitution.

4x **Formulation** vials for preparation of PROGEN's mRNA Delivery Nanoparticles.

4x sterile, non-toxic, pyrogenic-free polypropylene 1 ml syringes.

4x 21G ½ sterile needles (0.8 x 38 mm).

Storage

Before formulating, store the vials at -20°C. Once formulated, the mRNA-loaded nanoparticles should be stored at 2–8°C up to 48 h.

Required Material

- 1.5 ml sterile RNase-free microtubes
- 0.6 ml sterile RNase-free microtubes
- 2 ml sterile RNase-free microtubes
- 15 ml sterile RNase-free tube
- Amicon Ultra Centrifugal Filter
- 0.22 µm filter of PES membrane
- RNase contamination remover (e.g. RNaseZAP or RNase AWAY Surface Decontaminant)
- RNase-free water (Molecular Grade)
- Dulbecco's phosphate-buffered saline 1X (DPBS) (without calcium and magnesium)
- Ethanol (EtOH) 96%
- mRNA of interest

Citrate buffer 10 mM at pH3 (10 ml):

- Weigh 2.8 mg of Sodium Citrate Tribasic Dihydrate (CAS No: 6132-04-3) and 17.4 mg of Citric Acid Monohydrate (CAS No: 5949-29-1).
- Dissolve both components in 8 ml of RNase-free water (Molecular Grade).
- Measure the pH and, if necessary, adjust to pH3. Clean the sensor to avoid contamination by RNases.
- Adjust the final volume to 10 ml with RNase-free water.
- Store at 2–8°C in an RNase-free container.

Considerations Before Starting

- The following protocol is optimized for the formulation of 5 µg of mRNA, starting from one **mRNA Prep** vial. A protocol for the formulation of 1 µg of mRNA is also provided. The kit contains four **mRNA Prep** vials, allowing four separate nanoparticle preparations.

- PROGEN cannot guarantee optimal formulation performance if modifications in the protocol are introduced.

- It is recommended to use PROGEN's mRNA Delivery Nanoparticles within 48 hours after preparation for optimal performance.

- The transfection with PROGEN's mRNA Delivery Nanoparticles is stable in supplemented cell culture media for at least 24 h at 37°C.

- Do NOT use any buffer solution containing Triton-X, SDS, or Tween-20 for the preparation or manipulation of PROGEN's mRNA Delivery Nanoparticles.

- Once formulated, do NOT freeze PROGEN's mRNA Delivery Nanoparticles.

- Do NOT heat PROGEN's mRNA Delivery Nanoparticles over 90°C.

PROGEN's mRNA Delivery Nanoparticles

Protocol for the Encapsulation of 5 µg of mRNA

Clean the workspace and micropipettes before starting with 70% EtOH, followed by RNase contamination remover solution.

1. Add 300 µl of EtOH into the **mRNA Prep** by inserting the needle through the septum using the provided syringe and needle. Then vortex the vial.

   Note 1: DO NOT remove the metal cap from the vial to avoid spilling.

   Note 2: You will need the same syringe and needle for step 3. Do not discard them.

2. Add 895 µl of 10 mM Citrate Buffer (pH3) and 5 µl of mRNA to the **Formulation** vial.

   Note 1: Prepare this immediately before proceeding to the next step.

Note 2: The specified volumes are based on an mRNA stock concentration of 1 mg/ml. Please adjust accordingly for different stock concentrations.

