jetPEI®-Macrophage

in vitro DNA transfection reagent

PROTOCOL

DESCRIPTION

jetPEI®-Macrophage allows DNA transfection of macrophages and macrophage-like cells. It contains a mannose-conjugated linear polyethylenimine that enhances binding to cells expressing mannose receptors, such as macrophages. jetPEI®-Macrophage is able to condense DNA into compact particles similarly to jetPEI®. Publications using jetPEI®-Macrophage can be found on the Polyplus-transfection Database. The Polyplus-transfection Database gives transfection conditions over 400 cell lines and primary cells. This Database is available online at www.polyplus-transfection.com

1 TRANSFECTION OF PRIMARY MACROPHAGES

1.1 CELL CULTURE AND CELL SEEDING

For optimal transfection with jetPEI®-Macrophage, primary macrophages derived from blood monocytes should be cultured in the presence of GM-CSF or M-CSF (100 to 500 units/ml) for 5 to 10 days before transfection in order to express the mannose receptors at the cell surface.

For optimal transfection conditions with jetPEI®-Macrophage, cells should be 50-60% confluent. Typically for transfection in 24-well plates, 100 000 cells are seeded per well 5 to 7 days before transfection. (Refer to Table 1 for other culture formats).

Table 1. Recommended number of cells to seed

Culture vessel	Number of adherent cells to seed	Surface area per well (cm^2)	Volume of medium per well (ml)
24-well	100 000	1.9	1
12-well	200 000	3.8	2
6-well / 35 mm	400 000	9.4	4
60 mm / flask 25 cm^2	600 000	28	8

1.2 TRANSFECTION PROCEDURE FOR PRIMARY CELLS

The following conditions are given per well of a 24-well plate. For other culture format, please refer to Table 2.

1. Dilute 0.5 µg of DNA into 50 µl of 150 mM NaCl. Vortex gently and spin down briefly.
2. Dilute 1 µl of jetPEI®-Macrophage into 50 µl of 150 mM NaCl. Vortex gently and spin down briefly.
3. Add the 50 µl jetPEI®-Macrophage solution to the 50 µl DNA solution at once (Avoid the reverse order).
4. Vortex-mix the solution immediately and spin down briefly.
5. Incubate for 15 to 30 minutes at room temperature.
6. While complexes are forming, remove the growth medium from the cells. Add 1 ml of cell growth medium (with serum and antibiotics if needed).
7. Add the 100 µl jetPEI®-Macrophage/DNA complexes to each well and homogenize by gently swirling the plate.
8. Transfection experiments are usually analyzed after 24 hours and reporter gene activity is measured.

Table 2. DNA transfection guidelines according to the cell culture vessel per well

Culture Vessel	Amount of DNA (µg)	Volume of NaCl to dilute DNA (µl)	Volume of jetPEI®-Macrophage (µl)	Volume of NaCl to dilute jetPEI®-Macrophage (µl)	Total volume of complexes added per well
24-well	0.5	50	1	50	100
12-well	1	50	2	50	100
6-well / 35 mm	1.5	100	3	100	200
60 mm / flask 25 cm^2	2.5	250	5	250	500

2 TRANSFECTION OF ESTABLISHED CELL LINES

2.1 CELL CULTURE AND CELL SEEDING

For established cell lines such as RAW 264.7, maturation with GM-CSF or M-CSF is not required.

For optimal transfection conditions with jetPEI®-Macrophage, cells should be 50 to 60% confluent at the time of transfection. In order to transfect semi-adherent cell lines in 24-well plates, seed 50 000 to 100 000 cells per well the day before transfection. (Refer to Table 1 for other culture formats).

2.2 TRANSFECTION PROCEDURE FOR ESTABLISHED CELL LINES (e.g. RAW 264.7)

The following protocol is given for a 24-well plate using 2 µg of DNA per well (Refer to Table 3 for other culture formats).

