in vivo-jetPEI®-Man

in vivo DNA & siRNA/miRNA transfection reagent

PROTOCOL

DESCRIPTION

***in vivo*-jetPEI®-Man** is a mannose-conjugated linear polyethylenimine derivative, synthesized and purified at Polyplus-transfection® for effective and reproducible *in vivo* nucleic acid delivery (DNA, shRNA, siRNA, oligonucleotides …). Given the presence of a chemically coupled mannose ligand, it enhances delivery to cells expressing mannose-specific membrane receptors, such as macrophages and dendritic cells, while keeping the cell viability intact. Similarly to *in vivo*-jetPEI®, *in vivo*-jetPEI®-Man is able to condense DNA into compact particles. The enhanced cell targeting is due to specific binding of the mannose residue to its cell surface receptor, leading to internalization of the *in vivo*-jetPEI®-Man/nucleic acid complexes.
Publications using *in vivo*-jetPEI®-Man can be found in the Polyplus transfection® citation database at:

http://www.polyplus-transfection.com/resources/cell-transfection-database/

1 TRANSFECTION PROTOCOL

1.1 REAGENTS REQUIRED

We recommend using the 10% isotonic glucose solution (w/v) provided. This is required in order to form small and stable nucleic acids/*in vivo*-jetPEI®-Man complexes.

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 ddH_2O and a stock solution of 3-7 µg/µL.
For si/miRNA, it is preferable to use high quality grade si/miRNA (PAGE or HPLC purification) and a stock concentration of 5-10 µg/µL.

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 volume should not exceed **0.5 µg/µL**.

The volume of reagent is defined by the N/P ratio and is calculated according to the formula on page 9.
As a general guideline, we recommend using: **N/P = 6 – 8**. (*i.e.* 0.12 to 0.16 µL of *in vivo*-jetPEI®-Man per µg of nucleic acid). Prior to injections, ensure that *in vivo*-jetPEI®-Man 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 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 500 µL 5%glucose	100 - 200 µg (4 - 8 mg/kg)	400 – 600 µL
	Subcutaneous (s.c)	20 µg nucleic acid 3.2 µL reagent 100 µL of 5% glucose	20 - 30 µg	100 - 200 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

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

For other administration routes, please contact our Scientific Support team 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 with ***in vivo*-jetPEI®-Man** are available **from our scientific specialists** for many animal models **such as chicken, quail, sheep, dog, monkey etc.**

1.3 PROTOCOL

The preparation of the *in vivo*-jetPEI®-Man/nucleic acid complexes should be performed in a laminar flow hood using the 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 in the injection volume should not exceed 0.5 µg/µL.
- Choose the N/P ratio and calculate the corresponding volume of *in vivo*-jetPEI®-Man (Table 2).

Table 2. Volumes of *in vivo*-jetPEI®-Man 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®-Man		
	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