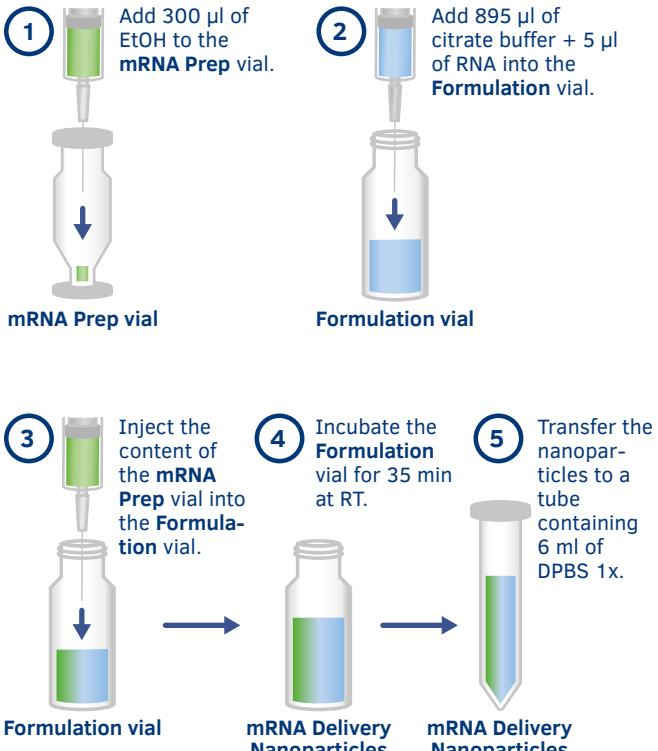
3. Remove the metal cap from the **mRNA Prep** vial. Take the volume from the **mRNA Prep** vial reusing the same syringe and needle from step 1. Before injection, ensure an approximately 0.3 ml air gap in the syringe. Then, inject the volume into the **Formulation** vial containing the mRNA with a sudden, vigorous downward motion, resulting in a final volume of 1.2 ml of PROGEN's nanoparticles.

4. Leave the **Formulation** vial open for 35 min at room temperature (RT).

5. Transfer the 1.2 ml of PROGEN 's nanoparticles from the **Formulation** vial to a 15 ml sterile RNase-free tube containing 6 ml of DPBS 1X at 2–8°C.

   Subsequently, either proceed with the *Concentration Protocol* or store the diluted formulation at 2–8°C for later use.

Figure 1. mRNA Delivery Nanoparticles Formulation Protocol.



Protocol for the Encapsulation of 1 µg of mRNA

Please note that the variability in the formulation increases when working with smaller mRNA quantities. Ensure careful handling to maintain consistency in nanoparticle formation and performance.

1. Inject 310 µl of EtOH into the **mRNA Prep** vial by inserting the needle through the septum using the provided syringe and needle. Then vortex the vial.

   Note: DO NOT remove the metal cap from the vial to avoid spilling.

2. Remove the metal cap from the **mRNA Prep** vial. Divide the volume of **mRNA Prep** vial into 5 aliquots of 60 µl in 0.6 ml sterile RNase-free microtubes.

   Note: Unused **mRNA Prep** aliquots can be stored at 4°C for up to 2 weeks. Ensure the microtubes are tightly closed to prevent evaporation of ethanol.

3. Add 175 µl of 10 mM Citrate Buffer (pH 3) and 5 µl of mRNA in 0.6 ml sterile RNase-free microtubes.

   Note 1: Prepare this immediately before proceeding to the next step.

Note 2: The specified volumes are based on an mRNA stock concentration of 0.2 mg/ml. Please adjust accordingly for different stock concentrations.

4. Using a 200 µl micropipette, add 60 µl from the previously reconstituted **mRNA Prep** vial into the 0.6 ml sterile RNase-free microtube containing the citrate buffer solution and the mRNA, resulting in a final volume of 240 µl of PROGEN's nanoparticles.

   Note: Before adding the volume from the **mRNA Prep** vial into the microtube, set the micropipette at the maximum volume and add the solution with a sudden, vigorous downward motion. Pipette up and down several times with confidence to ensure proper mixing and nanoparticle formation.

5. Leave the microtube containing PROGEN's nanoparticles open for 35 min at room temperature (RT).

6. Transfer 240 µl of PROGEN's nanoparticles to a 2 ml sterile RNase-free microtube containing 1.760 ml of DPBS 1X. Ensure that the mixture is mantained at 2–8°C.

   Subsequently, either proceed with the *Concentration Protocol* or store the diluted formulation at 2–8°C for later use.

Concentration Protocol

Select the most appropriate Amicon Ultra Centrifugal Filter.

