



***in vivo*-jetPEI™**

Cationic polymer transfection reagent

In vivo Transfection Protocol

201-10	0.1 ml		(sufficient for transfection of 1 mg of DNA at N/P=5)
201-20	0.2 ml		(sufficient for transfection of 2 mg of DNA at N/P=5)
201-50	0.5 ml		(sufficient for transfection of 5 mg of DNA at N/P=5)
201-10G	0.1 ml	5 ml of 10% glucose*	(sufficient for transfection of 1 mg of DNA at N/P=5)
201-20G	0.2 ml	5 ml of 10% glucose*	(sufficient for transfection of 2 mg of DNA at N/P=5)
201-50G	0.5 ml	2 x 5 ml of 10% glucose*	(sufficient for transfection of 5 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

Contents

0.1 ml of *in vivo*-jetPEI™ is sufficient to perform up to 20 intravenous injection in mouse (50 µg of DNA per injection at N/P = 5). Reagent for research use only. Not for use in humans. A 10 % glucose solution is included with catalog numbers 201-10G, 201-20G and 201-50G. This solution is suitable for high DNA concentration transfection.

Formulation and Storage

in vivo-jetPEI™ is provided as a 150 mM solution in sterile apyrogenic water (expressed as concentration of monomer nitrogen residues). *in vivo*-jetPEI™ is shipped at room temperature and should be stored at -20°C upon arrival. *in vivo*-jetPEI™ is stable for 1 year at -20°C. 10% Glucose solution should be stored at 4°C.

Description

In vivo-jetPEI™ is a linear polyethylenimine which is synthesized and purified by Polyplus-transfection for effective and reproducible *in vivo* gene and oligonucleotide

delivery with low toxicity¹. It performs better than other cationic polymers and lipids including the branched 25 KDa and 800 KDa PEI isomers available from chemicals manufacturers²⁻⁴. *In vivo*-jetPEI™ is a special brand developed by the team who discovered its remarkable properties^{5,6}. PEI is able to mediate efficient gene delivery to various tissues following intravenous^{2,3,7-11}, intracerebral¹²⁻¹⁴ or intraperitoneal injection^{15,16} as well as intratracheal instillation^{4,7}. The most efficient route in mice is systemic injection leading to very high levels of lung transfection^{7,10,11}. Many other organs are also transfected following *i.v.* injection although at levels lower than those observed in the lungs^{10,11}. Moreover, PEI is an effective vehicle for localized gene delivery in many tissues including the brain¹⁷. PEI efficiently delivers transgenes after intraventricular or intrathecal injections^{13,18}. Functional protein following transgene expression in neurons has been seen in genomic studies and therapeutic approaches¹⁸⁻²⁰. Other delivery routes and target organs are summarized in Table 2.

In vivo-jetPEI™ condenses DNA into positively charged particles capable of interacting with anionic proteoglycans at the cell surface and entering cells by endocytosis²¹. It possesses 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^{5,6}.

Definition of N/P ratio

Effective cell entry depends on cationic particles. The ionic balance of *in vivo*-jetPEI™ cations and DNA anions should thus be cationic.

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*-jetPEI™ per DNA phosphate. Not every nitrogen atom of PEI being a cation, electroneutrality of *in vivo*-jetPEI™ /DNA complexes is reached for $N/P = 2 - 3$. In practice, the best transfection results are obtained for $N/P = 5 - 10$. The optimal ratio can easily be determined for each new application.

In vivo-jetPEI™ 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*-jetPEI™ 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) :

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

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

Amount of DNA	Volume (μl) of <i>in vivo</i> -jetPEI™ at	Volume (μl) of <i>in vivo</i> -jetPEI™ at	Volume (μl) of <i>in vivo</i> -jetPEI™ at	Volume (μl) of <i>in vivo</i> -jetPEI™ at	Volume (μl) of <i>in vivo</i> -jetPEI™ 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*-jetPEI™/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 5 or 10% glucose (w/v) solution is strongly recommended to dilute *in vivo*-jetPEI™ and DNA in order to obtain a final concentration of 5% glucose. A 10 % glucose solution is provided with catalog numbers 201-10G, 201-20G and 201-50G.

