jetPEI® *in vitro* DNA Transfection Protocol

DESCRIPTION

jetPEI® is a powerful reagent that ensures robust, effective and reproducible DNA transfection into mammalian cells with low toxicity. jetPEI® is mainly composed of a linear polyethylenimine manufactured at Polyplus-transfection. This reagent has been shown to provide superior *in vitro* transfection when compared to other cationic lipids and polymers. jetPEI® is particularly recommended for High Throughput Screening (HTS) as it guarantees run to run and batch to batch reproducibility.

Over 500 publications using jetPEI® can be found in Polyplus-transfection Database. In addition this Database, available on the Polyplus website, www.polyplus-transfection.com, gives transfection conditions for over 400 cell lines and primary cells.

1. MECHANISM OF TRANSFECTION USING JETPEI®

jetPEI® compacts DNA into positively charged particles - called complexes - capable of interacting with anionic proteoglycans on 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®. 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®/DNA complexes is therefore crucial for efficient transfection. It is determined by the reagent to DNA 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® per phosphate (P) of DNA. To obtain positively charged complexes, a N/P>3 is required. In order to calculate the N/P ratio, use the following formula taking into account the volume of jetPEI® reagent used for a given amount of DNA.

$$\text{N/P ratio} = \frac{7.5^{*} \times \mu\text{l of jetPEI}^{®}}{3^{\lozenge} \times \mu\text{g of DNA}}$$

* concentration of nitrogen residues in jetPEI®

◊ nmoles of phosphate per µg of DNA

2. TRANSIENT TRANSFECTION PROTOCOL FOR ADHERENT CELLS (FORWARD)

2.1 CELL CULTURE AND CELL SEEDING

In this protocol, the cells are seeded the day before transfection and the complexes are added subsequently to the cells in serum-containing medium. This standard protocol is referred to as forward protocol and is recommended for routine experiments.

For optimal transfection conditions with jetPEI® we recommend using cells 50-70% confluent on the day of transfection. Typically, for transfection in 24-well plates, 50 000 to 100 000 cells are seeded per well 24 hours prior to transfection. Change medium the next morning before performing the experiment and add 1 ml of medium per well. jetPEI® is stable in the presence of serum therefore you may use serum containing medium during the entire experiment. For other culture formats, refer to Table 1 for the recommended number of cells to seed the day before transfection.

Table 1. Recommended number of cells to seed the day before transfection.

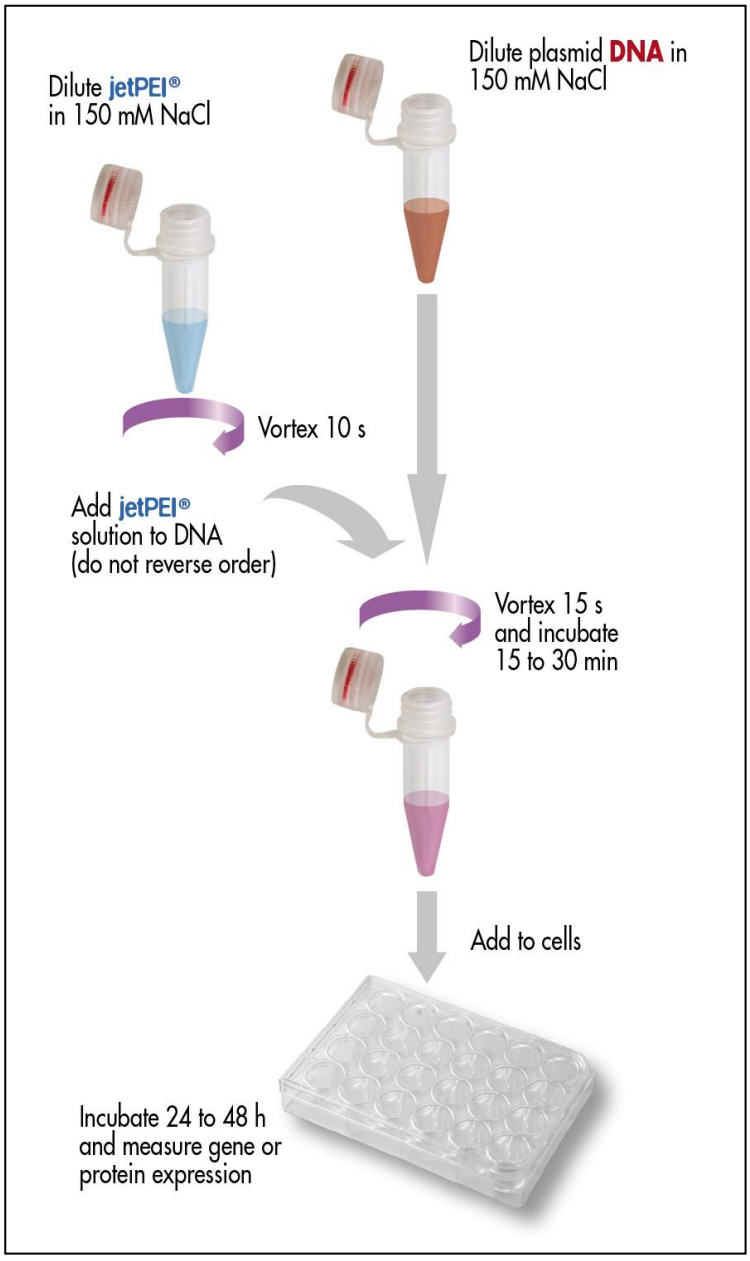
Culture vessel	Number of adherent cells to seed	Surface area per well (cm^2)	Volume of medium per well or plate (ml)
384-well	5 000 - 10 000	0.075	0.05 - 0.1
96-well	10 000 - 17 000	0.3	0.1 - 0.2
48-well	25 000 - 50 000	1	0.25 - 0.5
24-well	50 000 - 100 000	1.9	0.5 - 1
12-well	80 000 - 200 000	3.8	1 - 2
6-well/35 mm	200 000 - 400 000	9.4	2 - 4
6 cm/flask 25 cm^2	400 000 - 800 000	28	5 - 10
10 cm/flask 75 cm^2	2 000 000 - 4 000 000	78.5	10 - 15
14 cm/flask 175 cm^2	4×10^6 - 8×10^6	153 - 175	20 -30

