



jetPEI™-RGD
Cationic polymer transfection reagent

In vitro Transfection Protocol

104-02	0.2 ml		50 -100 transfections in 24-well plates 25 - 30 transfections in 35 mm plates
104-02N	0.2 ml	50 ml of 150 mM NaCl	50 -100 transfections in 24-well plates 25 - 30 transfections in 35 mm plates
104-05	0.5 ml		125 -250 transfections in 24-well plates 60 - 80 transfections in 35 mm plates
104-05N	0.5 ml	50 ml of 150 mM NaCl	125 -250 transfections in 24-well plates 60 - 80 transfections in 35 mm plates

Content

0.5 ml of jetPEI™-RGD transfection reagent is sufficient to perform ca. 125 to 250 transfections in 24-well plates or 30 to 40 transfections in 60-mm dishes.

Formulation and Storage

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

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

jetPEI™-RGD is stable for 1 year at 4°C.

Description

jetPEI™-RGD is a RGD peptide-conjugated polyethylenimine derivative, synthesized and purified by PolyPlus-transfection. jetPEI™-RGD is designed for selective transfection of epithelial and endothelial cells expressing integrins, including α₅β₁ and most α_v-containing proteins, which bind multiple Arg-Gly-Asp (RGD) peptide sequences¹. jetPEI™-RGD, as regular jetPEI™, is able to condense DNA into compact particles. Specific interactions between jetPEI™/DNA complexes and the cell are obtained by chemically coupling a short synthetic RGD-containing peptides that mimic the integrin's natural ligands^{2,3,4}. The specific interaction triggers internalization of the

complexes by endocytosis. Once inside endosomal vesicles, jetPEI™-RGD expresses the unique property of acting as a "proton sponge" that buffers the endosomal pH and protects DNA from degradation. The continuous proton influx also induces endosome osmotic swelling and rupture which provides an escape mechanism for DNA particles to the cytoplasm^{5,6,7}.

Quality control

Functional analysis: every batch of jetPEI™-RGD is tested by transfection into human epithelial-derived cell line, HeLa cells. Typically, transfection in the presence of serum and with a firefly luciferase gene (under the control of the CMV promoter) gives 10⁹ RLU (relative light unit)/ mg of protein (10⁹ RLU correspond to ca. 100 ng of luciferase protein).

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 jetPEI™-RGD per DNA phosphate. Approximately one in three nitrogen atoms of PEI is a cation, therefore electroneutrality of jetPEI™-RGD/DNA complexes is reached for N/P = 2 - 3. In practice, the best transfection of hepatocytes is obtained for N/P = 5-10. jetPEI™-RGD is supplied as a 7.5 mM solution (expressed in nitrogen residues) and 1 µg of DNA contains 3 nmoles of anionic phosphate.

The amount of jetPEI™-RGD solution to be mixed with DNA in order to obtain the desired N/P ratio is given in table 1 and can be calculated using the following formula :

$$\mu\text{l of jetPEI}^{\text{TM}}\text{-RGD to be used} = \frac{(\mu\text{g of DNA} \times 3) \times \text{N/P ratio}}{7.5}$$

Transfection Protocols

Reagent required

A 150mM NaCl sterile solution is required to dilute jetPEI™-RGD and DNA. This solution is provided with references N°104-02N and 104-05N.

Cell seeding

For optimal transfection conditions with jetPEI™-RGD, the cells should be 50-60% confluent. Typically, for transfection in 24-well plates, 50 000 to 100 000 cells are seeded per well. For other culture formats, refer to table 2 for the recommended number of cells to seed the day before transfection.

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

Amount of DNA	Vol (µl) of jetPEI™-RGD at	Vol (µl) of jetPEI™-RGD at	Vol (µl) of jetPEI™-RGD at	Vol (µl) of jetPEI™-RGD at
	N/P = 3	N/P = 5	N/P = 8	N/P = 10
1 µg	1.2	2	3.2	4
2 µg	2.4	4	6.4	8
4 µg	4.8	8	12.8	16
6 µg	7.2	12	19.2	24
8 µg	9.6	16	25.6	32
10 µg	12	20	32	40

Table 2 . Recommended number of cells to seed before transfection

Culture vessel	Number of adherent cells to seed	Surface area per well or plate (cm ²)	Volume of medium per well or plate
96-well	10 000 - 17 000	0.3	0.2 ml
48-well	25 000 - 50 000	1	0.5 ml
24-well	50 000 - 100 000	1.9	1 ml
12-well	80 000 - 200 000	3.8	2 ml
6-well	200 000 - 400 000	9.4	4 ml
35 mm	200 000 - 400 000	9.4	4 ml
60 mm	400 000 - 600 000	28	8 ml

Preparation of the complexes

We recommend using jetPEI™-RGD at N/P = 5. Refer to table 1 for other N/P ratios. The following protocol is given for 24-well plate transfections, refer to table 3 for transfection in other culture formats.

