

jetPEI™-Hepatocyte *in vitro* DNA Transfection Protocol

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Company Information

Technical Assistance and Scientific Advice

Contact the friendly Polyplus technical support *via*:
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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™-Hepatocyte is a galactose-conjugated linear polyethylenimine derivative, manufactured by Polyplus-transfection. jetPEI™-Hepatocyte has been specifically designed to increase transfection of cells bearing galactose-specific membrane lectins, such as the asialoglycoprotein receptor (ASGP-R or Gal/GalNAc receptor). jetPEI™-Hepatocyte is able to condense DNA into compact particles similarly to jetPEI™. Publications using jetPEI™-Hepatocyte can be searched on the Product Citation Database. The 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™-Hepatocyte Reagent	150 mM NaCl solution	Number of transfections
102-01N	0.1 ml	5 ml	30 transfections in 24-well plates
102-05N	0.5 ml	50 ml	160 transfections in 24-well plates

Content

0.5 ml of jetPEI™-Hepatocyte DNA transfection reagent is sufficient to perform ca. 160 transfections in 24-well plates or 30 transfections in 60-mm dishes.

Reagent use and Limitations

For research use only. Not for use in humans.

Quality control

Every batch of jetPEI™-Hepatocyte 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.

Formulation and Storage

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

jetPEI™-Hepatocyte is shipped at room temperature but should be stored at 4°C upon arrival to ensure long term stability. jetPEI™-Hepatocyte, as guaranteed by the Certificate of Analysis, will be valid for at least one year when stored appropriately.

Mechanism of transfection using jetPEI™-Hepatocyte

jetPEI™-Hepatocyte 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™-Hepatocyte. 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™-Hepatocyte/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™-Hepatocyte 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™-Hepatocyte reagent used for a given amount of DNA.

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

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

Note : jetPEI™-Hepatocyte was formerly named jetPEI™-Gal.

1. DNA transfection of adherent cells

1.1 Cell seeding

For optimal transfection conditions with jetPEI™-Hepatocyte, the cells should be at 50-60% confluency. Typically, for transfection in 24-well plates, 50 000 hepatocytes are seeded per well one day before transfection. For primary hepatocytes, we recommend seeding 100 000 cells per well in 24-well plate two days before transfection and change the culture medium every day. (Refer to Table 1 for other culture formats).

Table 1. Recommended number of cells to seed one or two days before transfection

Culture vessel	Number of hepatocyte cells to seed one day before	Number of primary hepatocytes to seed two days before	Surface area per well (cm ²)	Volume of medium per well (ml)
96-well	10 000	20 000	0.3	0.2
48-well	25 000	50 000	1	0.5
24-well	50 000	100 000	1.9	1
12-well	80 000	200 000	3.8	2
6-well / 3.5 cm	200 000	400 000	9.4	4
6 cm / flask 25 cm ²	400 000	600 000	28	8

1.2 Preparation of the complexes and transfection procedure

A 150 mM NaCl sterile solution is required to dilute jetPEI™-Hepatocyte and DNA. This solution is provided with jetPEI™-Hepatocyte catalogue number 102-01N and 102-05N.

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

1. Dilute 1 µg of DNA into 50 µl of 150 mM NaCl provided with 102-01N and 102-05N. Vortex gently and spin down briefly.
2. Dilute 3.2 µl of jetPEI™-Hepatocyte into 50 µl of 150 mM NaCl. Vortex gently and spin down briefly.
3. Add the 50 µl jetPEI™-Hepatocyte solution to the 50 µl DNA at once (Avoid reverse order)

4. Mix the solution immediately by vortexing and centrifuge briefly.
5. Incubate for 15 to 30 minutes at room temperature.
6. Add the 100 µl jetPEI™-Hepatocyte/DNA complexes to each well and homogenize by gently swirling the plate.
7. Generally, the volume of the jetPEI™-Hepatocyte/DNA mix represents one tenth of the total volume of culture medium.
8. Transfection experiments are usually analysed after 24 hours and reporter gene activity is measured.

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

Culture vessel	Amount of DNA (µg)	Volume of 150 mM NaCl to dilute DNA (µl)	Volume of jetPEI™-Hepatocyte (µl)	Volume of 150 mM NaCl to dilute jetPEI™-Hepatocyte (µl)	Total volume of complexes added per well
96-well	0.25	10	0.8	10	20
48-well	0.5	25	1.6	25	50
24-well	1	50	3.2	50	100
12-well	2	50	6.4	50	100
6-well / 3.5 cm	3	100	9.6	100	200
6 cm / flask 25 cm ²	5	250	16	250	500

2. Advantages of jetPEI™-Hepatocyte

The performance of jetPEI™-Hepatocyte is not affected by the presence of serum or antibiotics. As a result the protocol for jetPEI™-Hepatocyte is straightforward as no medium changes are required. The jetPEI™-Hepatocyte/DNA complexes can therefore be added directly to the complete medium, a significant advantage for sensitive cells such as primary hepatocytes.

3. Improving transfection efficiency

Transfection efficiencies can be improved by reducing the volume of medium indicated in Table 2 by half and/or by centrifugation of the culture plate (5 min at 180g at room temperature). For fragile cells, you may replace medium 2 to 4 hours after transfection.

4. Stable transfection

For stable transfection, perform transfection in 6-well plates or 60 mm plates according to the above protocol. Start selection with the appropriate antibiotic 24 to 48 h after transfection.

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Troubleshooting

Observations	Actions
Low silencing 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™-Hepatocyte/DNA ratio by adding more reagent.
	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.
Cellular toxicity	Decrease the amount of plasmid DNA used in the transfection assay keeping the jetPEI™-Hepatocyte/DNA ratio constant.
	Check DNA concentration and ensure that jetPEI™-Hepatocyte/DNA ratio is not higher than 3.2 µl of jetPEI™-Hepatocyte per 1 µg of DNA.
	Reduce the incubation time of the complexes jetPEI™-Hepatocyte/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.