2. Preparation of the complexes with 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*-jetPEI™ at N/P = 6 - 10. The following protocol is given for intravenous injection of 50 µg of DNA condensed with *in vivo*-jetPEI™ at N/P = 10. Refer to table 1 for other DNA amounts and other N/P ratios.

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

Let the *in vivo*-jetPEI™ solution thaw to room temperature before use.

Under sterile conditions:

- Dilute 50 µg of DNA into 200 µl of 5% glucose (w/v). Vortex gently and spin down briefly.
- Dilute 10 µl of *in vivo*-jetPEI™ reagent into 200 µl of 5% glucose (w/v). Vortex gently and spin down briefly.
- Add the 200 µl *in vivo*-jetPEI™ solution to the 200 µl DNA solution all at once (important: do not mix the solution in the reverse order).
- 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 10 % glucose solution

The following protocol is given for intravenous injection via the mice tail vein of 50 µg of DNA condensed with *in vivo*-jetPEI™ at N/P = 10 and a final total volume of injection of 400 µl. Refer to table 1 for other DNA amounts and other N/P ratios. To prevent precipitation of *in vivo*-jetPEI™ /DNA complexes, the final concentration of DNA in the total volume should not exceed 0.5 µg/µl.

Let the *in vivo*-jetPEI™ and glucose solution thaw to room temperature before injecting into the animal.

Under sterile conditions:

- Dilute 50 µg of DNA into 100 µl of glucose solution 10% (provided with references 201-10G, 201-20G and 201-50G) 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™ in 100 µl of glucose 10% solution and add 90 µl of pure sterile water (to obtain a final concentration of 5% glucose). Vortex gently and spin down briefly.
- Add the 200 µl *in vivo*-jetPEI™ solution to the 200 µl DNA solution all at once (important: do not mix the solution in the reverse order).
- Vortex-mix the solution immediately and spin down gently and briefly just to ensure that no liquid remains on the sides of the tube.
- 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, depending on the mode of injection and the organ targeted.

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

Animal	Site of injection	Suggested amount of DNA	N/P ratio	Maximum injection volume
Adult mouse	Tail vein	50 µg ^{11, 22} 100 µg ^{9, 10, 23}	10 4	200 to 400µl 400 to 500 µl
	Retroorbital	40 µg 60 µg ²²	8 10	200 µl 200 µl
	Portal vein	50 µg ¹¹ 100 µg ²⁴	10 10	1 ml 0.5 ml
	Intraperitoneal	100 µg ¹⁶ 100 µg ¹⁵	5 7	1 ml 600 µl
	Brain ventricle	2.5 µg ^{12, 13} 1 µg ¹⁹	6	5 µl
	Heart	50 µg ¹¹	10	200 µl
	Lung instillation	20 µg ²⁵ 50 µg ²	10 10	200 µl (150 mM NaCl) 300 to 650 µl (5% glucose)
	Subcutaneous tumor	50 µg ²⁶ 20 µg ²⁷ 10 µg ^{8, 28}	6 10 10	100 µl 100 µl 100 µl
Newborn mouse	Brain ventricle	1 µg ^{12, 13}	6	2 µl
	Tail vein	5 µg ²⁹	10	

References

- Demeneix, B., J. Behr, O. Boussif, M.A. Zanta, B. Abdallah, and J. Remy (1998) Gene transfer with lipospermines and polyethylenimines. *Adv Drug Deliv Rev.* **30**, 1-3, p85-95.
- Bragonzi, A., A. Boletta, A. Biffi, A. Muggia, G. Sersale, S.H. Cheng, C. Bordinon, B.M. Assael, and M. Conese (1999) Comparison between cationic polymers and lipids in mediating systemic gene delivery to the lungs. *Gene Ther.* **6**, 12, p1995-2004.

