
jetPEI™-RGD

in vitro DNA Transfection Protocol

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Company Information

Technical Assistance and Scientific Advice

Contact the friendly Polyplus technical support *via*:

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Intellectual Property

The use of polyethylenimine (PEI) or polypropylenimine (PPI) or cationic polymers similar in structure thereto for transfecting cells, as well as compositions comprising these cationic polymers and at least one nucleic acid, are the subject matter of U.S. Patent No. 6,013,240, EP Patent No. 0770140 and foreign equivalents, for which Polyplus-transfection™ is the worldwide exclusive licensee.

Trademarks

jetPEI and Polyplus-transfection are registered trademarks of Polyplus-transfection.

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Product Information

jetPEI™-RGD is a RGD peptide-conjugated linear polyethylenimine. It enhances efficient DNA transfection of integrin-expressing epithelial and endothelial cells. Integrins are adhesion molecules involved in cell-cell and cell-matrix adhesion processes. Many integrins, including alpha5β1 and most alphaV-containing proteins, bind multiple Arg-Gly-Asp (RGD) peptide sequences present in serum and extracellular matrix proteins. Publications using Polyplus reagents can be found on the Product Citation Database. In addition a Cell Transfection Database gives transfection conditions over 400 cell lines and primary cells. Both database are available on the Polyplus website, www.polyplus-transfection.com under the Products/Technical Resources heading.

Ordering information

Cat #	jetPEI™-RGD Reagent	150 mM NaCl solution	Number of transfections
104-01N	0.1 ml	5 ml	50 transfections in 24-well plates
104-05N	0.5 ml	50 ml	250 transfections in 24-well plates

Content

0.5 ml of jetPEI™-RGD DNA transfection reagent is sufficient to perform ca. 250 transfections in 24-well plates or 80 transfections in 60-mm dishes.

Reagent use and Limitations

For research use only. Not for use in humans.

Quality control

Every batch of jetPEI™-RGD is tested in a transfection assay. Typically, transfection of a firefly luciferase gene under the control of the CMV promoter gives 10⁹ RLU (relative light unit)/mg of protein. The value for each batch is indicated on the Certificate of Analysis.

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 but should be stored at 4°C upon arrival to ensure long term stability. jetPEI™-Macrophage, as guaranteed by the Certificate of Analysis, will be valid for at least one year when stored appropriately.

Mechanism of transfection using jetPEI™-RGD

jetPEI™-RGD compacts DNA into positively charged particles - called complexes - capable of interacting with anionic proteoglycans at the cell surface. 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. The specific interaction triggers internalization of the complexes by endocytosis. Once inside the endosomal compartment, the DNA is protected from degradation by jetPEI™-RGD. This is in part due to the unique property of this polymer to act as a "proton sponge", which in turn buffers the pH within the endosome. This mechanism ultimately leads to rupture of the endosome and release of the DNA and the complexes into the cytoplasm, thereby allowing nuclear transport for subsequent transcription.

The overall charge of the jetPEI™-RGD/DNA complexes is therefore crucial for efficient transfection. It is determined by the DNA to reagent ratio. This ratio, which represents the ionic balance within the complexes, is classically defined as the N/P ratio, referring to the number of nitrogen residues (N) in the jetPEI™-RGD per phosphate (P) of DNA. To obtain positively charged complexes, an N/P>3 is required. In order to calculate the N/P ratio, use the following formula taking into account the volume of jetPEI™-RGD reagent used for a given amount of DNA.

$$\text{N/P ratio} = \frac{7.5^* \times \mu\text{l of jetPEI}^{\text{TM}}\text{-RGD}}{3^{\diamond} \times \mu\text{g of DNA}}$$

* concentration of nitrogen residues in jetPEI™-RGD
 ◊ nmoles of phosphate per μg of DNA

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1. Transfection protocol

1. 1. 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 the day before transfection. (Refer to Table 1 for other culture formats).

Table 1. Recommended number of cells to seed the day before transfection

Culture vessel	Number of adherent cells to seed	Surface area per well or plate (cm ²)	Volume of medium per well
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/35 mm	200 000 - 400 000	9.4	4 ml
60 mm	400 000 - 600 000	28	8 ml

1.2. Preparation of the complexes and transfection procedure

The following protocol is given for 24-well plate transfections. (Refer to Table 2 for transfection in other formats).

1. Dilute 1 µg of DNA into 50µl of 150 mM NaCl (provided with 104-01N and 104-05N). Vortex gently and spin down briefly.
2. Dilute 2 µl of jetPEI™-RGD into 50 µl of 150 mM NaCl. Vortex gently and spin down briefly.
3. Add the 50 µl jetPEI™-RGD to the 50 µl DNA solution at once (important: do not mix solutions in the reverse order)
4. Vortex-mix the solution immediately and spin down briefly.
5. Incubate for 15 to 30 minutes at room temperature.
6. Add the 100 µl jetPEI™-RGD/DNA mix to each well and homogenize by gently swirling the plate.
7. The volume of the jetPEI™-RGD/DNA mix usually represents one tenth of the total volume of culture medium.

8. Transfection experiments are usually analysed after 24 hours and reporter gene activity assessed.

2. Advantages of jetPEI™-RGD

The performance of jetPEI™-RGD is not affected by the presence of serum or antibiotics. As a result the protocol for jetPEI™-RGD is straightforward.

The jetPEI™-RGD/DNA complexes can therefore be added directly to the complete medium, a significant advantage for sensitive cells.

3. 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 280g at room temperature).

For fragile cells, we recommend a medium exchange 2 to 4 hours after transfection.

Table 2. Preparation of the complexes for different cell culture formats

Culture vessel	Amount of DNA (µg)	Volume of 150 mM NaCl to dilute DNA (µl)	Volume of jetPEI™-RGD (µl)	Volume of 150 mM NaCl to dilute jetPEI™-RGD (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/ 35 mm	3	100	6	100	200
60 mm	5	250	10	250	500

4. 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.

Troubleshooting

Observations	Actions
Low transfection efficiency	Optimize the amount of plasmid DNA used in the transfection assay.
	Use high-quality plasmid preparation, free of RNA (the OD260/280 ratio should be greater than 1.8).
	Ensure that adherent cells are 50-60% confluent on the day of transfection.
	Optimize the jetPEI™- RGD/DNA ratio starting from 1 µl 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-Galactosidase expressed from commercially available plasmid).
	Decrease the volume of culture medium.
Cellular toxicity	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 not higher than 4 µl of jetPEI™-RGD per 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.
	Ensure that the plasmid preparation is endotoxin-free

Notes