

***in vivo*-jetPEI™-Gal**

Cationic polymer transfection reagent

In vivo Transfection Protocol

202-10	0.1 ml		(sufficient for transfection of 1 mg of DNA at N/P=5)
202-20	0.2 ml		(sufficient for transfection of 2 mg of DNA at N/P=5)
202-10G	0.1 ml	10 ml of 10% glucose*	(sufficient for transfection of 1 mg of DNA at N/P=5)
202-20G	0.2 ml	10 ml of 10% glucose*	(sufficient for transfection of 2 mg of DNA at N/P=5)

*** CAUTION**

NEW - Glucose concentration of the provided solution is now 10% instead of 5 %. This concentration is more suitable for transfection of highly concentrated DNA - NEW

Content

0.1 ml of *in vivo*-jetPEI™-Gal transfection reagent is sufficient to perform 20 intravenous injection in mouse (50 µg of DNA per injection at N/P=5). Reagent for research use only. Not for use in human. A 10% glucose solution is included with catalog numbers 202-10G and 202-20G. This solution is more suitable for transfection of highly concentrated DNA.

Formulation and Storage

In vivo-jetPEI™-Gal is provided as a 150 mM solution in sterile apyrogenic water (expressed as concentration of monomer nitrogen residues). *In vivo*-jetPEI™-Gal and 10% glucose solution are shipped at room temperature and should be stored at -20°C upon arrival.

Description

In vivo-jetPEI™-Gal is a galactose-conjugated linear polyethylenimine derivative which is synthesized and purified by PolyPlus-transfection for effective and reproducible gene and oligonucleotide delivery to cells expressing galactose-specific membrane receptors, such as as hepatocytes bearing the asialoglycoprotein receptor (ASGP-R or Gal/GalNAc receptor). *in vivo*-jetPEI™-Gal, as regular *in vivo*-jetPEI™, is able to condense DNA into

compact particles. Cell targeting is the result of the specific binding of the galactose residue to its cell surface receptor, leading to internalization of the *in vivo*-jetPEITM-Gal/DNA complexes ¹. After entering cells by receptor-mediated endocytosis, *in vivo*-jetPEITM-Gal expresses the unique property of acting as a "proton sponge" that buffers the endosomal pH and protects DNA from degradation. Continuous proton influx also induces endosome osmotic swelling and rupture which provides an escape mechanism for DNA particles to the cytoplasm ^{2,3}.

Definition of N/P ratio

The *N/P ratio* is a measure of the ionic balance of the complexes. It refers to the number of nitrogen residues of *in vivo*-jetPEITM-Gal per DNA phosphate. Not every nitrogen atom of PEI being a cation, electroneutrality of *in vivo*-jetPEITM-Gal/DNA complexes is reached for N/P = 2 - 3. In practice, the best *in vivo* delivery results are obtained for N/P = 5 - 10. The optimal ratio can easily be determined for each new application.

In vivo-jetPEITM-Gal is provided as a 150 mM solution (expressed as nitrogen residues) and 1 µg of DNA contains 3 nmoles of anionic phosphate.

The amount of *in vivo*-jetPEITM-Gal solution to be mixed with DNA in order to obtain the desired N/P ratio can thus be calculated using the following formula (typical conditions are also given in Table 1) :

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

Table 1 . Volumes of *in vivo*-jetPEITM-Gal solution and amounts of DNA for various N/P ratios.

Amount of DNA	Volume (µl) of <i>in vivo</i> -jetPEI TM -Gal at	Volume (µl) of <i>in vivo</i> -jetPEI TM -Gal at	Volume (µl) of <i>in vivo</i> -jetPEI TM -Gal at	Volume (µl) of <i>in vivo</i> -jetPEI TM -Gal at	Volume (µl) of <i>in vivo</i> -jetPEI TM -Gal at
	N/P = 4	N/P = 5	N/P = 6	N/P = 8	N/P = 10
5 µg	0.4	0.5	0.6	0.8	1
10 µg	0.8	1	1.2	1.6	2
50 µg	4	5	6	8	10
100 µg	8	10	12	16	20

Protocols

1. Reagent required

Formation of small and stable *in vivo*-jetPEITM-Gal/DNA complexes is only possible in the absence of high salt concentrations. Ionic solutions such as PBS or cell culture media are thus prohibited. A sterile isotonic glucose solution is strongly recommended to dilute *in vivo*-jetPEITM-Gal and DNA in order to obtain a final concentration of 5% glucose. A 10% glucose solution is provided with catalog numbers 202-10G and 202-20G.

