

in vivo-jetPEI®

DNA & siRNA Delivery Protocol

PROTOCOL

DESCRIPTION

in vivo-jetPEI® is a linear polyethylenimine, which mediates efficient nucleic acid (DNA, shRNA, siRNA, oligonucleotides, ...) delivery to a wide range of tissues using various delivery routes: intravenous (IV), intraperitoneal (IP), intratumoral, subcutaneous, topical, intrathecal, etc. Upon IV administration, high levels of nucleic acid delivery are achieved into the lung. Other organs such as salivary glands, heart, spleen and liver are also targeted following IV injection. In addition *in vivo*-jetPEI® is also an effective carrier for local gene and siRNA delivery such as intratumoral or topical application on the skin.

Previous publications using *in vivo*-jetPEI® can be found in the Polyplus transfection citation database at: www.polyplus-transfection.com/products/technical-resources/product-citations

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1 IN VIVO DELIVERY PROTOCOL

1.1 REAGENTS REQUIRED

We recommend using the 10% sterile isotonic glucose solution (w/v) provided in the kit. This is required in order to form small and stable nucleic acids/*in vivo*-jetPEI® complexes. The use of ionic buffers such as PBS or cell culture media for complex preparation should be avoided.

Furthermore, the nucleic acid should be resuspended in low salt buffer since high salt content in the nucleic acid preparation may lead to precipitation upon complexes formation.

For DNA, the best results are achieved with high quality endotoxin free DNA resuspended in ddH₂O.

For siRNA, order high quality grade siRNA (PAGE or HPLC purification).

1.2 RECOMMENDED AMOUNT OF NUCLEIC ACID AND INJECTION VOLUME

The amount of nucleic acid to deliver should be determined according to the animal model, the administration route and the targeted organ. Recommendations for delivery of DNA, siRNA, oligonucleotides and shRNA-expressing plasmids in rodents are given in Table 1.

The concentration of nucleic acid in the final injection solution should not exceed **0.5 µg/µl**.

Furthermore, to avoid precipitation, the nucleic acid should be resuspended in water or low salt buffer at high concentration (if possible for DNA 3-7 µg/µl and for siRNA 5-10 µg/µl).

The volume of reagent is defined by the N/P ratio and is calculated according to the formula on page 8.

As a general guideline, we recommend using: **N/P = 6 – 8**. (so 0.12 to 0.16 µl of *in vivo*-jetPEI® per µg DNA).

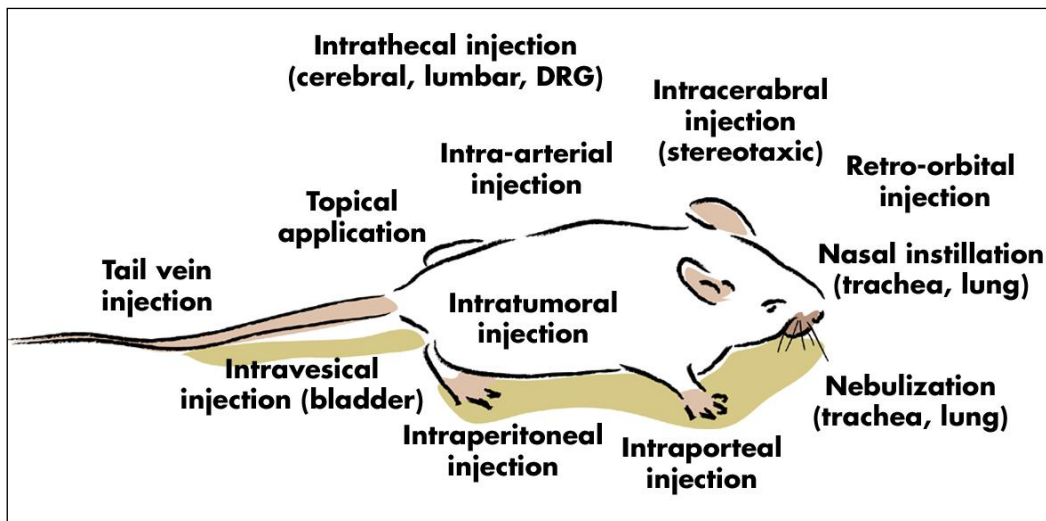
Prior to injections, ensure that *in vivo*-jetPEI® and glucose solution are equilibrated at room temperature.

