Instructions for Use

in vivo-jetRNA®+

Transfection Reagent for *In Vivo* Delivery of mRNA (or saRNA, circRNA, gRNA)



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1 Product Content

In vivo-jetRNA®+ is available in one pack size:

- 1 mL in vivo-jetRNA®+ & 60 mL mRNA Buffer

This pack size enables at least 50 intravenous injections or 100 intramuscular injections in mice.

2 Intended Use and Safety

For research use only. Not for use in humans.

In vivo-jetRNA®+ is a ready-to-use lipid-based nanoparticles solution for *in vivo* delivery of mRNA (as well as saRNA, circRNA, gRNA) into a wide range of tissues using various delivery routes: intravenous, intraperitoneal, intramuscular, intratumoral, etc.

It can be used for various applications such as genetic animal immunization, mRNA-based vaccination, immuno-oncology studies, as well as genome editing using CRISPR method or even mRNA-based protein replacement.

3 In vivo Transfection Protocol

3.1 Recommended Amount of mRNA and Injection Volume

The amount of mRNA to deliver must be determined according to the animal model, the administration route and the targeted organ. Recommendations for mRNA delivery in mouse are given in Table 1.

To ensure optimal results, it is crucial to utilize high-quality nucleic acid resuspended in double-distilled water (ddH2O). The recommended stock solution concentration is between 1-2 µg/µL. All calculations provided in this document are based on a stock solution concentration of 1 µg/µL.

For mRNA, we strongly recommend utilizing commercial mRNA for its reliability and consistency. If you are using home-made mRNA, employ the phenol-chloroform precipitation method followed by isopropanol purification, and avoid salt-based precipitation techniques. In addition, ensure additional washing steps are performed to eliminate residual salts, preventing precipitation issues. Additionally, mRNA must be diluted and aliquoted in RNAse-free water to maintain its integrity.

We recommend using a ratio mRNA | in vivo-jetRNA®+ of 1:2 (μg_{mRNA}:$\mu L_{reagent}$). Of note, the use of a lower ratio than 1:1.5 must be avoided.

Table 1: Recommended conditions for most common injection routes in mice

Animal	Route of Administration	Recommended Starting conditions	mRNA optimization range	in vivo-jetRNA®+ optimization range	Final injection volume
Mouse	Intravenous (IV) Tail Vein | Retro-orbital	10 µg mRNA 20 µL in vivo-jetRNA®+	10 – 20 µg	20 – 40 µL	200 µL
	Intraperitoneal (IP)	20 µg mRNA 40 µL in vivo-jetRNA®+	10 – 20 µg	20 – 40 µL	500 µL
	Subcutaneous (SC)	5 µg mRNA 10 µL in vivo-jetRNA®+	5 – 10 µg	10 – 20 µL	100 µL
	Intradermal (ID)	2 µg mRNA 4 µL in vivo-jetRNA®+	2 – 5 µg	4 – 10 µL	50 µL
	Intramuscular (IM)	5 µg mRNA 10 µL in vivo-jetRNA®+	5 – 10 µg	10 – 20 µL	50 µL

Depending on the application, multiple injections may be required.

For other administration routes and | or further experimental guidelines with different animal models, please contact our Technical Support team (CGT-techsupport@Sartorius.com).

3.2 Transfection Protocol

Define the experimental protocol and parameters:

- Set the injection volume of complexes to be prepared per animal (Table 1).
- Define the amount of mRNA to be delivered per injection (Table 1).
- Define the corresponding volume of in vivo-jetRNA®+ to prepare complexes (Table 1).
- Prepare the complexes under sterile conditions (e.g. laminar flow hood) using the supplied mRNA buffer.
- Prepare a mastermix for homogeneous complex formation, with a minimum volume of 20 µL.
- Note: the final concentration of mRNA in the injection volume must not exceed 0.3 µg/µL.

