PROTOCOL

in vivo-jetPEI®-Gal
in vivo DNA & siRNA/miRNA transfection reagent

DESCRIPTION

in vivo-jetPEI®-Gal is a galactose-conjugated linear polyethylenimine derivative, synthesized and purified at Polyplus-transfection® for effective and reproducible *in vivo* nucleic acid transfection (DNA, shRNA, siRNA, oligonucleotides …). It enhances delivery to cells expressing galactose-specific membrane receptors, such as hepatocytes bearing the asialoglycoprotein receptor (ASGP-R or Gal/GalNAc receptor).
Similarly to *in vivo*-jetPEI®, *in vivo*-jetPEI®-Gal, is able to condense nucleic acids into compact particles. The enhanced cell targeting is due to specific binding of the galactose residue to its cell surface receptor, leading to internalization of the *in vivo*-jetPEI®-Gal/nucleic acid complexes.

Publications using *in vivo*-jetPEI® derivatives can be found in the Polyplus-transfection database, available online at
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 acid/*in vivo*-jetPEI®-Gal 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 ddH2O 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 solution 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 6.
As a general guideline, we recommend using: N/P = 6 – 8. (i.e. 0.12 to 0.16 µL of *in vivo*-jetPEI®-Gal per µg of nucleic acid). Prior to injections, ensure that *in vivo*-jetPEI®-Gal 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 500 µL of 5% glucose	100 – 200 µg	400 – 600 µL
Rat	IV	150 µg nucleic acid 24 µL reagent 1 mL of 5% glucose	150 – 300 µg	1 – 1.5 mL

For other administration routes such as delivery into the hepatic vein, please contact our scientific 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 with *in vivo*-jetPEI®-Gal for other animal models such as chicken, quail, sheep, dog, monkey etc. are available from our scientific specialists.

1.3. Protocol

The preparation of the *in vivo*-jetPEI®-Gal/nucleic acid complexes should be performed in a laminar flow hood using a 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®-Gal (Table 2).

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

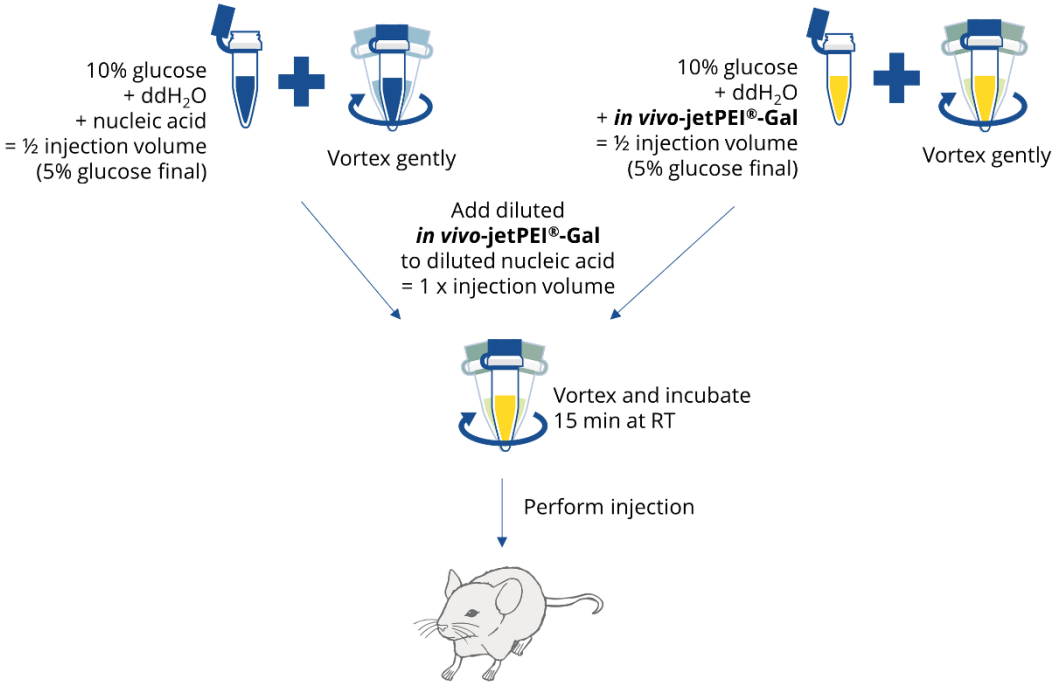
1. Dilute the nucleic acid using the 10 % glucose stock solution (provided) and sterile water to prepare a solution of ½ the injection volume of 5 % glucose. Vortex gently or mix by pipetting up and down.
2. Vortex *in vivo*-jetPEI®-Gal reagent for 5 sec and spin down before use.
3. Dilute the *in vivo*-jetPEI®-Gal 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®-Gal 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®-Gal 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®-Gal into 50 µL of 10% glucose; add sterile water to 100 µL, vortex gently and spin down.
3. Add the diluted *in vivo*-jetPEI®-Gal 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®-Gal 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®-Gal 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

Part N°	*in vivo*-jetPEI®-Gal Reagent	10% Glucose solution, sterile filtered 0.2 µm
101000047	0.1 mL	10 mL

3.2. Content

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

3.5. Formulation and Storage

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

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

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

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

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.

3.7. Trademarks

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

How to cite us: "*in vivo*-jetPEI®-Gal (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.