



jetPEI™-Hepatocyte
Cationic polymer transfection reagent

In vitro Transfection Protocol

102-01	0.1 ml		30 transfections in 24-well plates
102-05	0.5 ml		160 transfections in 24-well plates
102-05N	0.5 ml	50 ml of 150 mM NaCl	160 transfections in 24-well plates

Content

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

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 and should be store at 4°C upon arrival.

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

Description

jetPEI™-Hepatocyte is a galactose-conjugated linear polyethylenimine derivative, synthesized and purified by Polyplus-transfection. jetPEI™-Hepatocyte has been especially designed to transfect cells bearing galactose-specific membrane lectins, such as hepatocytes expressing the asialoglycoprotein receptor (ASGP-R or Gal/GalNAc receptor). jetPEI™-Hepatocyte is able to condense DNA into compact particles similarly to jetPEI™. Chemical coupling of jetPEI™ to a galactose ligand permit pecific interactions between jetPEI™/DNA complexes and a galactose receptor recognized on the cells ^{1,2}. This interaction triggers internalization of the complexes by endocytosis.

Once package into endosomal vesicles, jetPEI™-Hepatocyte expresses the unique property of acting as a "proton sponge" that buffers the endosomal pH and protects DNA from degradation. The continuous proton influx induces endosome osmotic swelling leading to rupture which provides an escape mechanism for DNA particles to the cytoplasm ^{3,4,5}.

Quality control

Functional analysis: every batch of jetPEI™-Hepatocyte is tested by transfection into HeLa cells. Typically, transfection in the presence of serum and with a firefly luciferase gene (under the control of the CMV promoter) gives 10⁹ RLU (relative light unit)/ mg of protein.

Definition of N/P ratio

The *N/P ratio* is a measure of the ionic balance of the complexes. It refers to the number of nitrogen residues of jetPEI™-Hepatocyte per number of phosphate in the DNA. Approximately one in three nitrogen atoms of PEI is cationic, therefore electroneutrality of jetPEI™-Hepatocyte/DNA complexes is reached at N/P = 2 - 3. In practice, best results for transfection of hepatocytes are obtained at N/P = 8. jetPEI™-Hepatocyte is supplied as a 7.5 mM solution (expressed in nitrogen residues). Note that 1 µg of DNA contains 3 nmoles of anionic phosphate.

The amount of jetPEI™-Hepatocyte solution to be mixed with DNA in order to obtain the desired N/P ratio is given in table 1 and is calculated using the following formula :

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

Transfection Protocols

Reagent required

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

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 prior to transfection. For primary hepatocytes, we recommend seeding 100 000 cells per well in 24-well plate two days before transfection and change culture medium every day. For other culture formats, refer to table 2 for the recommended number of cells to seed the day before transfection.

Table 1 . Volumes of jetPEI™-Hepatocyte and amount of DNA required for various N/P ratios.

Amount of DNA (µg)	Vol (µl) of jetPEI™ - Hepatocyte for	Vol (µl) of jetPEI™ - Hepatocyte for	Vol (µl) of jetPEI™ - Hepatocyte for	Vol (µl) of jetPEI™ - Hepatocyte for
	N/P = 3	N/P = 5	N/P = 8	N/P = 10
1 µg	1.2	2	3.2	4
2 µg	2.4	4	6.4	8
4 µg	4.8	8	12.8	16
6 µg	7.2	12	19.2	24
8 µg	9.6	16	25.6	32
10 µg	12	20	32	40

Table 2 . Recommended number of cells to seed before transfection

Culture vessel	Number of hepatocyte cell lines to seed one day before	Number of primary hepatocytes to seed two days before	Surface area per well (cm²)	Volume of medium per well (ml)
96-well	10 000	17 000	0.3	0.2 ml
48-well	25 000	50 000	1	0.5 ml
24-well	50 000	100 000	1.9	1 ml
12-well	80 000	200 000	3.8	2 ml
6-well / 35 mm	200 000	400 000	9.4	4 ml
60 mm	400 000	600 000	28	8 ml

Preparation of complexes

We recommend using jetPEI™-Hepatocyte at N/P = 8. Refer to table 1 for other N/P ratios. The following protocol is given for transfection in a 24-well plate, refer to table 3 for transfection in other culture formats.