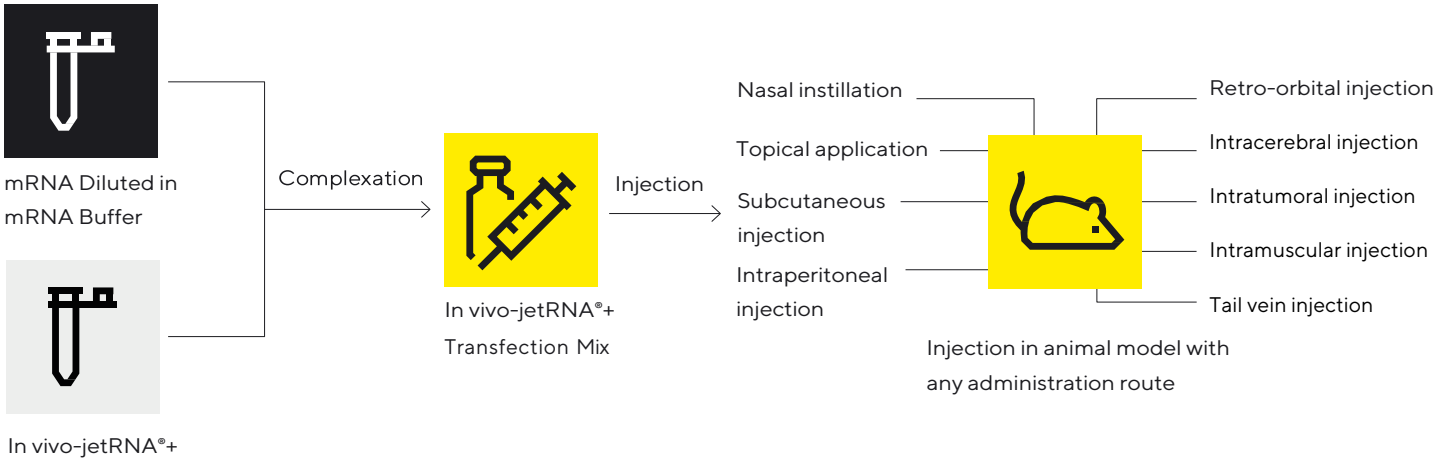


Figure 1. Two-step protocol for mRNA | in vivo-jetRNA®+ complexes preparation.

Protocol:

1. On the day of injection, equilibrate in vivo-jetRNA®+ and mRNA buffer at room temperature for optimal complexes preparation and thaw mRNA on ice.
2. Dilute the mRNA into mRNA Buffer. The volume of buffer to dilute the mRNA is equal to the final volume of injection minus the volume of mRNA and in vivo-jetRNA®+ reagent to add in step 5. Mix by pipetting up and down.
    - Example of IV injection: Dilute 10 µg of mRNA (1 µg/µL) in 170 µL of mRNA buffer and add 20 µL of in vivo-jetRNA®+.
3. Vortex in vivo-jetRNA®+ reagent for 5 seconds.
4. Add the in vivo-jetRNA®+ reagent (following a ratio mRNA | in vivo-jetRNA®+ of 1:2 μg_{mRNA}:$\mu L_{reagent}$) to the diluted mRNA all at once and homogenize by gently pipetting up and down. Do not vortex. Some turbidity can occur, but the transfection mix can be safely administrated as long as no precipitates are observed.
5. Incubate for 15 minutes at room temperature.
6. Perform injections into animals using solution equilibrated at room temperature. Complexes are stable at 4°C up to a week (do not incubate the complexes on ice).
7. Analyze gene expression 6 - 72h after the injection.

4 In Vitro Transfection Protocol (for mRNA Validation)

4.1 Cell Seeding

For optimal mRNA transfection conditions, we recommend using cells which are 60 to 80% confluent at the time of transfection. Typically, for experiments in 24-well plates, between 40 000 to 100 000 adherent cells are seeded per well in 0.5 mL of cell growth medium 24 h prior to transfection.

