in vivo-jetPEI™-FluoF

DNA & siRNA Delivery Protocol

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Company Information

Technical Assistance and Scientific Advice

Contact the friendly Polyplus technical support *via*:
The Polyplus website: www.polyplus-transfection.com
Email: support@polyplus-transfection.com
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Intellectual Property

The use of polyethylenimine (PEI) or polypropylenimine (PPI) or cationic polymers similar in structure thereto for transfecting cells, as well as compositions comprising these cationic polymers and at least one nucleic acid, are the subject matter of U.S. Patent No. 6,013,240, EP Patent No. 0770140 and foreign equivalents, for which Polyplus-transfection™ is the worldwide exclusive licensee.

Trademarks

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

Related reagents

in vivo-jetPE™, *in vivo*-jetPEI-Man™, *in vivo*-jetPEI-Gal™, *in vivo*-jetPEI-FluoR™

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Product Information

***in vivo*-jetPEI™-FluoF** is a fluorescein-conjugated *in vivo*-jetPEI™ (excitation at 490 nm; emission at 520 nm), which is a linear polyethylenimine synthesized and purified by Polyplus-transfection for effective and reproducible in vivo nucleic acid delivery (DNA, shRNA, siRNA, oligonucleotides …) Please note that due to auto-florescence of certain organs, it can sometimes be difficult to visualize ***in vivo*-jetPEI™-FluoF** depending on the target organ.
in vivo-jetPEI™-FluoF mediates efficient nucleic acid delivery to a wide range of tissues using various delivery routes: intravenous (IV), intraperitoneal (IP), intratumoral, intracardiac, sub-cutaneous, topical, etc. Publications using *in vivo*-jetPEI™ derivates can be found in the Polyplus transfection citation database at:
www.polyplus-transfection.com/products/technical-resources/product-citations

Ordering information

Cat #	*in vivo*-jetPEI™-FluoF Reagent	10% Glucose solution, sterile filtered 0.2µm.
205-10G	0.1 ml	10 ml

Content

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

Reagent use and Limitations

For research use only. Not for use in humans.

Formulation and Storage

in vivo-jetPEI™-FluoF is provided at 150 mM (expressed as the concentration of nitrogen residues) in sterile apyrogenic water. *In vivo*-jetPEI™-FluoF and 10% glucose are shipped at room temperature and stored at -20°C upon arrival for long term storage. *in vivo*-jetPEI™-FluoF is stable for at least 1 year at -20°C, as indicated on the certificate of analysis enclosed with the reagent when delivered.

Definition of N/P ratio

The ionic balance within *in vivo*-jetPEI™-FluoF /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™-FluoF 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™-FluoF 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™-FluoF to be mixed with DNA in order to obtain a specific N/P ratio is calculated using the following formula:

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

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.

In vivo Delivery Protocol

1. Reagents required

We recommend using the 10% sterile isotonic glucose solution (w/v) provided. This is required in order to form small and stable nucleic acids/*in vivo*-jetPEI™-FluoF 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 DNA prepared in water.
For siRNA, use PAGE or HLPC purification.

2. Recommended amount of nucleic acid and injection volume

The amount of nucleic acid to deliver is 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 volume 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 4.
As a general guideline, we recommend using: **N/P** = **6 – 8**.

Prior to injections, ensure that *in vivo*-jetPEI™-FluoF 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 optimisation range	Injection volume Optimisation 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 (1.6 - 2.4 mg/kg)	200 - 400 µl
	IP	100 µg nucleic acid 16 µl reagent 1 ml 5%glucose	100 - 200 µg (4 - 8 mg/kg)	1 ml
	Intratumoral	10 µg nucleic acid 1.2 µl reagent 20 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
	Intraventricular (Brain ventricle)	0.5 µg nucleic acid 0.06 µl reagent 1 µl of 5% glucose	0.5 - 1 µg	1 - 2 µl
Rat	IV	150 µg nucleic acid 24 µl reagent 1 ml of 5% glucose	150 - 300 µg (0.6 -1.2 mg/kg)	1 - 1.5 ml
	Intraventricular/ intracerebral	1 µg nucleic acid 0.12 µl reagent 2 µl of 5% glucose	1 – 2 µg	2-3 µl

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For other administration routes 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/

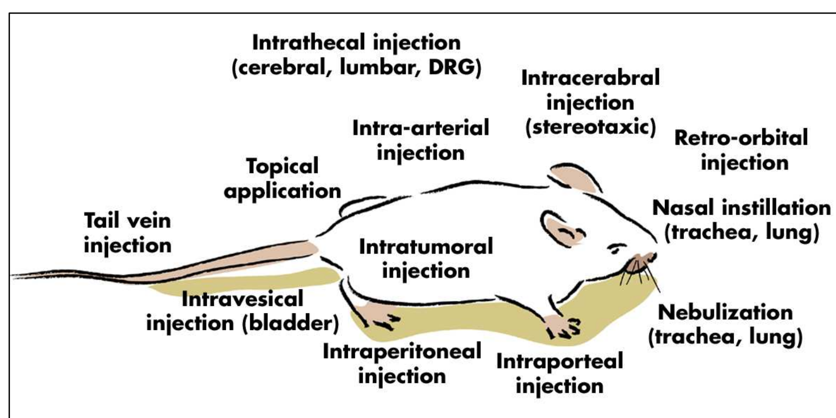


Figure 1. Successfully delivery routes using *in vivo*-jetPEI™ in mouse

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

3. Protocol

The preparation of the *in vivo*-jetPEI™-FluoF/nucleic acid complexes should be performed in a laminar flow hood using the sterile 10 % glucose solution provided. 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 is the injection volume should not exceed 0.5 µg/µl.
- The N/P ratio and calculate the corresponding volume of in vivo-jetPEI™-FluoF (Table 2).

