

**jetPEI™**

DNA transfection reagent

In vitro DNA Oligonucleotides Transfection Protocol**Product information**

	101-01N	0.1 ml	5 ml NaCl	50 transfections in 24-well plates
jetPEI™ + 150 mM NaCl	101-05N	0.5 ml	50 ml NaCl	250 transfections in 24-well plates
	101-10N	1 ml	50 ml NaCl	500 transfections in 24-well plates
jetPEI™ x4 concentrated	101-30	1ml	-	200 transfections in 24-well plates

Content

1 ml of jetPEI™ transfection reagent (7.5 mM) is sufficient to perform ca. 2000 transfections in 96-well plates (with 0.25 µg of oligonucleotide) or 500 transfections in 24-well plates (with 1 µg of oligonucleotide).

1 ml of jetPEI™ transfection reagent (30 mM) is sufficient to perform ca. 200 transfections in 24-well plates (with 10 µg of oligonucleotide). For research use only.

Formulation and Storage

jetPEI™ is provided as a 7.5 mM or 30 mM solution in sterile and apyrogenic water (expressed as concentration of monomer nitrogen residues).

jetPEI™ is shipped at room temperature and should be stored at 4°C upon arrival.

jetPEI™ is stable for 1 year at 4°C.

Description

Antisense oligonucleotides and ribozymes are selective inhibitors or modulators of gene expression. Their use requires oligonucleotide delivery systems to achieve cellular uptake.

jetPEI™ is an efficient delivery vehicle for oligonucleotides ensuring effective and reproducible oligonucleotides delivery with low toxicity¹. jetPEI™ is a linear polyethylenimine, synthesized and purified by PolyPlus-transfection.

jetPEI™ forms stable complexes with oligonucleotides (ON)^{2,3}. The resulting positively charged particles are able to interact with anionic proteoglycans at the cell surface and to enter cells by endocytosis⁴. jetPEI™ possesses the unique property of acting as a "proton sponge" that buffers the endosomal pH^{2,5} and protects oligonucleotides from degradation³. The continuous proton influx also induces endosome osmotic swelling and rupture which provides an escape mechanism for oligonucleotides to the cytoplasm.

Note : jetPEI™ is able to interact with anionic oligonucleotides but not with uncharged oligonucleotides such as peptide nucleic acid molecules (PNA) or methylphosphonate oligonucleotides.

Definition of N/P ratio

Efficient cell entry requires cationic particles. The ionic balance of jetPEI™ cations and oligonucleotide anions should thus be in favor of the cations ⁶.

The *N/P ratio* is a measure of the ionic balance of the complexes. It refers to the number of nitrogen residues of jetPEI™ per oligonucleotide phosphate. Approximately one in three nitrogen atom of PEI is a cation, therefore electroneutrality of jetPEI™ /oligonucleotide complexes is reached for N/P = 2 - 3. The best transfection results are obtained for N/P = 3 - 5. jetPEI™ concentration is expressed in nitrogen residues molarity and 1 µg of oligonucleotide contains 3 nmoles of anionic phosphate.

The volume of jetPEI™ solution to be mixed with oligonucleotides to obtain a desired N/P ratio is given in Table 1 and calculated using Formula 1:

Formula 1 :

$$\mu\text{l of jetPEI}^{\text{TM}} \text{ to be used} = \frac{(\mu\text{g of oligonucleotide} \times 3) \times \text{N/P ratio}}{\text{jetPEI}^{\text{TM}} \text{ concentration in nitrogen residues (mM)}}$$

To calculate the volume of jetPEI™ solution to be mixed with oligonucleotides (ON) according to the ON size, use formula 2:

Formula 2 :

$$\mu\text{l of jetPEI}^{\text{TM}} \text{ to be used} = \frac{(\text{ON base number}) \times (\text{pmoles of ON}) \times \text{N/P ratio} \times 10^{-3}}{\text{jetPEI}^{\text{TM}} \text{ concentration in nitrogen residues (mM)}}$$