1. Dilute 2 µg of DNA into 50 µl of 150 mM NaCl. Vortex gently and spin down briefly.
2. Dilute 6.4 µl of jetPEI®-Macrophage into 50 µl of 150 mM NaCl. Vortex gently and spin down briefly.
3. Add the 50 µl jetPEI®-Macrophage solution to the 50 µl DNA solution at once (Avoid 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®-Macrophage/DNA complexes to each well and homogenize by gently swirling the plate.
7. Transfection experiments are usually analyzed after 24 hours and reporter gene activity is measured.

Table 3. DNA transfection guidelines according to the cell culture vessel

Culture Vessel	Amount of DNA (µg)	Volume of NaCl to dilute DNA (µl)	Volume of jetPEI®-Macrophage (µl)	Volume of NaCl to dilute jetPEI®-Macrophage (µl)	Total volume of complexes added per well
96-well	0.5	10	1.6	10	20
24-well	2	50	6.4	50	100
12-well	4	50	12.8	50	100
6-well / 35 mm	6	100	19.2	100	200

3 ADVANTAGES OF JETPEI®-MACROPHAGE

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

The jetPEI®-Macrophage/DNA complexes can therefore be added directly to complete medium, a significant advantage for sensitive cells such as primary macrophages.

4 IMPROVING TRANSFECTION EFFICIENCY

Transfection efficiencies can be improved by reducing the volume of medium indicated in Table 1 by half or/and by centrifugation of the culture plate (5 min at 180 g at room temperature).

For fragile cells, you may replace medium 2 to 4 hours after transfection.

5 TROUBLESHOOTING

Observations	Actions
Low DNA transfection efficiency	Optimize the amount of DNA used in the transfection assay.
	Use high-quality plasmid preparation, free of RNA ($OD_{260/280}$ > 1.8).
	Ensure the cells are NOT in OptiMEM® during transfection.
	Ensure that adherent cells are 50-60% confluent on the day of transfection.
	Optimize the jetPEI®-Macrophage/DNA ratio starting from 1 µl jetPEI®-Macrophage/µg DNA up to 4 µl jetPEI®-Macrophage/µg DNA.
	Perform a positive control transfection experiment with a well-characterized reporter gene (Luciferase or β-Galactosidase expressed from commercially available plasmid).
	Perform the transfection in culture medium without supplements; 2 to 4 hours later, replace the transfection medium with fresh growth medium.
	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	Decrease the amount of plasmid DNA used in the transfection assay (keeping the jetPEI®-Macrophage/DNA ratio constant).
	Check DNA concentration and ensure that jetPEI®-Macrophage/DNA ratio is not higher than 3.2 µl of jetPEI®-Macrophage per µg of DNA.
	Reduce the incubation time of the complexes jetPEI®-Macrophage/DNA with the cells.
	Continuously use complete medium with M-CSF or GM-CSF during the entire experiment (before and after the transfection).
	Ensure that the plasmid preparation is endotoxin-free.
	Verify the toxicity of the expressed protein. If the expressed protein is toxic for the cells, reduce the amount of plasmid DNA.

Contact the friendly Polyplus technical support *via*:

- The Polyplus website: www.polyplus-transfection.com
- Email: support@polyplus-transfection.com
- Phone: + 33 (0)3 90 40 61 87

6 PRODUCT INFORMATION

6.1 ORDERING INFORMATION

Ref. N°	jetPEI®-Macrophage Reagent	NaCl 150 mM solution
103-05N	0. 5 ml	50 ml

6.2 CONTENT

0.5 ml of jetPEI®-Macrophage DNA transfection reagent is sufficient to perform ca. 500 transfections in 24-well plates or 160 transfections in 60 mm dishes.

6.3 REAGENT USE AND LIMITATIONS

For research use only. Not for use in humans.

6.4 QUALITY CONTROL

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

6.5 FORMULATION AND STORAGE

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

jetPEI®-Macrophage 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.

Polyplus-transfection® has been awarded ISO 9001 Quality Management System Certification since 2002, which ensures that the company has established reliable and effective processes for manufacturing, quality control, distribution and customer support.

How to cite us: “jetPEI®-Macrophage (Polyplus-transfection S.A, Illkirch, France)”