3. Chemin, I., D. Moradpour, S. Wieland, W.B. Offensperger, E. Walter, J.P. Behr, and H.E. Blum (1998) Liver-directed gene transfer: a linear polyethylenimine derivative mediates highly efficient DNA delivery to primary hepatocytes in vitro and in vivo. *J Viral Hepat.* **5**, 6, p369-75.
4. Ferrari, S., A. Pettenazzo, N. Garbati, F. Zacchello, J.P. Behr, and M. Scarpa (1999) Polyethylenimine shows properties of interest for cystic fibrosis gene therapy. *Biochim Biophys Acta.* **1447**, 2-3, p219-25.
5. Behr, J.P. (1997) The proton sponge - A trick to enter cells the viruses did not exploit. *CHIMIA.* **51**, p34-36.
6. Boussif, O., F. Lezoualc'h, M.A. Zanta, M.D. Mergny, D. Scherman, B. Demeneix, and J.P. Behr (1995) A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine. *Proc Natl Acad Sci U S A.* **92**, 16, p7297-301.
7. Bragonzi, A., G. Dina, A. Villa, G. Calori, A. Biffi, C. Bordignon, B.M. Assael, and M. Conese (2000) Biodistribution and transgene expression with nonviral cationic vector/DNA complexes in the lungs. *Gene Ther.* **7**, 20, p1753-60.
8. Coll, J.L., P. Chollet, E. Brambilla, D. Desplanques, J.P. Behr, and M. Favrot (1999) In vivo delivery to tumors of DNA complexed with linear polyethylenimine. *Hum Gene Ther.* **10**, 10, p1659-66.
9. Goula, D., N. Becker, G.F. Lemkine, P. Normandie, J. Rodrigues, S. Mantero, G. Levi, and B.A. Demeneix (2000) Rapid crossing of the pulmonary endothelial barrier by polyethylenimine/DNA complexes. *Gene Ther.* **7**, 6, p499-504.
10. Goula, D., C. Benoist, S. Mantero, G. Merlo, G. Levi, and B.A. Demeneix (1998) Polyethylenimine-based intravenous delivery of transgenes to mouse lung. *Gene Ther.* **5**, 9, p1291-5.
11. Zou, S.M., P. Erbacher, J.S. Remy, and J.P. Behr (2000) Systemic linear polyethylenimine (L-PEI)-mediated gene delivery in the mouse. *J Gene Med.* **2**, 2, p128-34.
12. Demeneix, B.A., M. Ghorbel, and D. Goula (2000) Optimizing polyethylenimine-based gene transfer into mammalian brain for analysis of promoter regulation and protein function. *Methods Mol Biol.* **133**, p21-35.
13. Goula, D., J.S. Remy, P. Erbacher, M. Wasowicz, G. Levi, B. Abdallah, and B.A. Demeneix (1998) Size, diffusibility and transfection performance of linear PEI/DNA complexes in the mouse central nervous system. *Gene Ther.* **5**, 5, p712-7.
14. Ouatas, T., S. Le Mevel, B.A. Demeneix, and A. de Luze (1998) T3-dependent physiological regulation of transcription in the *Xenopus* tadpole brain studied by polyethylenimine based in vivo gene transfer. *Int J Dev Biol.* **42**, 8, p1159-64.
15. Aoki, K., S. Furuhashi, K. Hatanaka, M. Maeda, J.S. Remy, J.P. Behr, M. Terada, and T. Yoshida (2001) Polyethylenimine-mediated gene transfer into pancreatic tumor dissemination in the murine peritoneal cavity. *Gene Ther.* **8**, 7, p508-14.
16. Louis, M.H., S. Dutoit, Y. Denoux, P. Erbacher, E. Deslandes, J.P. Behr, P. Gauduchon, and L. Poulain (2005) Intraperitoneal linear polyethylenimine (L-PEI)-mediated gene delivery to ovarian carcinoma nodes in mice. *Cancer Gene Ther.* *in press*.