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

Animal	Site of injection	Starting conditions	Nucleic acid optimization range	Injection volume optimization range (5% glucose)
Mouse	IV Tail vein/retro-orbital	40 µg nucleic acid 6.4 µl reagent 200 µl of 5% glucose	40 - 60 µg	200 - 400 µl
	IP	100 µg nucleic acid 16 µl reagent 1 ml 5% glucose	100 - 200 µg	1 ml
	Intratumoral	10 µg nucleic acid 1.2 µl reagent 50 µl of 5% glucose	5 - 15 µg	20 - 100 µl
	Subcutaneous (s.c)	5 µg nucleic acid 0.6 µl reagent 10 µl of 5% glucose	3 - 5 µg	5 - 15 µl
	Intracerebral	1.5 µg nucleic acid 0.18 µl reagent 5 µl of 5% glucose	1 - 2 µg	4 - 5 µl
Rat	IV	150 µg nucleic acid 24 µl reagent 1 ml of 5% glucose	100 - 300 µg	1 - 1.5 ml
	Intracerebral	3 µg nucleic acid 0.36 µl reagent 10 µl of 5% glucose	2 - 4 µg	8 - 10 µl

For siRNA delivery and DNA immunization protocols, multiple injections may be required.

For other administration routes (e.g. Fig. 1) such as intravitreal, nasal instillation, intra-arterial, intradermal, intracortical (kidney), bladder instillation, intratesticular etc., please contact our technical support at support@polyplus-transfection.com for advice or browse the literature on our website www.polyplus-transfection.com/products/technical-resources/product-citations/

**Figure 1. Successful delivery routes in mouse**

Experimental guidelines for other animal models **such as chicken, quail, sheep, dog, monkey etc.** are available **from our *in vivo* specialists**. You will be amazed by the wide range of animal models we have developed protocols for.

1.3 PROTOCOL

The preparation of the *in vivo*-jetPEI®/nucleic acid complexes should be performed in a laminar flow hood using a sterile 10 % glucose solution (provided with catalog number 201-10G, 201-20G and 201-50G). The final concentration of glucose in the injection volume should be 5 %.

Define the experimental protocol:

The injection volume of complexes to be prepared per animal (Table 1).

Note: the final concentration of glucose in the injection volume is 5%.

The amount of nucleic acid to be delivered per injection (Table 1)

Note: the final concentration of nucleic acid in the injection volume should not exceed 0.5 µg/µl.

The N/P ratio and calculate the corresponding volume of *in vivo*-jetPEI® (Table 2). When increasing the N/P ratio, ensure to decrease the nucleic acid amount.

As a general guideline, we recommend using: **N/P = 6 – 8 (so 0.12 to 0.16 µl of *in vivo*-jetPEI® per µg DNA).**

Table 2. Volumes of *in vivo*-jetPEI® to be used according to the N/P ratio and the amount of DNA required

Amount of DNA (µg)	Volume (µl) of <i>in vivo</i> -jetPEI®	
	N/P = 6	N/P = 8
1	0.12	0.16
5	0.6	0.8
10	1.2	1.6
40	4.8	6.4
50	6	8
100	12	16

Protocol overview

1. Dilute the nucleic acid using the 10% glucose stock solution (provided) and sterile water to prepare a solution of ½ the injection volume of 5 % glucose. Vortex gently or mix by pipetting up and down.
2. Dilute the *in vivo-jetPEI*® reagent using the 10% glucose stock solution (provided) and sterile water to prepare a solution of ½ the injection volume of 5 % glucose. Vortex gently and spin down.
3. Add the diluted *in vivo-jetPEI*® to the diluted nucleic acid all at once, vortex gently and spin down.
4. Incubate for 15 minutes at room temperature. From this time point, the complexes are stable 2 h at room temperature and for 24 h if stored at 4 °C.
5. Perform injections into animals using complexes equilibrated at room temperature.

For siRNA and DNA immunization protocols, repeat injections up to 3 times a week if required with freshly prepared complexes each time.