- Dilute 1 µg of DNA into 50µl of 150mM NaCl (provided with references N°104-02N and 104-05N). Vortex gently and spin down briefly.
 - Dilute 2 µl of jetPEI™-RGD solution into 50 µl of 150mM NaCl. Vortex gently and spin down briefly.
 - Add the 50 µl jetPEI™-RGD solution to the 50 µl DNA solution all at once (important: do not mix solutions in the reverse order)
 - Vortex-mix the solution immediately and spin down briefly.
 - Incubate for 15 to 30 minutes at room temperature.
 - Add the 100µl jetPEI™-RGD/DNA mixture to each well and homogenize the mixture by gently swirling the plate.
- Generally, the volume of the jetPEI™-RGD/DNA mixture represents one tenth of the total volume of culture medium.
- Transfection experiments are usually stopped after 24 hours and reporter gene activity assessed.

Factors affecting transfection efficiency

- In contrast to other transfection reagents, jetPEI™-RGD is not affected by the presence of serum during transfection. Therefore, the jetPEI™-RGD/DNA complexes can be added directly to the serum-containing medium ¹.
- Usually, transfection efficiencies can be improved by using smaller volumes of medium (half the quantity indicated in table 2) or/and by centrifugation of the culture plate (5 min at 280g at room temperature) ⁸.
- For especially fragile cells, the transfection complexes can be removed after a 2-4 hour incubation period. In this case, aspirate the medium containing complexes and replace it with fresh serum-containing medium.

Table 3. Complexes preparation for different cell culture formats

Culture vessel	Amount of DNA (µg)	Volume of the NaCl dilution solution (µl)	Volume of jetPEI™-RGD reagent (µl)	Volume of jetPEI™-RGD dilution solution (µl)	Total volume of complexes per well
96-well	0.25	10	0.5	10	20
48-well	0.5	25	1	25	50
24-well	1	50	2	50	100
12-well	2	50	4	50	100
6-well	3	100	6	100	200
35 mm	3	100	6	100	200
60 mm	5	250	10	250	500

Stable transfection

For stable transfection, perform transfection in 6-well plates or 60 mm plates according to the above protocol.

Start selection with appropriate antibiotic 24 – 48 h after transfection.

References

1. Erbacher P., J. S. Remy and J. P. Behr (1999) Gene transfer with synthetic virus-like particles via the integrin- mediated endocytosis pathway. *Gene Ther* **6**, 138-45
2. Pierschbacher M. D. and E. Ruoslahti (1984) Cell attachment activity of fibronectin can be duplicated by small fragments of the molecule. *Nature* **309**, 30-33
3. Akiyama S. K. and K. M. Yamada (1985) Synthetic peptides competitively inhibit both direct binding to fibroblasts and functional biological assays for the purified cell-binding domain of fibronectin. *J. Biol. Chem.* **260**, 10402-10405
4. Ruoslahti E. and M. D. Pierschbacher (1987) New perspectives in cell adhesion: RGD and integrins. *Science* **238**, 491-7.
5. 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
6. Behr J. (1996) L'éponge à protons: un moyen d'entrer dans une cellule auquel les virus n'ont pas pensé. *Medecine&Science* **12**, 56-58
7. Behr J. P. (1997) The Proton Sponge - A Trick to Enter Cells the Viruses Did Not Exploit. *CHIMIA* **51**, 34-36
8. 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

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

Troubleshooting

NOTES

Problems	Comments and Suggestions
Low transfection efficiency	<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).• Ensure that adherent cells are 50-60% confluent the day of transfection.• Optimize the jetPEI™-RGD/DNA ratio starting from 1µl jetPEI™-RGD/µg DNA up to 4µl jetPEI™-RGD/µg DNA.• Perform a positive control transfection experiment with a well-characterized reporter gene (Luciferase or β-Gal from commercially available plasmid).• 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 plasmid DNA used in the transfection assay (keeping the jetPEI™-RGD/DNA ratio constant).• Check DNA concentration and ensure that jetPEI™-RGD/DNA ratio is no more than 2µl of jetPEI™-RGD for 1 µg of DNA.• Reduce the incubation time of the complexes jetPEI™-RGD/DNA with the cells.• If the expressed protein is toxic for the cells, reduce the amount of plasmid DNA used in the transfection assay.• Make sure that the plasmid preparation is endotoxin-free.