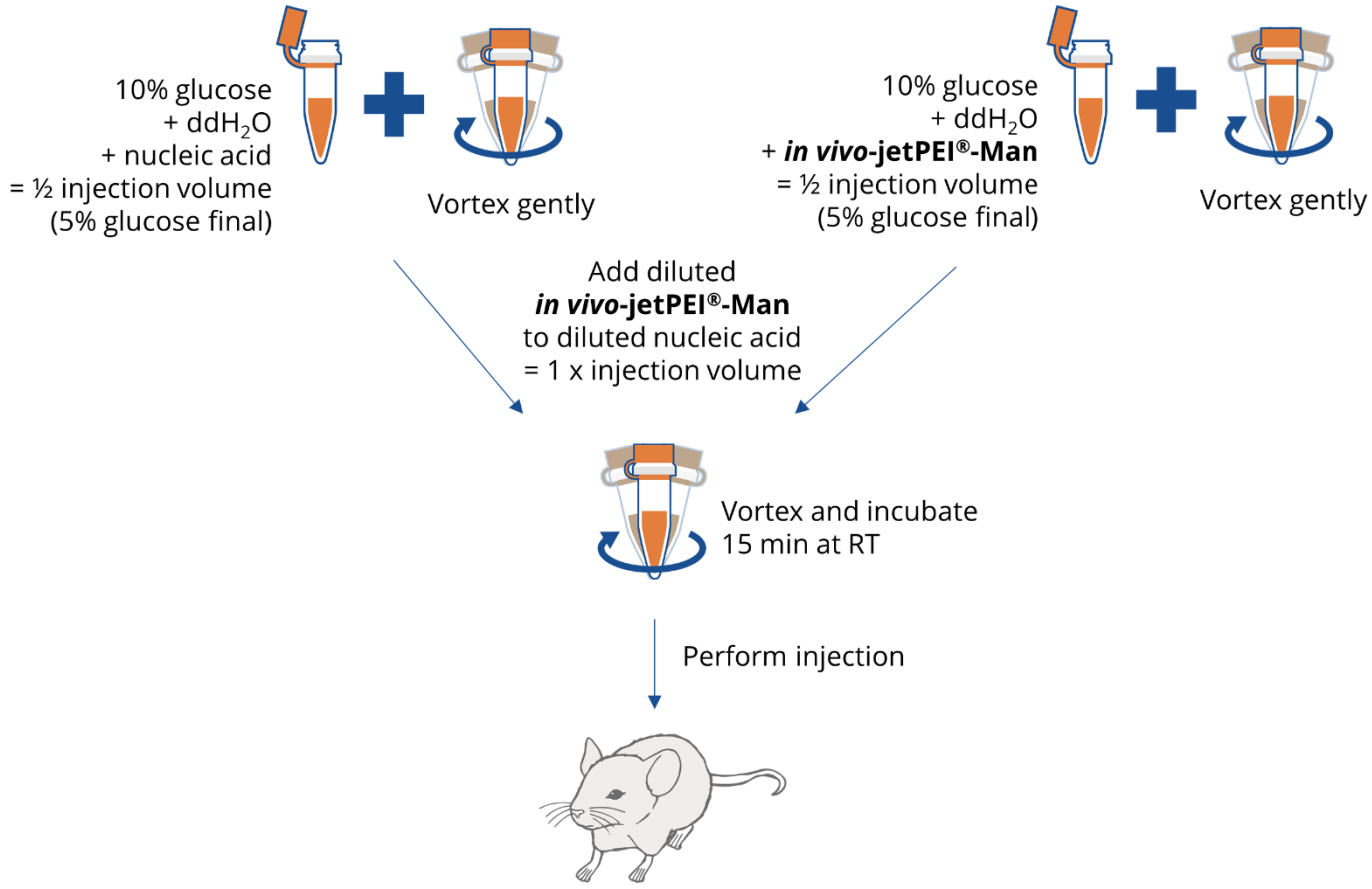
Protocol overview

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. Vortex *in vivo*-jetPEI®-Man reagent for 5 sec and spin down before use.
3. Dilute the *in vivo*-jetPEI®-Man 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.
4. Add the diluted *in vivo*-jetPEI®-Man to the diluted nucleic acid all at once, vortex gently, spin down.
5. 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 ºC.
6. Perform injections into animals using complexes equilibrated at room temperature.
   For siRNA and DNA immunization protocols, repeat injections several times if required with freshly prepared complexes each time.
7. Monitor gene expression as required at the appropriate time point 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®-Man 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®-Man into 50 µL of 10% glucose the *in vivo*-jetPEI®-Man reagent; add sterile water to 100 µL, vortex gently, spin down.
3. Add the diluted *in vivo*-jetPEI®-Man 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®-Man complexes preparation



2 TROUBLESHOOTING

Observations	Actions
Unsatisfactory results	• 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. • Check that the nucleic acid is efficient *in vitro*. • Ensure that the complexes are prepared in glucose 5%. • Ensure that both nucleic acid and *in vivo*-jetPEI®-Man 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 DNA is resuspended in water.

3 PRODUCT INFORMATION

3.1 ORDERING INFORMATION

Ref. N°	*in vivo*-jetPEI®-Man Reagent	10% Glucose solution, sterile filtered 0.2µm
203-10G	0.1 mL	10 mL

3.2 CONTENT

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

3.3 REAGENT USE AND LIMITATIONS

For research use only. Not for use in humans.

3.4 QUALITY CONTROL

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

3.5 FORMULATION AND STORAGE

in vivo-jetPEI®-Man is provided at 150 mM (expressed as the concentration of nitrogen residues) in sterile apyrogenic water. *in vivo*-jetPEI®-Man and 10% glucose should be stored at -20°C upon arrival for long term storage. When stored appropriately, *in vivo*-jetPEI®-Man is stable at least for 1 year at -20°C, as indicated on the Certificate of Analysis enclosed with the reagent when delivered.

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®-Man /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®-Man per nucleic acid phosphate. Approximately one in three nitrogen atoms within the PEI is cationic, therefore electroneutrality of *in vivo*-jetPEI®-Man/nucleic acid complexes is reached at N/P > 2 - 3.

in vivo-jetPEI®-Man 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®-Man to be mixed with DNA in order to obtain a specific N/P ratio is calculated using the following formula:

$$\text{µL of } \textit{in vivo}\text{-jetPEI®-Man to be used} = \frac{(\text{µg of DNA} \times 3) \times \text{N/P ratio}}{150}$$

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.

3.7 TRADEMARKS

Polyplus-transfection and *in vivo*-jetPEI®-Man are registered trademarks of Polyplus-transfection S.A.

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

3.8 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
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Please note that the Polyplus-transfection support is available by phone from 9:00 am to 5:00 pm CEST.