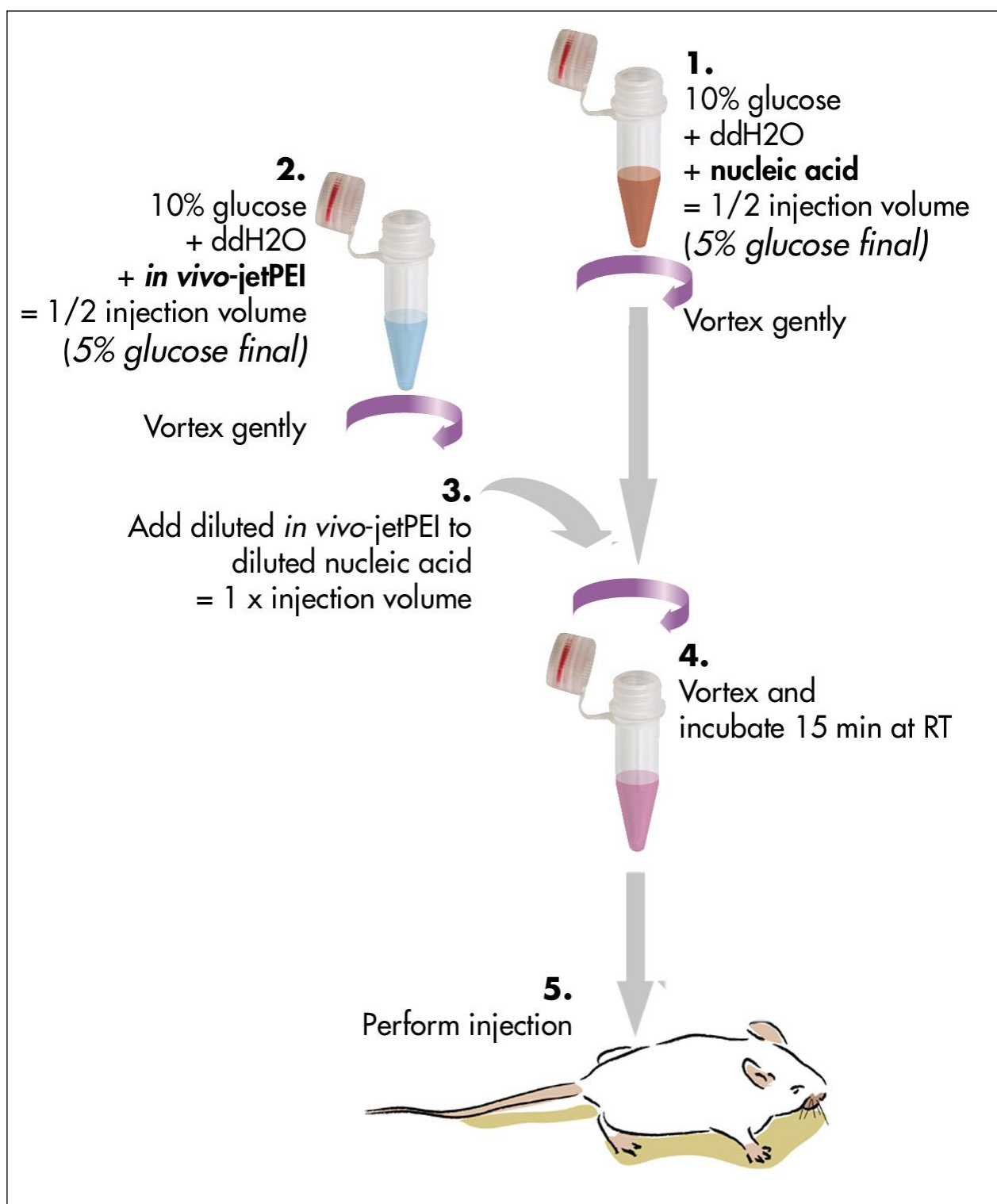
6. Monitor gene expression as required at the appropriate time point (6 – 72 h after the last injection) depending on the mode of injection and the targeted organ.

Example: IV injection in mouse

Preparation of 200 µl injection volume of 5 % glucose containing 40 µg of plasmid DNA and in vivo-jetPEI® at N/P = 8

1. Dilute 40 µg of DNA into 50 µl of 10% glucose; add sterile water to 100 µl, vortex gently and spin down,
2. Dilute 6.4 µl of *in vivo-jetPEI*® into 50 µl of 10% glucose; add sterile water to 100 µl, vortex gently and spin down.
3. Add the diluted *in vivo-jetPEI*® to the diluted DNA at once, vortex briefly and spin down.
4. Incubate for 15 minutes at room temperature.
5. Perform injections into animals using complexes equilibrated at room temperature.
6. Monitor gene expression.

