



**jetPEI™-HUVEC**  
Cationic polymer transfection reagent

**In vitro Transfection Protocol**

108-05	0.5 ml		(125 transfections in 24-well plates)
108-05N	0.5 ml	50 ml of 150 mM NaCl	(125 transfections in 24-well plates)

**Content**

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

**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 and should be store at 4°C upon arrival.

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

**Description**

jetPEI™-HUVEC is a derivative of the powerful jetPEI™<sup>1</sup>. jetPEI™-HUVEC has been developed and optimized for the transfection of primary human endothelial cells such as HUVEC. Transfection efficiencies up to 50% have been reached with this reagent<sup>2</sup>. jetPEI™-HUVEC is also recommended for the transfection of vascular endothelial cells of various origins and appears to be adapted for such fragile primary cells.

**Quality control**

Functional analysis: every batch of jetPEI™-HUVEC is tested by transfection into cells.

**Definition of N/P ratio**

The *N/P ratio* refers to the number of nitrogen residues of jetPEI™-HUVEC per DNA phosphate. jetPEI™-HUVEC is supplied as a 7.5 mM solution (expressed in nitrogen residues) and 1 µg of DNA contains 3 nmoles of anionic phosphate. For the transfection of HUVEC cells, we recommend to use N/P = 5.

However, the amount of jetPEI™-HUVEC solution to be mixed with DNA in order to obtain another N/P ratio can be calculated using the following formula:

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

**Transfection Protocols**

**1. Transient transfection of adherent HUVEC cells**

**1.1. Reagent required**

A 150 mM NaCl sterile solution is required to dilute jetPEI™-HUVEC and DNA. This solution is provided with references N° 108-05N.

**1.2. Cell culture**

Human Umbilical Vein Endothelial Cells (HUVECs) are cultured in a mixture of M199 containing 25 mM HEPES and RPMI 1640 medium (v/v), supplemented with 30% of heat inactivated human serum and 0.1% gentamicin. Cells are grown in flasks coated with 10 µg/ml of fibronectin and maintained at 37°C in a 5% CO<sub>2</sub> humidified atmosphere.

**1.3. Cell seeding**

For optimal transfection conditions with jetPEI™-HUVEC, the cells should be 50-60% confluent. For transfection in 24-well plates, 35 000 cells are seeded per well, 24 hours before transfection in a fibronectin-coated 24 multi-well tissue culture plate in 1 ml of fresh complete medium containing 30% of human serum and antibiotics. On the day of

transfection, cells are rinsed with PBS and 500 µl per well of DMEM-Glutamax medium containing 2% of human serum is added. For other culture formats, refer to table 1 for the recommended number of cells to seed the day before transfection.

**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 <sup>2</sup> )	Volume of medium per well or plate
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 <sup>2</sup>	300 000	28	5 ml - 10 ml
10 cm/flask 75 cm <sup>2</sup>	800 000	78.5	10 ml – 20 ml
14 cm/flask 153 cm <sup>2</sup>	1.6 x 10 <sup>6</sup>	153	20 ml – 40 ml

#### 1.4. Preparation of the complexes and transfection procedure

We recommend using jetPEI™-HUVEC at N/P = 5 or N/P = 10 (2 µl of jetPEI™-HUVEC for 1 µg of DNA or 4 µl of jetPEI™-HUVEC for 1 µg of DNA). The following protocol is given for transfection in 24-well plates, refer to table 2 for transfection in other culture formats.

- For each well, dilute 2 µg of DNA into 50 µl of 150 mM NaCl (provided with references N° 108-05N). Vortex gently and spin down briefly.
- For each well, dilute 4 or 8 µl of jetPEI™-HUVEC solution into 50 µl of 150 mM NaCl. Vortex gently and spin down briefly.
- Add the 50 µl jetPEI™-HUVEC solution to the 50 µl DNA solution all at once (**important: do not mix the solutions in the reverse order**)

- Vortex-mix the solution immediately and spin down briefly to bring drops to the bottom of the tube.
- Incubate for 30 minutes at room temperature.
- Add the 100 µl jetPEI™-HUVEC/DNA mixture drop-wise onto the transfection medium in each well and homogenize the mixture by gently swirling the plate.
- Incubate at 37°C and 5% CO<sub>2</sub> in a humidified atmosphere for 4 hours.
- Remove the transfection medium and replace with HUVEC growth medium containing 30% FBS plus antibiotics for the next 24 hours.

**Table 2.** Complexes preparation for different cell culture formats at N/P of 5 or 10.

Culture vessel	Amount of DNA (µg)	Volume of the NaCl dilution solution (µl)	Volume of jetPEI™-HUVEC reagent (µl)	Volume of jetPEI™-HUVEC dilution solution (µl)	Total volume of complexes per well (µl)
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

Problems	Comments and Suggestions
Low transfection efficiency	<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>• Ensure that adherent cells are 50-60% confluent the day of transfection.</li> <li>• Optimize the jetPEI™-HUVEC/DNA ratio starting from 2 µl jetPEI™-HUVEC/µg DNA up to 4 µl jetPEI™-HUVEC/µg DNA.</li> <li>• Perform a positive control transfection experiment with a well-characterized reporter gene (Luciferase or β-Gal from commercially available plasmid).</li> <li>• Decrease the culture medium volume.</li> <li>• Gently centrifuge the culture plates (if the cells can withstand it), usually 5 min at 280g.</li> </ul>
Cellular toxicity	<ul style="list-style-type: none"> <li>• Decrease the amount of plasmid DNA used in the transfection assay (keeping the jetPEI™-HUVEC/DNA ratio constant).</li> <li>• Reduce the incubation time of the complexes jetPEI™-HUVEC/DNA with the cells.</li> <li>• Verify the toxicity of the expressed protein. If the expressed protein is toxic for the cells, reduce the amount of plasmid DNA used in the transfection assay.</li> <li>• Make sure that the plasmid preparation is endotoxins-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](mailto:support@polyplus-transfection.com)

Telephone: + 33 (0) 3 90 40 61 87

## Related compounds

jetPEI™, for *in vitro* applications

*In vivo*-jetPEI™ for *in vivo* applications

## References

1. 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
2. Zaric V., Weltin D., Erbacher P., Remy J-S., Behr J-P. and Stephan D. (2004) Effective polyethylenimine-mediated gene transfer into human endothelial cells. *The Journal of Gene Medicine* **6**, 176-184

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