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

Amount of DNA (µg)	Volume (µl) of *in vivo*-jetPEI™-FluoF		
	N/P = 6	N/P = 7	N/P = 8
1	0.12	0.14	0.16
5	0.6	0.7	0.8
10	1.2	1.4	1.6
40	4.8	5.6	6.4
50	6	7	8
100	12	14	16

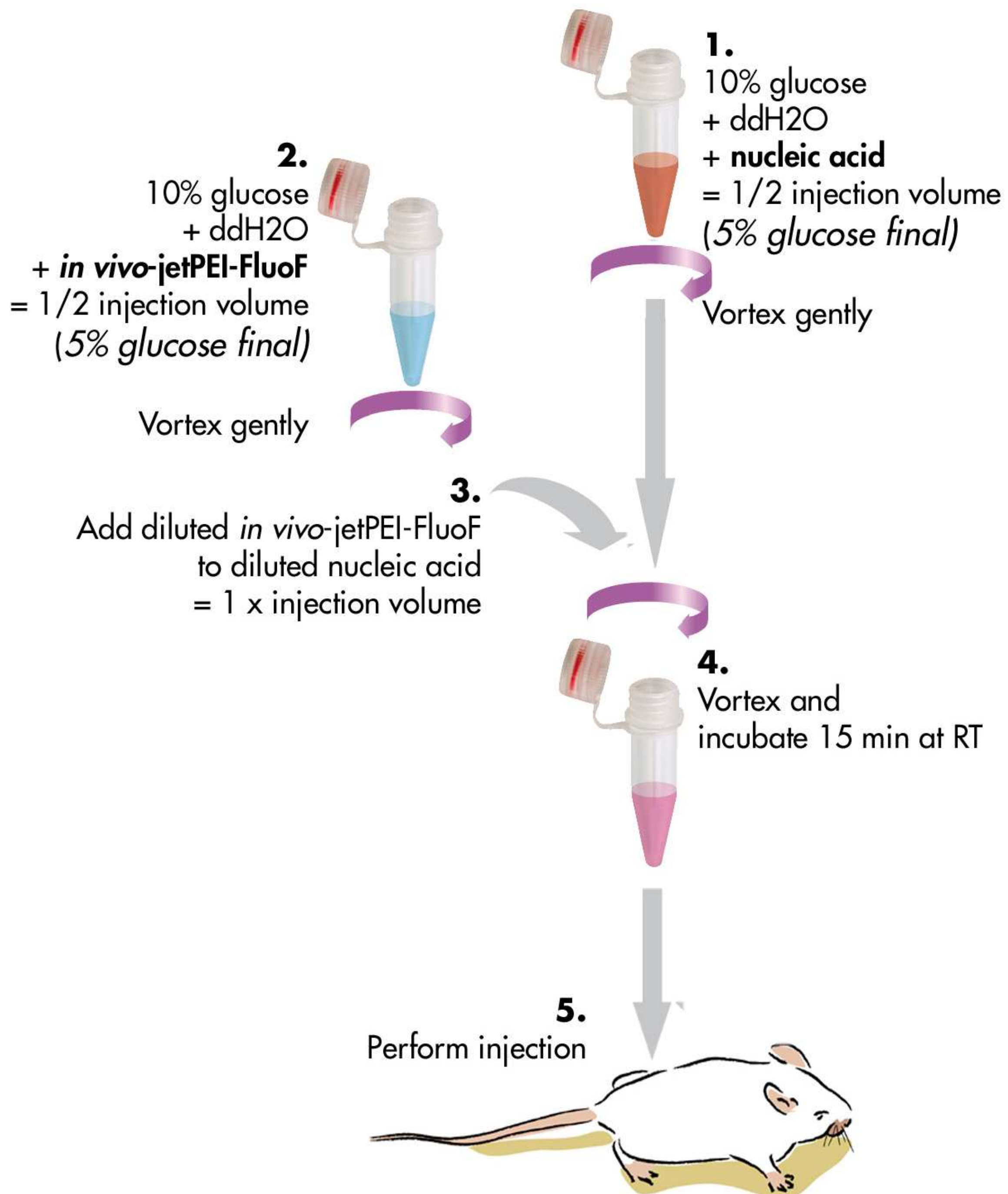
Protocol overview

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™-FluoF 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, spin down.
3. Add the diluted *in vivo*-jetPEI™-FluoF to the diluted nucleic acid all at once, vortex gently, spin down.
4. Incubate for 15 minutes at room temperature. From this time point, the complexes are stable 2h at room temperature and for 24 h if stored at 4 °C.
5. Perform injections into animals using complexes equilibrated at room temperature.
6. The biodistribution of *in vivo*-jetPEI-FluoF can be followed as early as 1 to 4 h following injection, using biofluorescence of the whole animal or slices of fixed tissues depending on the target organ.
7. Monitor gene expression as required at the appropriate time point (1 – 96 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™-FluoF at N/P = 8

1. Dilute 40 µg of DNA into 50 µl of 10% glucose; add sterile water to 100 µl, vortex gently, spin down,
2. Dilute 6.4 µl of *in vivo*-jetPEI™-FluoF into 50 µl of 10% glucose the in vivo-jetPEI™-FluoF reagent; add sterile water to 100 µl, vortex gently, spin down.
3. Add the diluted *in vivo*-jetPEI™-FluoF 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*-jePEI™-FluoF complexes preparation



Troubleshooting

Observations	Comments and Suggestions
Unsatisfactory results	• Check the auto-fluorescence of your target organ. • Optimize the amount of plasmid DNA, siRNA or shRNA used in the transfection 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. • Optimize the N/P ratio.
Toxicity	• Decrease the amount of nucleic acid but 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.

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Notes