jetPEI™-HUVEC
in vitro DNA Transfection Protocol

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Company Information

Technical Assistance and Scientific Advice

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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™-HUVEC is a powerful transfection reagent optimized for the transfection of primary endothelial cells such as HUVEC (Human umbilical vein endothelial cells). DNA Transfection efficiencies up to 50% have been obtained with this reagent. jetPEI™-HUVEC is recommended for the transfection of vascular endothelial cells of various origins and is well-suited for such fragile primary cells. Publications using Polyplus transfection 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 databases are available on the Polyplus website, www.polyplus-transfection.com under the Products/Technical Resources heading.

Ordering information

Cat #	jetPEI™-HUVEC Reagent	150 mM NaCl solution	Number of transfections
108-01N	0.1 ml	5 ml	25 transfections in 24-well plates
108-05N	0.5 ml	50 ml	125 transfections in 24–well plates

Content

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

Reagent use and Limitations

For research use only. Not for use in humans.

Quality control

Every batch of jetPEI™-HUVEC is tested in a transfection assay. Typically, transfection of a firefly luciferase gene under the control of the CMV promoter gives 10^9 RLU (relative light unit)/mg of protein. The value for each batch is indicated on the Certificate of Analysis.

Formulation and Storage

jetPEI™-HUVEC is provided as a 7.5 mM solution in sterile and apyrogenic water (expressed as concentration of nitrogen residues).
jetPEI™-HUVEC is shipped at room temperature but should be stored at 4°C upon arrival to ensure long term stability. jetPEI™-HUVEC, as guaranteed by the Certificate of Analysis, will be valid for at least one year when stored appropriately.

Mechanism of transfection using jetPEI™-HUVEC

jetPEI™-HUVEC compacts DNA into positively charged particles - called complexes - capable of interacting with anionic proteoglycans at the cell surface. Upon binding to the cell membrane, the complexes are internalized *via* endocytosis. Once inside the endosomal compartment, the DNA is protected from degradation by jetPEI™-HUVEC. 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™-HUVEC/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™-HUVEC 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™-HUVEC reagent used for a given amount of DNA.

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

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

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1.Transient Transfection Protocol for adherent HUVEC

1. 1. Cell seeding

In order to achieve optimal transfection efficiency with jetPEI™-HUVEC, the cells should be 50-60% confluent at the time of transfection. For this purpose plate out 35 000 cells per well in a 24-well plate coated with fibronectin (10 µg/ml) the day before transfection. The cell should preferably be seeded in complete medium containing 30% human serum and antibiotics. Before transfection, wash the cells with PBS and add 500 µl of DMEM-Glutamax medium containing 2% human serum. Alternatively use the media of your choice. 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	0.3	0.2 ml
48-well	20 000	1	0.5 ml
24-well	35 000	1.9	0.5 ml - 1 ml
12-well	70 000	3.8	1 ml - 2 ml
6-well/ 35 mm	150 000	9.4	2 ml - 4 ml
6 cm/flask 25 cm^2	300 000	28	5 ml - 10 ml
10 cm/flask 75 cm^2	800 000	78.5	10 ml – 20 ml
14 cm/flask 153 cm^2	1.6×10^6	153	20 ml – 40 ml

1.2. Preparation of the complexes and transfection procedure

We recommend using jetPEI™-HUVEC at N/P = 5 or N/P = 10. These ratios are equivalent to 2 µl of jetPEI™-HUVEC per µg of DNA or 4 µl of jetPEI™-HUVEC per µg of DNA respectively.

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

1. Dilute 2 µg of DNA into 50 µl of 150 mM NaCl provided with catalogue numbers 108-01N and 108-05N. Vortex gently and spin down briefly.

2. Dilute 4 or 8 µl of jetPEI™-HUVEC into 50 µl of 150 mM NaCl. Vortex gently and spin down briefly.
3. Add the 50 µl containing the jetPEI™-HUVEC to the 50 µl DNA solution at once (important: do not mix the solutions in the reverse order).
4. Vortex-mix the solution immediately and spin down briefly to bring drops to the bottom of the tube.
5. Incubate for 30 minutes at room temperature.
6. Add the 100 µl jetPEI™-HUVEC/DNA mix drop-wise onto the well and homogenize by gently swirling the plate.
7. Incubate at 37°C and 5% CO_2 in a humidified atmosphere for 4 hours.
8. Remove the transfection medium and replace with HUVEC growth medium containing 30% FBS and antibiotics.
9. Analyse the experiment after 24 hours or as required.

Table 2. Amount of DNA and volumes of reagent to be used for the preparation of complexes at N/P=5 and N/P=10 in different cell culture formats

Culture vessel	Amount of DNA (µg)	Volume of NaCl to dilute DNA (µl)	Volume of jetPEI™-HUVEC (µl)		Volume of NaCl to dilute jetPEI™-HUVEC (µl)	Total volume of complexes added per well (µl)
			N/P=5	N/P=10		
96-well	0.4	10	0.8	1.6	10	20
48-well	1	25	2	4	25	50
24-well	2	50	4	8	50	100
12-well	4	50	8	16	50	100
6-well / 35 mm	6	100	12	24	100	200
6 cm	8	250	16	32	250	500
10 cm	14	500	28	56	500	1000
14 cm	20	1000	40	80	1000	2000

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™- HUVEC/DNA ratio starting from 1µl jetPEI™-HUVEC/µg DNA up to 4µl jetPEI™-HUVEC/µg DNA.
	Perform a positive control transfection experiment with a well-characterized reporter gene (Luciferase or ß-Galactosidase expressed from commercially available plasmid).
	Decrease the volume of culture medium.
	Gently centrifuge the cell culture plates for 5 min at 180g if the cells can withstand it.
Cellular toxicity	Decrease the amount of plasmid DNA used in the transfection assay (keeping the jetPEI™-HUVEC/DNA ratio constant).
	Check DNA concentration and ensure that jetPEI™-HUVEC/DNA ratio is not higher than 4 µl of jetPEI™-HUVEC per 1 µg of DNA.
	Reduce the incubation time of the complexes jetPEI™-HUVEC/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