- Dilute 1 µg of DNA into 50 µl of 150 mM NaCl (provided with reference N° 102-05N). Vortex gently and spin down briefly.
 - Dilute 3.2 µl of jetPEI™-Hepatocyte solution into 50 µl of 150 mM NaCl. Vortex gently and spin down briefly.
 - Add the 50 µl jetPEI™-Hepatocyte solution to the 50 µl DNA at once (important: do not mix solutions in the reverse order)
 - Vortex-mix the solution immediately and spin down briefly.
 - Incubate for 15 to 30 minutes at room temperature.
 - Add the 100 µl jetPEI™-Hepatocyte/DNA mixture to each well and homogenize the mixture by gently swirling the plate.
- Generally, the volume of the jetPEI™-Hepatocyte/DNA mixture represents one tenth of the total volume of culture medium.
- Transfection experiments are usually stopped after 24 hours and reporter gene activity assessed.

Factors affecting transfection efficiency

- In contrast to other transfection reagents, jetPEI™-Hepatocyte is not affected by the presence of serum during transfection. Therefore, the jetPEI™-Hepatocyte/DNA complexes can be added directly to the complete medium ¹.
- Usually, transfection efficiencies can be improved by using smaller volumes of medium (half the quantity indicated in table 2) or/and by centrifugation of the culture plate (5 min at 280g at room temperature) ⁶.
- For especially fragile cells, the transfection complexes can be removed after a 2-4 hour incubation period. In this case, aspirate the medium containing the complexes and replace it with fresh serum-containing medium.

Table 3: Complexes preparation for different cell culture formats

Culture vessel	Amount of DNA (µg)	Final volume of 150 mM NaCl to dilute the DNA (µl)	Volume of jetPEI™-Hepatocyte reagent (µl)	Final volume of 150 mM NaCl to dilute the jetPEI™-Hepatocyte (µl)	Total volume of complexes 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 / 35 mm	3	100	9.6	100	200
60 mm	5	250	16	250	500

Stable transfection

For stable transfection, perform transfection in 6-well plates or 60 mm plates according to the above protocol.

Start selection with appropriate antibiotic 24 – 48 h after transfection.

References

1. Zanta, M. A., Boussif, O., Adib, A. & Behr, J. P. In-Vitro Gene Delivery to Hepatocytes with Galactosylated Polyethylenimine. *Bioconj. Chem.* **8**, 839-844 (1997).
2. Bettinger, T., Remy, J. S. & Erbacher, P. Size reduction of galactosylated PEI/DNA complexes improves lectin- mediated gene transfer into hepatocytes. *Bioconjug Chem* **10**, 558-61 (1999).
3. Boussif, O. et al. 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 (1995).
4. Behr, J. L'éponge à protons: un moyen d'entrer dans une cellule auquel les virus n'ont pas pensé. *Medecine&Science* **12**, 56-58 (1996).
5. Behr, J. P. The Proton Sponge - A Trick to Enter Cells the Viruses Did Not Exploit. *CHIMIA* **51**, 34-36 (1997).
6. Boussif, O., Zanta, M. A. & Behr, J. P. Optimized Galenics Improve in-Vitro Gene-Transfer with Cationic Molecules Up to 1000-Fold. *Gene Therapy* **3**, 1074-1080 (1996).

Troubleshooting

Problems	Comments and Suggestions
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™-Hepatocyte/DNA ratio starting from 1µl jetPEI™-Hepatocyte/µg DNA up to 4µl jetPEI™-Hepatocyte/µg DNA. • Perform a positive control transfection experiment with a well-characterized reporter gene (Luciferase or β-Gal from commercially available plasmid). • Decrease the volume of culture medium. • Gently centrifuge the culture plates (if the cells can withstand it), usually 5 min at 280g.
Cellular toxicity	<ul style="list-style-type: none"> • 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. • Make sure that the plasmid preparation is endotoxin-free.

NOTES**Technical Assistance**

Contact the Polyplus technical support service via:

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Related compounds

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