jetPEI®-Hepatocyte
in vitro DNA transfection reagent
PROTOCOL

DESCRIPTION

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 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 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 / 35 mm	200 000	400 000	9.4	4
60 mm / flask 25 cm^2	400 000	600 000	28	8

1.2 PREPARATION OF THE COMPLEXES AND TRANSFECTION PROCEDURE

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

1. Dilute 1 µg of DNA into 50 µl of 150 mM NaCl (provided). Vortex gently and spin down briefly.
2. Vortex jetPEI®-Hepatocyte reagent for 5 sec and spin down before use.
3. Dilute 3.2 µl of jetPEI®-Hepatocyte into 50 µl of 150 mM NaCl. Vortex gently and spin down briefly.
4. Add the 50 µl jetPEI®-Hepatocyte solution to the 50 µl DNA at once (Avoid reverse order).
5. Mix the solution immediately by vortexing and centrifuge briefly.
6. Incubate for 15 to 30 minutes at room temperature.
7. Add the 100 µl jetPEI®-Hepatocyte/DNA complexes to each well and homogenize by gently swirling the plate.
8. Transfection experiments are usually analysed 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 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 / 35 mm	3	100	9.6	100	200
60 mm / flask 25 cm^2	5	250	16	250	500

2 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.

3 TROUBLESHOOTING

Observations	Actions
Low transfection efficiency	Optimize the amount of plasmid DNA used in the transfection assay
	Ensure that adherent cells are 50-60% confluent on the day of transfection.
	Optimize the jetPEI®-Hepatocyte/DNA ratio by adding more reagent.
	Use a plasmid containing a common reporter gene such as Luciferase or GFP as positive control.
	Decrease the volume of culture medium.
	Perform the transfection in culture medium without supplements; 4 hours later, replace the transfection medium with fresh growth medium.
	Gently centrifuge the cell culture plates for 5 min at 180g if the cells can withstand it.
	Use high-quality plasmid preparation, free of proteins and RNA ($OD_{260/280}$ > 1.8).
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.

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

4 PRODUCT INFORMATION

4.1 ORDERING INFORMATION

Ref. N°	jetPEI®-Hepatocyte Reagent	150 mM NaCl Solution	Number of transfections
102-05N	0.5 ml	50 ml	150 transfection in 24-well plates

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

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

4.2 ADDITIONAL BUFFER

jetPEI®-Hepatocyte reagent is provided with a 150 mM NaCl solution. This solution must be used to ensure successful transfection experiments.

4.3 CONTENT

0.5 ml of jetPEI®-Hepatocyte DNA transfection reagent is sufficient to perform ca. 150 transfections in 24-well plates or 30 transfections in 60-mm dishes.

4.4 REAGENT USE AND LIMITATIONS

For research use only. Not for use in humans.

4.5 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 10^9 RLU (relative light unit)/mg of protein. The value for each batch is indicated on the Certificate of Analysis.

4.6 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.

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.