

jetPEI™-Macrophage *in vitro* DNA Transfection Protocol

Company Information.....	2
Product Information.....	3
Contact our Technical Assistance and Scientific Advice Service	4
1. Transfection of primary macrophages	5
1. 1. Cell culture and cell seeding	5
1. 2. Preparation of the complexes and transfection procedure for primary cells	5
2. Transfection of established cell lines (e.g. RAW 264.7)	6
2. 1. Cell culture and cell seeding	6
2. 3. Preparation of the complexes and transfection procedure for established cell lines (e.g. RAW 264.7)	6
3. Advantages of jetPEI™-Macrophage	7
4. Improving transfection efficiency	7
Troubleshooting	8

Company Information

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 (0)3 90 40 61 87

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.

POLYPLUS-TRANSFECTION SA

Bioparc
Boulevard Sébastien Brant
BP 90018
67401 ILLKIRCH cedex
FRANCE
Phone: +33 3 90 40 61 80
Fax: + 33 3 90 40 61 81
info@polyplus-transfection.com
www.polyplus-transfection.com

POLYPLUS-TRANSFECTION Inc.

1251 Ave of the Americas, 34th fl.
NEW YORK, NY 10020
USA
Phone: +1 508 315 9629

Product Information

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 also the reagent of choice for the transfection of cell lines such as RAW 264.7. jetPEI™-Macrophage is able to condense DNA into compact particles similarly to jetPEI™. Publications using Polyplus-transfection reagents can be found on the Product Citation Database. In addition an extensive 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™-Macrophage Reagent	150 mM NaCl solution	Number of transfections
103-01N	0.1 ml	5 ml	100 transfections in 24-well plates
103-05N	0.5 ml	50 ml	500 transfections in 24-well plates

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.

Reagent use and Limitations

For research use only. Not for use in humans.

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

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.

Mechanism of transfection using jetPEI™-Macrophage

jetPEI™-Macrophage compacts DNA into positively charged particles - called complexes - capable of interacting with anionic proteoglycans and mannose receptors 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™-Macrophage. 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™-Macrophage/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™-Macrophage 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™-Macrophage reagent used for a given amount of DNA.

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

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

Note : jetPEI™-Macrophage was formerly named jetPEI™-Man.

Contact our Technical Assistance and Scientific Advice Service

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

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 (100 to 500 units/ml) for 7 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 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 (ml)
24-well	100 000	1.9	1 ml
12-well	200 000	3.8	2 ml
6-well/35 mm	400 000	9.4	4 ml
60 mm	600 000	28	8 ml

1. 2. Preparation of the complexes and transfection procedure for primary cells

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

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. Add the 100 µl jetPEI™ -Macrophage/DNA complexes to each well and homogenize by gently swirling the plate.
7. Generally, the volume of the jetPEI™-Macrophage/DNA mixture represents 1/10 of the total volume of the culture medium.
8. Transfection experiments are usually analyzed after 24 hours and reporter gene activity is measured.

Table 2. Amounts of DNA and volumes to be used for the preparation of the complexes for primary macrophages in different cell culture formats

Culture vessel	Amount of DNA (µg)	Volume of the 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	2.5	250	5	250	500

2. Transfection of established cell lines (e.g. RAW 264.7)

2. 1. Cell culture and cell seeding

For established cell lines such as RAW 264.7, maturation with GM-CSF is not required. For optimal transfection conditions with jetPEI™-Macrophage, cells should be 50-60% confluent. 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 2 for other culture formats).

2. 3. Preparation of the complexes and 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 provided with catalogue numbers 103-01N and 103-05N. 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 (important: do not mix the solution 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™-Macrophage/DNA mix to each well and homogenize by gently swirling the plate.
7. Generally, the volume of the jetPEI™-Macrophage/DNA mix represents 1/10 of the total volume of the culture medium.
8. Transfection experiments are usually analyzed after 24 hours and reporter gene activity is measured.

Table 3. Amounts of DNA and volumes to be used for the preparation of complexes for established cell lines in different cell culture formats

Culture vessel	Amount of DNA (µg)	Volume of the 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 hepatocytes.

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.

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™- Macrophage/DNA ratio starting from 1µl jetPEI™-Macrophage/µg DNA up to 4µl 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).
	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™-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 1 µg of DNA.
	Reduce the incubation time of the complexes jetPEI™-Macrophage /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