Example: to deliver 150 pmoles of 20-mer ON at N/P = 5, use :

$$\frac{20 \times 150 \times 5 \times 10^{-3}}{7.5} = 2 \mu\text{l of jetPEI}^{\text{TM}} \text{ at } 7.5 \text{ mM}$$

Table 1. Volumes of jetPEI™ and amount of oligonucleotides for various N/P ratios.

jetPEI™ at 7.5 mM nitrogen residues (Catalogue number 101-01N; 101-05N; 101-10N)					
Amount of ONs (20-mer)	Amount of ONs	Volume of jetPEI™ (µl)	Volume of jetPEI™ (µl)	Volume of jetPEI™ (µl)	Volume of jetPEI™ (µl)
		N/P = 3	N/P = 5	N/P = 8	N/P = 10
37 pmoles	0.25 µg	0.3	0.5	0.8	1
75 pmoles	0.5 µg	0.6	1	1.6	2
100 pmoles	0.75 µg	0.9	1.5	2.4	3
150 pmoles	1 µg	1.2	2	3.2	4
300 pmoles	2 µg	2.4	4	6.4	8
600 pmoles	4 µg	4.8	8	12.8	16
900 pmoles	6 µg	7.2	12	19.2	24

jetPEI™ at 30 mM nitrogen residues (Catalogue number ref. 101-30)					
Amount of ONs (40-mer)	Amount of ONs	Volume of jetPEI™ (µl)	Volume of jetPEI™ (µl)	Volume of jetPEI™ (µl)	Volume of jetPEI™ (µl)
		N/P = 3	N/P = 5	N/P = 8	N/P = 10
375 pmoles	5 µg	1.5	2.5	4	5
750 pmoles	10 µg	3	5	8	10
1500 pmoles	20 µg	6	10	16	20
3000 pmoles	40 µg	12	20	32	40

Oligonucleotides Transfection Protocol

1. Reagent not provided

A 150 mM NaCl sterile solution is required to dilute jetPEI™ and oligonucleotides.

2. Cell seeding

For optimal oligonucleotides transfection conditions with jetPEI™, the recommended cell density is 50 to 60% of confluence. The optimal cell density should be determined for each cell type. For transfection in 24-well plates, 50 000 to 100 000 cells are seeded per well, 24 hours before transfection as a starting condition. Refer to table 2 for other culture formats.

Table 2 . Recommended number of cells to seed the day before ON transfection

Culture vessel	Number of adherent cells to seed	Number of suspension cells to seed	Volume of medium per well	Volume of jetPEI™/ON complexes per well (µl)
96-well	10 000 - 17 000	20 000 – 50 000	0.2 ml	20
48-well	25 000 - 50 000	50 000 – 100 000	0.5 ml	50
24-well	50 000 - 100 000	100 000 – 200 000	0.5 ml - 1 ml	100
12-well	80 000 - 200 000	200 000 – 500 000	1 - 2 ml	100 – 200
6-well	200 000 - 400	500 000 – 2 .10 ⁶	2 - 4 ml	200

3. Preparation of complexes (based on jetPEI™ at 7.5 mM)

We recommend using jetPEI™ at N/P = 5 as a starting point. Refer to Table 1 for other N/P ratios. The following protocol is given for transfection in 24-well plate. Refer to Table 2 for transfection in other culture formats.

- Dilute 1 µg of 20-mer oligonucleotide (150 pmoles) into 50 µl of 150 mM NaCl. Vortex gently and spin down briefly.
- Dilute 2 µl of jetPEI™ (7.5 mM) into 48 µl of 150 mM NaCl. Vortex gently and spin down briefly.
- Add the 50 µl jetPEI™ to the 50µl nucleic acids solution all at once (important: do not mix solutions in the reverse order)
- Vortex-mix the solution immediately and spin down lightly and briefly.
- Incubate for 15 minutes at room temperature.

4. Addition of complexes to the cells

4. 1. Serum-containing medium condition

- Add the 100 µl jetPEI™/oligonucleotide mix to the cells in 1 ml of medium supplemented with 10% serum and homogenize by gently swirling the plate.
- Incubate the cells at 37°C in a humidified 5% CO₂ incubator.

