



***in vivo*jetPEI™-FluoF**  
Cationic polymer transfection reagent

## **In vivo Transfection Protocol**

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205-10	0.1 ml		(sufficient for transfection of 1 mg of DNA at N/P=5)
205-10G	0.1 ml	10 ml of 10% glucose*	(sufficient for transfection of 1 mg of DNA at N/P=5)

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**\* 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™-FluoF transfection reagent 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 human. A 10% glucose solution is included with catalog number 205-10G. This solution is more suitable for transfection of highly concentrated DNA.

### **Formulation and Storage**

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

### **Description**

*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* gene and oligonucleotide delivery with low toxicity<sup>1</sup>. It performs better than other cationic polymers and lipids including the branched 25 KDa and 800 KDa PEI isomers available from chemicals manufacturers <sup>2-4</sup>. *In vivo*-jetPEI™ is a special brand developed by the team who

discovered its remarkable properties<sup>5, 6</sup>. PEI is able to mediate efficient gene delivery to various tissues following intravenous<sup>2, 3, 7-11</sup>, intracerebral<sup>12-14</sup> or intraperitoneal injection<sup>15, 16</sup> as well as intratracheal instillation<sup>4, 7</sup>. The most efficient route in mice is systemic injection leading to very high levels of lung transfection<sup>7, 10, 11</sup>. Many other organs are also transfected following *i.v.* injection although at levels lower than those observed in the lungs<sup>10, 11</sup>. Moreover, PEI is an effective vehicle for localized gene delivery in many tissues including the brain<sup>17</sup>. PEI efficiently delivers transgenes after intraventricular or intrathecal injections<sup>13, 18</sup>. Functional protein following transgene expression in neurons has been seen in genomic studies and therapeutic approaches<sup>18-20</sup>. Other delivery routes and target organs are summarized in Table 2.

*In vivo*-jetPEI<sup>TM</sup> condenses DNA into positively charged particles capable of interacting with anionic proteoglycans at the cell surface and entering cells by endocytosis<sup>21</sup>. 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<sup>5, 6</sup>.

### Definition of N/P ratio

Effective cell entry depends on cationic particles. The ionic balance of *in vivo*-jetPEI<sup>TM</sup>-FluoF 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<sup>TM</sup>-FluoF per DNA phosphate. Not every nitrogen atom of PEI being a cation, electroneutrality of *in vivo*-jetPEI<sup>TM</sup>-FluoF/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<sup>TM</sup>-FluoF 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<sup>TM</sup>-FluoF solution to be mixed with DNA in order to obtain a desired N/P ratio can thus be calculated using the following formula (typical conditions are also given in Table 1) :

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

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

Amount of DNA	Volume (µl) of <i>in vivo</i> -jetPEI™-FluoF at	Volume (µl) of <i>in vivo</i> -jetPEI™-FluoF at	Volume (µl) of <i>in vivo</i> -jetPEI™-FluoF at	Volume (µl) of <i>in vivo</i> -jetPEI™-FluoF at	Volume (µl) of <i>in vivo</i> -jetPEI™-FluoF 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™-FluoF/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*-jetPEI™-FluoF and DNA in order to obtain a final concentration of 5% glucose. A 10% glucose solution is provided with catalog number 205-10G.

### 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™-FluoF at N/P = 6 - 10. The following protocol is given for intravenous injection of 400 µl solution containing 50 µg of DNA condensed with *in vivo*-jetPEI™-FluoF at N/P = 10. Refer to table 1 for other DNA amounts and other N/P ratios.

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

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

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™-FluoF reagent into 200 µl of 5% glucose (w/v). Vortex gently and spin down briefly.
- Add the 200 µl *in vivo*-jetPEI™-FluoF 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 24h).
- 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 of 400 µl solution containing 50 µg of DNA condensed with *in vivo*-jetPEI™-FluoF at N/P = 10. Refer to table 1 for other DNA amounts and other N/P ratios.

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

Let the *in vivo*-jetPEI™-FluoF 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) solution provided with catalog number 205-10G 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™-FluoF 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™-FluoF 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 24h).
- 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 in mouse

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

## References

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## Troubleshooting

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

## Technical Assistance

Contact the PolyPlus assistance service via:

Internet address: [www.polyplus-transfection.com](http://www.polyplus-transfection.com)

Email: support@polyplus-transfection.com

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## Related compounds

JetPEI™, jetPEI™-FluoF, jetPEI™-FluoR for *in vitro* applications

*in vivo* jetPEI™-FluoF and *in vivo* jetPEI™-FluoR for *in vivo* applications