2. Preparation of the complexes with a 5% glucose solution

The amount of DNA as well as the injection volume should be adapted to the size of the animal and to the route of administration. Suggested amounts of DNA to be injected for a mouse are given in table 2. Usually, we recommend using *in vivo*-jetPEITM-Gal at N/P = 5 - 10. The following protocol is given for intravenous injection of 400 μ l solution containing 50 μ g of DNA condensed with *in vivo*-jetPEITM-Gal at N/P = 10. Refer to table 1 for other DNA amounts and other N/P ratios.

To prevent precipitation of *in vivo*-jetPEITM-Gal/DNA complexes, the final concentration of DNA in the total volume should not exceed 0.5 μ g/ μ l.

Let the *in vivo*-jetPEITM-Gal solution thaw to room temperature before use and vortex-mix to ensure homogeneity.

Under sterile conditions:

- Dilute 50 μ g of DNA into 200 μ l of 5% glucose (w/v). Vortex gently and spin down briefly.
- Dilute 10 μ l of *in vivo*-jetPEITM-Gal reagent into 200 μ l of 5% glucose (w/v). Vortex gently and spin down briefly.
- Add the 200 μ l *in vivo*-jetPEITM-Gal solution to the 200 μ l DNA solution all at once (important: do not reverse the order of addition).
- Vortex-mix the solution immediately and spin down lightly and briefly.
- Incubate for 15 minutes at room temperature (complexes are stable and can be used within the next 24 h).
- Inject animals
- Monitor transgene expression after the desired time period. Robust gene delivery and expression may require 12-48 h.

3. Preparation of the complexes with a 10% glucose solution

The following protocol is given for intravenous injection of 400 µl solution containing 50 µg of DNA condensed with *in vivo*-jetPEI™-Gal at N/P = 10. Refer to table 1 for other DNA amounts and other N/P ratios.

To prevent precipitation of *in vivo*-jetPEI™-Gal/DNA complexes, the final concentration of DNA in the total volume should not exceed 0.5 µg/µl.

Let the *in vivo*-jetPEI™-Gal solution thaw to room temperature before use and vortex-mix to ensure homogeneity.

Under sterile conditions:

- Dilute 50 µg of DNA into 100 µl of 10% glucose (w/v) provided with catalog numbers 202-10G and 202-20G and adjust the volume to 200 µl with pure sterile water in order to obtain a final concentration of 5% glucose. Vortex gently and spin down briefly.
- Dilute 10 µl of *in vivo*-jetPEI™-Gal reagent into 100 µl of 10% glucose (w/v) and add 90 µl of pure sterile water in order to obtain a final concentration of 5% glucose. Vortex gently and spin down briefly.
- Add the 200 µl *in vivo*-jetPEI™-Gal solution to the 200 µl DNA solution all at once (important: do not reverse the order of addition).
- Vortex-mix the solution immediately and spin down lightly and briefly.
- Incubate for 15 minutes at room temperature (complexes are stable and can be used within the next 24 h).
- Inject animals
- Monitor transgene expression after the desired time period. Robust gene delivery and expression may require 12-48 h.

Table 2. Suggested amounts of DNA according to the route of injection

Animal	Site of injection	Suggested amount of DNA	N/P ratio	Maximum injection volume
Adult mouse	Tail vein	50 µg ^{4,7}	10	200-400 µl
	Portal vein	50 µg ⁷	10	1 ml
	Heart	50 µg ⁷	10	200 µl
	Peritoneal cavity	50-100 µg	10	500-1000 µl
	Subcutaneous tumor	10 µg ⁸	10	100 µl

References

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Troubleshooting

Problems	Comments and Suggestions
Too low transfection level	<ul style="list-style-type: none">• Optimize the amount of plasmid DNA used in the transfection assay.• Use high-quality plasmid preparation, free of RNA (the OD_{260/280} ratio should be greater than 1.8).• Optimize the <i>in vivo</i>-jetPEI™-Gal/DNA ratio starting from 1µl <i>in vivo</i>-jetPEI™-Gal/10µg DNA up to 2µl <i>in vivo</i>-jetPEI™-Gal/10 µg DNA.
Mortality	<ul style="list-style-type: none">• Decrease the amount of plasmid DNA used in the transfection assay (keep the <i>in vivo</i>-jetPEI™-Gal/DNA ratio constant).• Make sure the plasmid preparation is endotoxin-free.

Technical Assistance

Contact the PolyPlus assistance service via:

Internet address: www.polyplus-transfection.com

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Related compounds

jetPEI™-Gal for *in vitro* applications

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