

jetPEI®-HUVEC

in vitro DNA transfection Protocol

DESCRIPTION

jetPEI®-HUVEC is a powerful transfection reagent optimized for transfection of primary endothelial cells such as HUVEC (Human umbilical vein endothelial cells). DNA transfection efficiencies up to 70 % have been obtained with this reagent. jetPEI®-HUVEC is recommended for 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 in the Polyplus-transfection® Database available on our website, www.polyplus-transfection.com

1. TRANSIENT TRANSFECTION OF 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 35 000 cells per well in a 24-well plate coated with fibronectin (10 µg/ml) the day before transfection. The cells should preferably be seeded in supplemented endothelial cell growth medium. Before transfection, wash the cells with PBS and add 500 µl of basal medium (without supplements). Please refer to Table 1 to seed cells in 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 (ml)
96-well	10 000	0.3	0.2
48-well	20 000	1	0.5
24-well	35 000	1.9	0.5 - 1
12-well	70 000	3.8	1 - 2
6-well / 35 mm	150 000	9.4	2 - 4
60 mm / flask 25 cm ²	300 000	28	5 - 10
10 cm / flask 75 cm ²	800 000	78.5	10 - 20
14 cm / flask 153 cm ²	1.6 x 10 ⁶	153	20 - 40

1.2 PREPARATION OF 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 1 µg of DNA or 4 µl of jetPEI®-HUVEC per 1 µg of DNA respectively.

The following protocol contains conditions to use 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 the reagent. Vortex gently and spin down briefly.
2. Vortex jetPEI®-HUVEC reagent for 5 sec and spin down before use.
3. Dilute 4 or 8 µl of jetPEI®-HUVEC into 50 µl of 150 mM NaCl. Vortex gently and spin down briefly.
4. 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).
5. Vortex the solution immediately and spin down briefly to bring drops to the bottom of the tube.
6. Incubate for 30 minutes at room temperature.
7. Remove the cell growth medium. Per well, add 0.5 – 1 mL of medium without supplements.
8. Add the 100 µl jetPEI®-HUVEC/DNA mix drop-wise onto the well and homogenize by gently swirling the plate.
9. Incubate at 37°C and 5 % CO₂ in a humidified atmosphere for 2-4 hours.
10. Remove the transfection medium and replace with supplemented endothelial cell growth medium.
11. Analyze the results 24 hours after transfection or as required.

Table 2. Recommended conditions to use for preparation of complexes in various 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
			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

2. TROUBLESHOOTING

Observations	Action (Contact us for tips and advices: support@polyplus-transfection.com)
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 on the day of transfection. ✦ Optimize the jetPEI®-HUVEC/DNA ratio starting from 1 µl jetPEI®-HUVEC/µg DNA up to 4 µl jetPEI®-HUVEC/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. ✦ Gently centrifuge the cell culture plates for 5 min at 180 g if the cells can withstand it.
Cellular toxicity	<ul style="list-style-type: none"> ✦ 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

3. PRODUCT INFORMATION

3.1 ORDERING INFORMATION

Ref #	jetPEI®-HUVEC Reagent	150 mM NaCl solution	Number of transfections
108-05N	0.5 ml	50 ml	125 transfections in 24-well plates

3.2 CONTENT

0.5 ml of jetPEI®-HUVEC DNA transfection reagent is sufficient to perform ca. 125 transfections in 24-well plates or 30 transfections in 60-mm dishes.

3.3 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.

3.4 REAGENT USE AND LIMITATIONS

For research use only. Not for use in humans.

3.5 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 10⁹ RLU (relative light unit)/mg of protein. The value for each batch is indicated on the Certificate of Analysis.

TRADEMARKS

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

How to cite us: "jetPEI®-HUVEC (Polyplus-transfection S.A, Illkirch, France)

TECHNICAL ASSISTANCE AND SCIENTIFIC ADVICE

Contact the friendly Polyplus technical support *via*:

- The Polyplus website: www.polyplus-transfection.com
- Email: support@polyplus-transfection.com
- Phone: +33 3 90 40 61 87