2.2 PREPARATION OF THE COMPLEXES AND TRANSFECTION

The following protocol is a standard protocol for transfection in a 24-well plate; refer to Table 2 for transfection in other culture formats.

The optimal transfection conditions for majority of adherent cell lines are given in the forward protocol described below. Check our extensive online Cell transfection database for optimized conditions for various cell lines (http://www.polyplus-transfection.com/resources/cell-transfection-database/).

FORWARD TRANSFECTION



Transfection procedure in a 24-well plate:

1. Per well, dilute 1 µg of DNA in 150 mM NaCl to a final volume of 50 µl. Vortex gently and spin down briefly.
2. Per well, dilute 2 µl of jetPEI® reagent in 150 mM NaCl to a final volume of 50 µl. Vortex gently and spin down briefly.
3. Add the 50 µl jetPEI® solution **to** the 50 µl DNA solution all at once. Please note that mixing the solutions in the reverse order may reduce transfection efficiency.
4. Vortex the solution immediately and spin down briefly.
5. Incubate for 15 to 30 minutes at room temperature.
6. Per well, add the 100 µl jetPEI®/DNA mix drop-wise to the cells in 1 ml of serum-containing medium and homogenize by gently swirling the plate.
7. Return the plates to the cell culture incubator.
8. Perform reporter gene assay 24 to 48 h after transfection.

We recommend using a ratio of 2 µl of jetPEI® per µg of DNA as a starting condition, however the amount of jetPEI® may be adjusted from 1 to 4 µl per µg of DNA depending on the cell line to be transfected.

When using other plate sizes, adjust the amounts and volumes according to Table 2.

Table 2. Complex preparation for transfection in different cell culture formats.

Culture vessel	Amount of DNA (µg)	Volume of jetPEI® reagent (µl)	Volume of NaCl solution for both DNA and jetPEI® (µl)	Total volume of complexes added per well (µl)
384-well	0.1	0.2	5	10
96-well	0.25	0.5	10	20
48-well	0.5	1	25	50
24-well	1	2	50	100
12-well	2	4	50	100
6-well/35 mm	3	6	100	200
6 cm/flask 25 cm^2	5	10	250	500
10 cm/flask 75 cm^2	10 - 20	20 - 40	250	500
14 cm/flask 175 cm^2	20 - 30	40 - 60	500	1000

3. TRANSIENT TRANSFECTION PROTOCOL FOR CELLS GROWN IN SUSPENSION (FORWARD)

3.1 CELL SEEDING

For optimal transfection conditions with jetPEI®, seed the appropriate number of suspension cells according to the culture vessel used (Table 3) and perform transfection right away.