Protocol for nucleic acid/*in vivo*-jetPEI® complexes preparation



2 TROUBLESHOOTING

Observations	Comments and Suggestions
Unsatisfactory results	• Optimize the amount of nucleic acids used in the delivery assay. • Optimize the injection volume. • Use high quality plasmid or siRNA preparation. Ensure they contain neither salt, RNA, protein or endotoxin. For plasmid DNA, OD_{260/280} ratio should be greater than 1.8. It is best to use DNA prepared in water. For siRNA, prefer HPLC or PAGE purified oligos. • Optimize the N/P ratio. • Check that the nucleic acid is efficient <i>in vitro</i>. • Ensure that the complexes are formed in glucose 5%. • Ensure that both nucleic acid and <i>in vivo-jetPEI</i>® are diluted in 5% glucose before mixing.
Toxicity	• Decrease the amount of nucleic acid, keeping the N/P ratio constant. • Decrease the N/P ratio, keeping the amount of nucleic acid constant. • If using plasmid DNA, ensure the preparation is endotoxin-free and in water. • Ensure that the N/P ratio is lower than 8 (0.16 µl <i>in vivo-jetPEI</i>® per µg DNA).

3 PRODUCT INFORMATION

3.1 ORDERING INFORMATION

Cat #	<i>in vivo-jetPEI</i> ® Reagent	10% Glucose solution, sterile filtered 0.2µm.
201-10G	0.1 ml	10 ml
201-50G	0.5 ml	2 x 10 ml

3.2 CONTENT

100 µl of *in vivo-jetPEI*® is sufficient to perform 15-25 intravenous injections in mouse. A 10 % sterile glucose solution is included to prepare the *in vivo-jetPEI*®/nucleic acid 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

DNA delivery (pCMV-Luciferase) is assessed in mouse lung for every batch of *in vivo*-jetPEI® reagent. The amount of Luciferase (ng) per mg of protein is indicated on the Certificate of Analysis for each batch.

3.5 FORMULATION AND STORAGE

in vivo-jetPEI® is provided at 150 mM (expressed as the concentration of nitrogen residues) in sterile apyrogenic water. *in vivo*-jetPEI® and 10 % glucose are shipped at room temperature and stored at -20°C upon arrival for long term storage. *in vivo*-jetPEI® is stable at least one year as guaranteed and indicated on the Certificate of Analysis, when stored appropriately.

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 DEFINITION OF N/P RATIO

The ionic balance within *in vivo*-jetPEI® /nucleic acid complexes is crucial. Indeed, for effective cell entry, the complexes should be cationic. The N/P ratio is a measure of the ionic balance within the complexes and is defined as the number of nitrogen residues of *in vivo*-jetPEI® per nucleic acid phosphate. Approximately one in three nitrogen atoms within the PEI is cationic, therefore electroneutrality of *in vivo*-jetPEI® /nucleic acid complexes is reached at $N/P > 2 - 3$.

in vivo-jetPEI® is provided as a 150 mM solution (expressed as nitrogen residues). Given that 1 µg of nucleic acid contains 3 nmoles of anionic phosphate, the amount of *in vivo*-jetPEI® to be mixed with DNA in order to obtain a specific N/P ratio is calculated using the following formula:

$$\mu\text{l of } in\ vivo\text{-jetPEI}^{\circledR} \text{ to be used} = \frac{(\mu\text{g of DNA} \times 3) \times N/P \text{ ratio}}{150}$$

For *in vivo* nucleic acid delivery experiments, we recommend $N/P = 6 - 8$. The optimal N/P ratio however should be determined for each new application, animal model and administration route. Please contact the Technical Support Team for any specific technical request, writing to support@polyplus-transfection.com or using the contact form available on Polyplus website.

3.7 TRADEMARKS

Polyplus-transfection and jetPEI are registered trademarks of Polyplus-transfection.

3.8 TECHNICAL ASSISTANCE AND SCIENTIFIC ADVICE

Contact the friendly Polyplus Technical Support *via*:

- The Polyplus website: www.polyplus-transfection.com
- Email: support@polyplus-transfection.com
- Phone: + 33 (0)3 90 40 61 87

The Technical Support will be delighted to provide guidelines adapted to your experiments.