Table 2: Recommended conditions for in vitro transfection of commonly used cell lines

Cell type	**Number of cells to seed per well of a 24-well plate**	**Amount of mRNA (µg)**	**Ratio ($\mu g_{mRNA}/\mu L_{reagent}$)**
Caco-2	40 000	0.5	1:2
A549	60 000	0.5	
HeLa	50 000	0.25	
HEK-293	50 000	0.25	
HepG2	100 000	0.5	

4.2 Transfection Protocol

1. On the day of transfection, equilibrate in vivo-jetRNA®+ and mRNA buffer at room temperature for optimal complexes preparation and thaw mRNA on ice.
2. Dilute 0.5 µg mRNA into 48.5 µL mRNA Buffer. Mix by pipetting up and down.
3. Vortex in vivo-jetRNA®+ reagent for 5 seconds.
4. Add 1 µL in vivo-jetRNA®+ reagent (following a ratio mRNA | in vivo-jetRNA®+ of 1:2 μg_{mRNA}:$\mu L_{reagent}$) to the diluted mRNA and homogenize by gently pipetting up and down.
5. Incubate for 15 minutes at room temperature. Complexes are stable at RT up to 3 days (do not incubate the complexes on ice).
6. Add 50 µL of transfection mix per well dropwise onto the cells in growth medium and | or additives and distribute evenly.
7. Gently rock the plate back and forth and from side to side.
8. Analyze gene expression 24 - 48 h after the transfection.

5 Troubleshooting

Table 3: Troubleshooting *in vivo* delivery

Observations	Actions
Unsatisfactory results	▪ Optimize the amount of mRNA used in the delivery assay. ▪ Optimize the injection volume. ▪ Use high quality mRNA preparation. The OD260 | 280 ratio must be greater than 2. ▪ Verify the transfection efficiency of mRNA in vitro. ▪ Ensure that the complexes are prepared in mRNA Buffer. ▪ Ensure that the quality of the mRNA is optimal. Preferably use mRNA purchased from an oligo supplier, instead of using homemade transcribed mRNA. ▪ Use a common reporter gene-encoding mRNA as a positive control (ex: Luciferase or GFP). ▪ The use of chemically 5′ capped and modified mRNA (Pseudouridine, 5′ Methylcytosine, 5′ methoxyuridine, etc...) could improve gene expression.
Precipitates in transfection mix	▪ Decrease the amount of mRNA without changing the ratio mRNA | in vivo-jetRNA®+. ▪ Use commercial mRNA or mRNA preparation free of residual salts. ▪ For home-made mRNA, use phenol-chloroform precipitation method followed by isopropanol purification and avoid using salt-based precipitation techniques.
Toxicity	▪ Decrease the amount of mRNA, while keeping the ratio mRNA | in vivo-jetRNA®+ constant. ▪ Decrease the volume of in vivo-jetRNA®+ reagent, while keeping the amount of mRNA constant. ▪ Ensure that the mRNA preparation is endotoxin-free.

Table 4: Troubleshooting *in vitro* delivery

Observations	Actions
Cellular Toxicity	▪ Replace medium 4 h after transfection. ▪ Analyze transfection at an earlier time point (e.g., at 24 h instead of 48 h). ▪ Decrease the amount of mRNA added per well.

For any troubleshooting assistance or additional inquiries, please reach out to our dedicated Technical Support team (CGT-techsupport@Sartorius.com) .

6 Storage and Stability

In vivo-jetRNA®+ and mRNA Buffer must be stored at 5 ± 3 °C upon arrival for long term storage. Do not freeze the product.

7 Quality Control

Each batch of in vivo-jetRNA®+ reagent is tested for conformity to established Quality Controls and relevant specifications. Certificates of Analysis are available online in your Customer Area: https://my.sartorius.com/.

8 Quality Assurance

Sartorius Polyplus has been awarded ISO 9001 Quality Management System Certification since 2002, which ensures that the company has established reliable and effective processes for manufacturing, quality control, distribution and customer support.

9 Ordering Information

Reference	in vivo-jetRNA®+	mRNA buffer
101000122	1 mL	60 mL

10 Trademark Information

In vivo-jetRNA®+ is a registered trademark of Sartorius Polyplus SAS. For more information on this registration, please visit our website at www.sartorius.com (patents-and-trademarks).

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