17. Abdallah, B., A. Hassan, C. Benoist, D. Goula, J.P. Behr, and B.A. Demeneix (1996) A powerful nonviral vector for in vivo gene transfer into the adult mammalian brain: polyethylenimine. *Hum Gene Ther.* **7**, 16, p1947-54.
18. Guissouma, H., S.M. Dupre, N. Becker, E. Jeannin, I. Seugnet, B. Desvergne, and B.A. Demeneix (2002) Feedback on Hypothalamic TRH Transcription Is Dependent on Thyroid Hormone Receptor N Terminus. *Mol Endocrinol.* **16**, 7, p1652-66.
19. Lemkine, G.F., S. Mantero, C. Migne, A. Raji, D. Goula, P. Normandie, G. Levi, and B.A. Demeneix (2002) Preferential transfection of adult mouse neural stem cells and their immediate progeny in vivo with polyethylenimine. *Mol Cell Neurosci.* **19**, 2, p165-74.
20. Wu, K., C.A. Meyers, J.A. Bennett, M.A. King, E.M. Meyer, and J.A. Hughes (2004) Polyethylenimine-mediated NGF gene delivery protects transected septal cholinergic neurons. *Brain Res.* **1008**, 2, p284-7.
21. Mislick, K.A. and J.D. Baldeschwieler (1996) Evidence for the role of proteoglycans in cation-mediated gene transfer. *Proc Natl Acad Sci U S A.* **93**, 22, p12349-54.
22. Ge, Q., L. Filip, A. Bai, T. Nguyen, H.N. Eisen, and J. Chen (2004) Inhibition of influenza virus production in virus-infected mice by RNA interference. *Proc Natl Acad Sci U S A.* **101**, 23, p8676-81.
23. Garzon, M.R., *et al.* (2005) Induction of gp120-specific protective immune responses by genetic vaccination with linear polyethylenimine-plasmid complex. *Vaccine.* **23**, 11, p1384-92.
24. Johansson, A., G. Nowak, C. Moller, and P. Harper (2004) Non-viral delivery of the porphobilinogen deaminase cDNA into a mouse model of acute intermittent porphyria. *Mol Genet Metab.* **82**, 1, p20-6.
25. Wiseman, J.W., C.A. Goddard, D. McLelland, and W.H. Colledge (2003) A comparison of linear and branched polyethylenimine (PEI) with DCChol/DOPE liposomes for gene delivery to epithelial cells in vitro and in vivo. *Gene Ther.* **10**, 19, p1654-62.
26. Ohana, P., *et al.* (2004) Regulatory sequences of the H19 gene in DNA based therapy of bladder cancer. *Gene Ther Mol Biol.* **8**, p181-192.
27. Ohlfest, J.R., P.D. Lobitz, S.G. Perkinson, and D.A. Largaespada (2004) Integration and long-term expression in xenografted human glioblastoma cells using a plasmid-based transposon system. *Mol Ther.* **10**, 2, p260-8.
28. Lavergne, E., C. Combadiere, M. Iga, A. Boissonnas, O. Bonduelle, M. Maho, P. Debre, and B. Combadiere (2004) Intratumoral CC chemokine ligand 5 overexpression delays tumor growth and increases tumor cell infiltration. *J Immunol.* **173**, 6, p3755-62.
29. Nickerson, H.D. and W.H. Colledge (2004) A LacZ-based transgenic mouse for detection of somatic gene repair events in vivo. *Gene Ther.* **11**, 17, p1351-7.

Trouble shooting

Problems	Comments and Suggestions
Too low transfection level	• Optimize the amount of plasmid DNA used in the transfection assay. • Use high-quality plasmid preparation, free of RNA and protein (the OD_{260/280} ratio should be greater than 1.8). • Optimize the <i>in vivo</i>-jetPEI™/DNA ratio starting from 1µl <i>in vivo</i>-jetPEI™/10µg DNA up to 2µl <i>in vivo</i>-jetPEI™/10 µg DNA.
Mortality	• Decrease the amount of plasmid DNA used in the transfection assay (keep the <i>in vivo</i>-jetPEI™/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

Email: support@polyplus-transfection.com

Telephone: + 33 (0)3 90 40 61 87

Related compounds

jetPEI™ for *in vitro* applications