Note: A molecular weight cut-off (MWCO) between 10 kDa and 100 kDa is recommended. The total volume of the diluted formulation will depend on the selected formulation protocol.

- **Protocol for the encapsulation of 5 µg of mRNA.** If possible, select a filter with a capacity greater than 7.2 ml. If the capacity is lower, the diluted formulation (from step 5, *Formulation Protocol*) can be added in multiple steps. In such cases, the formulation must always be kept at 2–8°C during the waiting period.

- **Protocol for the encapsulation of 1 µg of mRNA.** If possible, select a filter with a capacity greater than 2 ml. If the capacity is lower, the diluted formulation (from step 6, *Formulation Protocol*) can be added in multiple steps. In such cases, the formulation must always be kept at 2–8°C during the waiting period.

1. Equilibrate the membrane of the Amicon Ultra Centrifugal Filter by adding 1X DPBS at 4°C, ensuring the membrane is fully covered. Centrifuge at 2,500 RCF at 4°C (5–10 min) and discard the flow-through.

2. Add the diluted formulation and centrifuge at 2,500 RCF at 4°C.

   Note 1: Please check Table 1 for recommendations on final working concentrations.

   Note 2: The centrifugation time may vary depending on the molecular weight (MW) of the mRNA and the characteristics of the Amicon Ultra Centrifugal Filter, so adjust the centrifugation time accordingly.

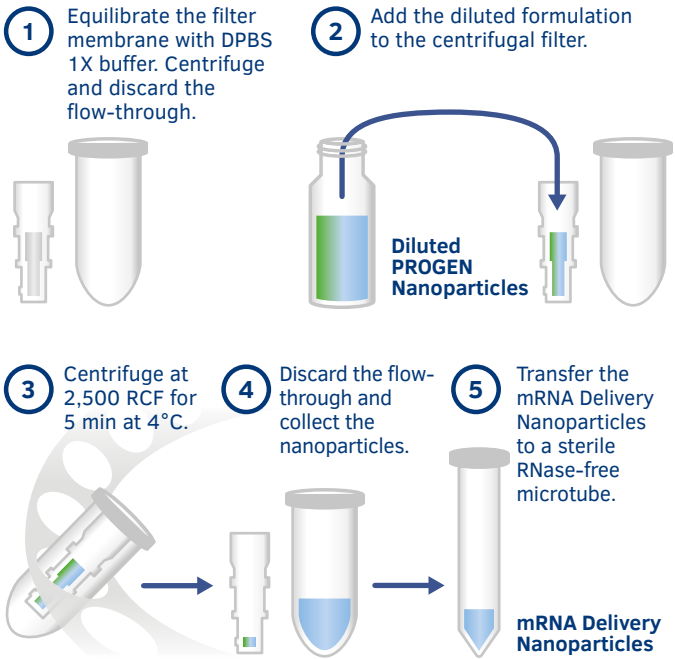
   As a reference, PROGEN's nanoparticles loaded with an mRNA of 1,922 nt using an Amicon Ultra Centrifugal Filter (15 ml-30 kDa) and centrifuged for 35 min resulted in approximately 200 µl.

   Note 3: Ensure that PROGEN's nanoparticles remain in suspension, and DO NOT allow them to dry completely.

3. Discard the flow-through and collect PROGEN's nanoparticles from the upper part of the Amicon Ultra Centrifugal Filter.

4. Transfer PROGEN's nanoparticles to a 0.6 ml sterile RNase-free microtube and store at 2–8°C until use.

Figure 2. mRNA Delivery Nanoparticles Concentration Protocol.



Transfection Assay

Example Protocol

1. Seed the recommended number of the cells in a 96-well plate with 100 µl of supplemented medium the day before the transfection assay.

   Note: Optimizations should be performed depending on the cell type and the length of the experiment.

2. Prepare PROGEN's nanoparticles for 5 µg of mRNA according to the provided *Formulation Protocol*. Concentrate it up to 250 µl for a final mRNA concentration of 20 µg/ml.

