in vivo-jetRNA®

mRNA delivery reagent

PROTOCOL

DESCRIPTION

1 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. Furthermore, mRNA should be diluted and aliquoted in RNAse-free water (or in low salt buffer).

We recommend to use a **ratio mRNA / *in vivo*-jetRNA® of 1:1** (μg_{mRNA}:$\mu L_{reagent}$).

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	IV Tail vein/retro-orbital	10 µg mRNA 10 µL reagent	10 – 20 µg	10 – 20 µL	200 µL
	IP	20 µg mRNA 20 µL reagent	10 - 20 µg	10 - 20 µL	500 µL
	Subcutaneous (s.c)	5 µg mRNA 5 µL reagent	5 – 10 µg	5 – 10 µL	100 µL
	Intradermal injection	2 µg mRNA 2 µL reagent	2 – 5 µg	2 – 5 µL	50 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 http://www.polyplus-transfection.com/resources/cell-transfection-database/

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

1.2 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.

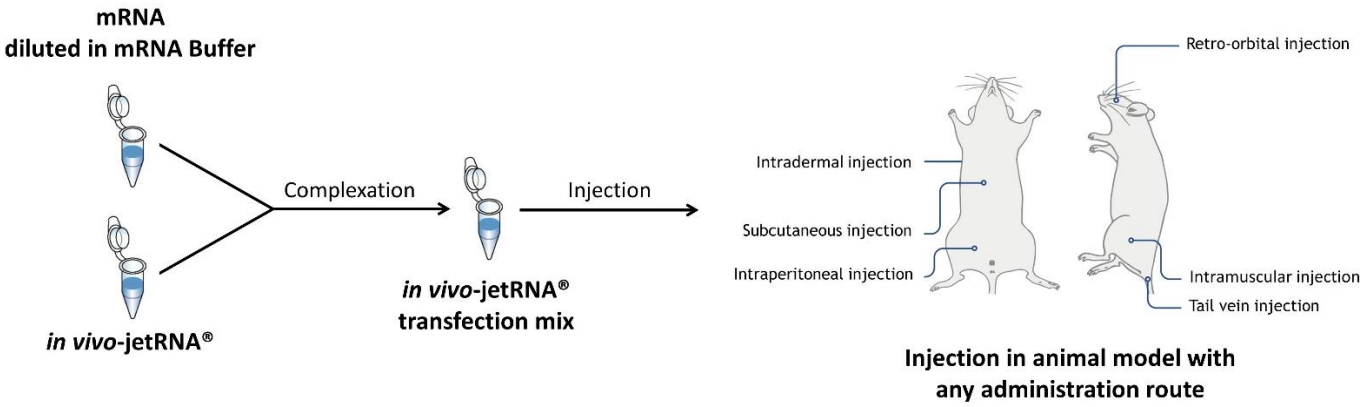
Define the experimental protocol and parameters:

- 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.

Protocol:

1. On the day of injection, 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 3. Mix by pipetting up and down.
   Example of IV injection: Dilute 10 µg of mRNA (1 µg/µL) in 180 µL of mRNA buffer and add 10 µL of in vivo-jetRNA®
2. Vortex *in vivo*-jetRNA® reagent for 5 seconds and spin down before use.
3. Add the *in vivo*-jetRNA® reagent (following a ratio **mRNA / *in vivo*-jetRNA® of 1:1** (μg_{RNA}:$\mu L_{reagent}$)) to the diluted mRNA all at once and homogenize by pipetting up and down.
4. Incubate for 15 minutes at room temperature.
5. Perform injections into animals using solution equilibrated at room temperature. Complexes are stable at RT up to 1h at RT.
6. Analyze gene expression 6 - 72h after the injection.

Protocol for mRNA/in vivo-jetRNA® complexes preparation



2 TROUBLESHOOTING

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*. • 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.
Cloudy solutions	• Decrease the amount of mRNA without changing the ratio mRNA / *in vivo*-jetRNA® of 1:1. • Use mRNA preparation with low amount of salt.
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 PRODUCT INFORMATION

3.1 ORDERING INFORMATION

Ref. N°	*in vivo*-jetRNA® reagent	mRNA buffer
204-03	0.3 mL	10 mL
204-10	1 mL	2 x 10 mL

3.2 CONTENT

The volume of 300 µL of *in vivo*-jetRNA® is sufficient to perform 15 – 30 intravenous injections in mouse. 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.

3.3 REAGENT USE AND LIMITATIONS

For research use only. Not for use in humans.

3.4 QUALITY CONTROL

Each batch of *in vivo*-jetRNA® reagent is tested for conformity to established Quality Controls and relevant specifications. A Certificate of Analysis (CoA) is provided with each vial of reagent.

3.5 FORMULATION AND STORAGE

in vivo-jetRNA® and mRNA Buffer are shipped with ice pack to reduce temperature variations and stored at 5 ± 3 °C upon arrival for long term storage.

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.

3.6 TRADEMARKS

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

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

3.7 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 the 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 is dedicated to help our Customers reach their goals by proposing different services such as: protocol optimization, personalized transfection conditions, tailored protocols, etc…

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-transfection S.A	Polyplus-transfection Inc.
Bioparc 850 Bd S. Brant 67400 Illkirch France	1251 Ave of the Americas 34th fl. New-York - NY 10020 United States

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