PROTOCOL

in vivo-jetRNA®+
in vivo mRNA transfection reagent

DESCRIPTION

in vivo-jetRNA®+ is the next generation transfection reagent that enables efficient mRNA delivery to a wide range of tissues using various delivery routes: intravenous (IV), intraperitoneal (IP), intramuscular (IM), intratumoral, subcutaneous, etc. Upon intraperitoneal injection, complexes are delivered in the spleen and lymph nodes, but also in other organs such as liver, lung, pancreas and uterus. *in vivo*-jetRNA®+ is highly efficient for mRNA delivery for various applications, from immunization purposes to anti-cancer studies as well as genome editing using CRISPR/Cas9 method or protein replacement.

1. *in vivo* transfection protocol

1.1 Recommended amount of mRNA and injection volume

The amount of mRNA to deliver should 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.

The concentration of mRNA in the final injection solution should not exceed **0.3 µg/µL**.

For optimal conditions, we recommend using chemically modified mRNA. When using non-commercial mRNA, extra washing steps must be performed in order to remove residual salts to prevent precipitation issues. Furthermore, mRNA should be diluted and aliquoted in RNAse-free water.
We recommend to use 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 should be avoided.

The use of commercial mRNA is highly encouraged. For home-made mRNA, we recommend using phenol-chloroform precipitation method followed by isopropanol purification and avoid using salt-based precipitation techniques.

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

Animal	Site of injection	Starting conditions	mRNA optimization range	*in vivo*-jetRNA®+ reagent 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 (s.c)	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	100 µL

Depending on the application, multiple injections may be required.

For other administration routes, please contact our technical support at support@polyplus-transfection.com for advice or browse the literature on our website: Transfection Database

Experimental guidelines for other animal models are available from our *in vivo* specialists.

1.2. Transfection protocol

The preparation of the mRNA/*in vivo*-jetRNA®+ complexes should be performed in sterile conditions (e.g. in a laminar flow hood) using the mRNA Buffer provided with the transfection reagent.
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).
- We recommend preparing a mastermix to ensure homogenous complex formation, the smallest mix being minimum 20 µL.
  Note: the final concentration of mRNA in the injection volume should not exceed 0.3 µg/µL.

The best results are achieved with high quality mRNA resuspended in ddH2O and a stock solution of 1-2 µg/µL.