3. Add PROGEN's nanoparticles at the desired transfection concentration in, at least, triplicates (e.g., 5 µl of nanoparticles in a final volume of 100 µl, achieving a final concentration in the well of 1 µg/ml of mRNA encapsulated in PROGEN's nanoparticles).

   Note: This concentration can be modified depending on the type of mRNA and the specific cells of interest, but a minimum amount of 1 µg/ml of mRNA is recommended for the first set of experiments.

4. The read out can be performed after different incubation times depending on the mRNA of interest.

For example:

- HEK293 cells transfected with 1 µg/ml FLuc mRNA encapsulated in PROGEN's nanoparticles can be analyzed 24 h post-transfection with the ONE-Glo™ Luciferase Assay (Promega (Ref.: E6120).

- HEK293 cells transfected with 1 µg/ml GFP mRNA encapsulated in PROGEN's nanoparticles can be analyzed 24 h post-transfection by Flow Cytometry Analysis, fluorescence or confocal microscopy.

Technical Notes

Table 1. Recommended Volume of PROGEN's mRNA Delivery Nanoparticles to Transfect 100 ng of mRNA in 100 µl Using a 96-well Plate.

mRNA Delivery Nanoparticles	mRNA in mRNA Delivery Nanoparticles	Final concentration	mRNA Delivery Nanoparticles to transfect
500 µl	5 µl	10 ng/µl	10 µl
250 µl		20 ng/µl	5 µl
100 µl		50 ng/µl	2 µl

Table 2. Recommended Volume of PROGEN's mRNA Delivery Nanoparticles to Transfect a Final Concentration of 1 µg/ml mRNA Starting from a Concentration of 20 µg/ml.

Cell culture vessel	Amount of mRNA/ well	mRNA Delivery Nanoparticles*	Medium	Final volume/ well
100 cm	5000 ng	250 µl	4.75 ml	5 ml
6-well	1000 ng	50 µl	950 µl	1 ml
12-well	500 ng	25 µl	475 µl	500 µl
24-well	250 ng	12.5 µl	237.5 µl	250 µl
96-well	100 ng	5 µl	95 µl	100 µl

*These volumes are recommended for an incubation time of 4 hours. If PROGEN's nanoparticles are incubated for longer than 4 hours, it is recommended to double the volumes of both the nanoparticles and the medium.

Table 3. Example of Cells Successfully Transfected Using PROGEN's mRNA Delivery Nanoparticles.

Immortalized Cells	Embryonic kidney cells (HEK293) Epithelial breast cancer cells (MDA-MB-231) Epithelial lung cancer cells (A549) Human monocytes (THP-1) Mouse macrophages (RAW264) Mouse fibroblasts (NIH/3T3) Mouse cardiomyocytes (HL-1) Human cardiomyocytes (AC10) Human fibroblasts (HFF-1)
Primary cells	Human primary monocytes-derived macrophages Cortical neurons
Organoids	From brain cells

Frequently Asked Questions

Can I use RNA encoding any protein?
Yes, PROGEN's mRNA Delivery Nanoparticles can be loaded with any mRNA encoding for your protein of interest.

What is the maximum amount of RNA to encapsulate?
The maximum amount of mRNA to encapsulate is 5 µg per **mRNA Prep** vial. However, higher amounts can be achieved as a customized formulation. For customized prototypes, contact PROGEN.

Can PROGEN's mRNA Delivery Nanoparticles be used for other types of nucleic acids?
No, but PROGEN can provide customized formulations for other types of nucleic acids. For specific customized prototypes, contact PROGEN.

Can I filter the formulation?
Yes, if necessary, PROGEN's mRNA Delivery Nanoparticles can be filtered using small 0.22 µm filters of PES membrane.

How can I measure the size of the final formulation?
Diameter size can be measured by Dynamic Light Scattering (DLS).

Can I use PROGEN mRNA Delivery Nanoparticles for *in vivo* studies?

Yes, PROGEN mRNA Delivery Nanoparticles can be used *in vivo* (not for use in humans). Contact PROGEN for specific recommendations for *in vivo* experiments.

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