The volume of the jetPEI™/oligonucleotide mix usually represents one tenth of the total volume of the culture medium.

- The experiment is usually analysed after 24 to 48 h.

4. 2. Serum-free medium condition

- Add the 100 µl jetPEI™/oligonucleotide mix to each well (containing 1 ml of serum-free medium) and homogenize the mix by gently swirling the plate.
- Incubate the cells at 37°C for 2 - 4 h in a humidified 5% CO₂ incubator.
- Add 100 µl of serum and rehomogenize the mixture
- Prolong incubation of the cells at 37°C for the desired time
- The experiment is usually analysed after 24 to 48 h..

5. Transfection of suspension cells

5. 1. Cell density

Seed with fresh complete medium at the cell density recommended in Table 2 just before transfection.

5. 2. Preparation of the complexes

Refer to the procedure given for adherent cell types (the protocol is given for transfection in 24-well plate).

6. Advantages of jetPEI™

- The performance of jetPEI™ is not affected by the presence of serum or antibiotics. As a result the protocol for jetPEI™ is straightforward.
- The jetPEI™/oligonucleotides complexes can therefore be added directly to complete medium⁸, a significant advantage for sensitive cells.

Improving transfection efficiency

- Transfection efficiencies can be improved by reducing the volume of medium indicated in Table 2 by half or/and by centrifugation of the culture plate (5 min at 280 g at room temperature)⁷.
- For fragile cells, we recommend a medium exchange 2 to 4 hours after transfection

References

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2. 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, 7297-301
3. Dheur S., N. Dias, A. van Aerschot, P. Herdewijn, T. Bettinger, J. S. Remy, C. Helene and E. T. Saison-Behmoaras (1999) Polyethylenimine but not cationic lipid improves antisense activity of 3'-capped phosphodiester oligonucleotides. *Antisense Nucleic Acid Drug Dev* 9, 515-25.
4. Mislick K. A. and J. D. Baldeschwieler (1996) Evidence for the role of proteoglycans in cation-mediated gene transfer. *Proc Natl Acad Sci USA* 93, 12349-12354
5. Behr J. P. (1997) The Proton Sponge - A Trick to Enter Cells the Viruses Did Not Exploit. *CHIMIA* 51, 34-36
6. Dheur S. and T. E. Saison-Behmoaras (2000) Polyethyleneimine-mediated transfection to improve antisense activity of 3'-capped phosphodiester oligonucleotides. *Methods Enzymol* 313, 56-73
7. Boussif O., M. A. Zanta and J. P. Behr (1996) Optimized Galenics Improve in-Vitro Gene-Transfer with Cationic Molecules Up to 1000-Fold. *Gene Therapy* 3, 1074-1080

Troubleshooting

	Comments and Suggestions
Low transfection efficiency	<ul style="list-style-type: none"> • Optimize the amount of oligonucleotide used in the transfection assay. • Ensure that adherent cells are 50-60% confluent the day of transfection. • Optimize the jetPEI™/oligonucleotide ratio. • Decrease the volume of culture medium. • Gently centrifuge the culture plates (if the cells can withstand it), usually 5 min at 280g.
Cellular toxicity	<ul style="list-style-type: none"> • Decrease the amount of ON used in the transfection assay (keeping the jetPEI™/oligonucleotide ratio constant). • Check oligonucleotide concentration and ensure that jetPEI™/oligonucleotide ratio is no more than 2 µl of jetPEI™ for 1 µg of oligonucleotide. • Reduce incubation time of the jetPEI™/oligonucleotide complexes with the cells.

Technical Assistance

Contact the Polyplus technical support via:
The Polyplus website: www.polyplus-transfection.com
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Related product

in vivo-jetPEI™ for *in vivo* applications
jetPEI™-Macrophage for delivery into macrophages cells
jetPEI™-Hepatocyte for delivery into hepatocytes
jetPEI™-RGD for delivery into epithelial and endothelial cells
jetPEI™-FluoF,-FluoR and –Biotin for tracking transfection complexes

NOTES