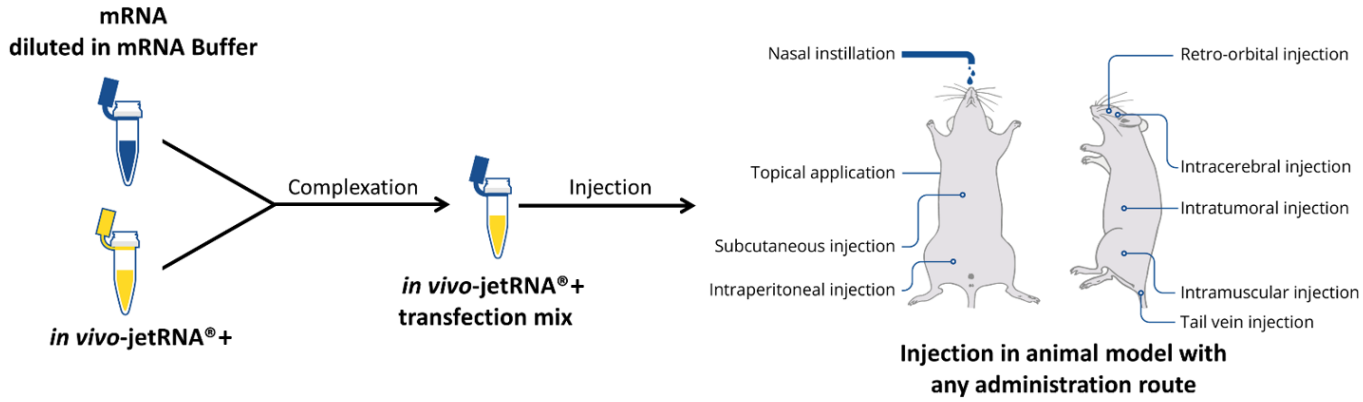

Figure 1. Two-step protocol for mRNA/*in vivo*-jetRNA®+ complexes preparation

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.
3. *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®+.
4. Vortex *in vivo*-jetRNA®+ reagent for 5 seconds.
5. Add the *in vivo*-jetRNA®+ reagent (following a ratio **mRNA / *in vivo*-jetRNA®+ of 1:2** (μg_{RNA}:$\mu L_{reagent}$)) 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.
6. Incubate for 15 minutes at room temperature.
7. Perform injections into animals using solution equilibrated at room temperature. Complexes are stable at RT up to 3 days (do not incubate the complexes on ice).
8. Analyze gene expression 6 - 72h after the injection.

2. Troubleshooting (*in vivo*)

Observations	Actions
Unsatisfactory results	• Optimize the amount of mRNA used in the delivery assay. • Optimize the injection volume. • Use high quality mRNA preparation. The $OD_{260/280}$ ratio should 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 the 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.

3. *in vitro* transfection protocol

3.1 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.

Cell type	Cells	Number of cells to seed per well of a 24-well plate	Amount of mRNA (ng)	Ratio
Epithelial	Caco-2	40,000	500	1:2
	A549	60,000	500	1:2
	HeLa	50,000	250	1:2
	HEK-293	50,000	250	1:2
Hepatocyte	HepG2	100,000	500	1:2

3.2 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_{RNA}:$\mu L_{reagent}$)) 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.

4. Troubleshooting (*in vitro*)

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.

5. Product Information

5.1 Ordering Information

Part N°	*in vivo*-jetRNA®+ Reagent	mRNA Buffer
101000122	1 mL	60 mL

5.2 Content

The volume of 1 mL of *in vivo*-jetRNA®+ is sufficient to perform at least 50 intravenous injections or 100 intramuscular injections in mice. An mRNA Buffer is provided with the reagent to prepare the *in vivo*-jetRNA®+ / mRNA complexes. This buffer should be used to ensure successful delivery experiments.

5.3 Reagent use and limitations

For research use only. Not for use in humans.

5.4 Quality control

Each batch of *in vivo*-jetRNA®+ reagent is tested for conformity to established Quality Controls and relevant specifications. Certificate of Analysis is available online in your Customer Area: https://myaccount.polyplus-transfection.com/wp-login.php

5.5 Formulation and storage

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

Polyplus-transfection® 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.

5.6 Trademarks

Polyplus® and *in vivo*-jetRNA®+ are registered trademarks of Polyplus-transfection S.A.

How to cite us: "*in vivo*-jetRNA®+ (Polyplus-transfection S.A, Illkirch, France)".

5.7 Contact information

Do you have any technical question regarding your product?

- Website: www.polyplus-transfection.com
- Email: support@polyplus-transfection.com
- Phone: +33 3 90 40 61 87

Contact our friendly Scientific Support team which is composed of highly educated scientists, PhDs and Engineers, with extensive hands-on experience in cell culture and transfection. The Scientific Support team is dedicated to help our customers reach their goals by offering various services such as: protocol optimization, personalized transfection conditions, tailored protocols, etc.

Please note that the Polyplus-transfection® support is available by phone from 9:00 am to 5:00 pm CEST.

For any administrative question, feel free to contact our administration sales team:

- Reception Phone: +33 3 90 40 61 80
- Fax: +33 3 90 40 61 81
- Addresses:

Polyplus® locations	Addresses
Headquarter Transfection reagent manufacturing site	75, rue Marguerite Perey 67400 Illkirch France
Plasmid design site	80 Rue du Dr Yersin 59120 Loos France
US Sales Office	1251 Ave of the Americas 34th fl. New-York - NY 10020 United States
China Sales Office	Room 1506, Tower B, Sunyoung Center No. 28 Xuanhua Road Changning District, Shanghai China