Table 3. Recommended number of cells, amount of DNA and jetPEI® volume for transfection of cells grown in suspension.

Culture vessel	Number of cells in suspension to seed	Volume of medium containing the cells (ml)	Amount of DNA (µg)	Volume of jetPEI® (µl)	Volume of NaCl solution for both DNA and jetPEI® (µl)	Total volume of complexes added per well (µl)
96-well	2×10^4 - 5×10^4	0.2	0.2 - 0.4	0.4 - 0.8	10	20
48-well	5×10^4 - 10^5	0.5	0.5 - 1	1 - 2	25	50
24-well	10^5 - 2×10^5	0.5 - 1	1 - 2	2 - 4	50	100
12-well	2×10^5 - 5×10^5	1 - 2	2 - 4	4 - 8	50	100
6-well/35 mm	5×10^5 - 1×10^6	2 - 4	6 - 12	12 - 24	100	200
6 cm/flask 25 cm2	1×10^6 - 2×10^6	5 - 10	10 - 20	20 - 40	250	500
10 cm/flask 75 cm2	3×10^6 - 6×10^6	10 - 15	30 - 60	60 - 120	500	1000

We recommend using the lower amounts of DNA as starting conditions and a ratio of 2 µl of jetPEI® per µg of DNA, however the amount of jetPEI® may be adjusted from 1 to 4 µl per µg of DNA depending on the cell line to be transfected.

3.2 PREPARATION OF THE COMPLEXES AND TRANSFECTION

The optimal conditions of transfection for most cell lines in suspension are given below. For other cell lines, check the online Cell Transfection database.

Transfection procedure in a 24-well plate:

1. Per well, dilute 2 µg of DNA in 150 mM NaCl to a final volume of 50 µl. Vortex gently and spin down briefly.
2. Per well, dilute 4 µl of jetPEI® in 150 mM NaCl to a final volume of 50 µl. Vortex gently and spin down briefly.
3. Add the 50 µl jetPEI® solution **to** the 50 µl DNA solution all at once. Please note that mixing the solutions in the reverse order may reduce transfection efficiency.
4. Vortex the solution immediately and spin down briefly.
5. Incubate for 15 to 30 minutes at room temperature.
6. Add the 100 µl jetPEI®/DNA mixture drop-wise onto the cells in 1 ml of serum-containing medium, homogenize the mixture by gently swirling the plate.
7. Return the plates to the cell culture incubator.
8. Perform reporter gene assay 24 to 48 h following transfection.

4. REVERSE TRANSFECTION PROTOCOL FOR HTS

The following protocol has been developed for reproducible and efficient reverse transfection in 96-well plates for high throughput screening (HTS). The reverse transfection protocol is time-saving compared to the forward protocol.

For reverse transfection in 384-well plates, please refer to Tables 4 and 5. For forward protocol in HTS, refer to Section 2.

Table 4. Recommended conditions for reverse transfection relative to the cell culture vessel (per well).

Culture vessel	Amount of DNA (µg)	Volume of jetPEI® (µl)	Volume of NaCl solution for both DNA and jetPEI® (µl)	Number of cells
384-well	0.1	0.3 - 0.4	5	2 500 - 5 000
96-well	0.2	0.6 - 0.8	25	10 000 - 20 000

Briefly, a large volume of complexes is prepared by mixing the DNA with jetPEI® transfection reagent. The complexes are then distributed into 96-well plates and the cells are added afterwards.

As starting conditions, we recommend testing two ratios of jetPEI®/DNA:

- 3 µl of jetPEI® per 1 µg of DNA

- 4 µl of jetPEI® per 1 µg of DNA

4.1 COATING OF THE PLATES

In order to facilitate adhesion of the complexes to the plate, we recommend pre-coating the wells with fibronectin (2-5 µg/cm^2). For this purpose, prepare a solution of fibronectin at 20 µg/ml, add to wells to cover the bottom. Leave for 30 min, aspirate and leave to dry.

4.2 PREPARATION OF THE COMPLEXES

1. Dilute 0.6 µl jetPEI® in 150 mM NaCl to a final volume of 25 µl. Vortex briefly.
2. Dilute 200 ng of DNA in 150 mM NaCl to a final volume of 25 µl. Vortex briefly.
3. Add the 25 µl of jetPEI® reagent **to** the 25 µl of DNA all at once.

   Note: mixing the solutions in the reverse order may reduce transfection efficiency.

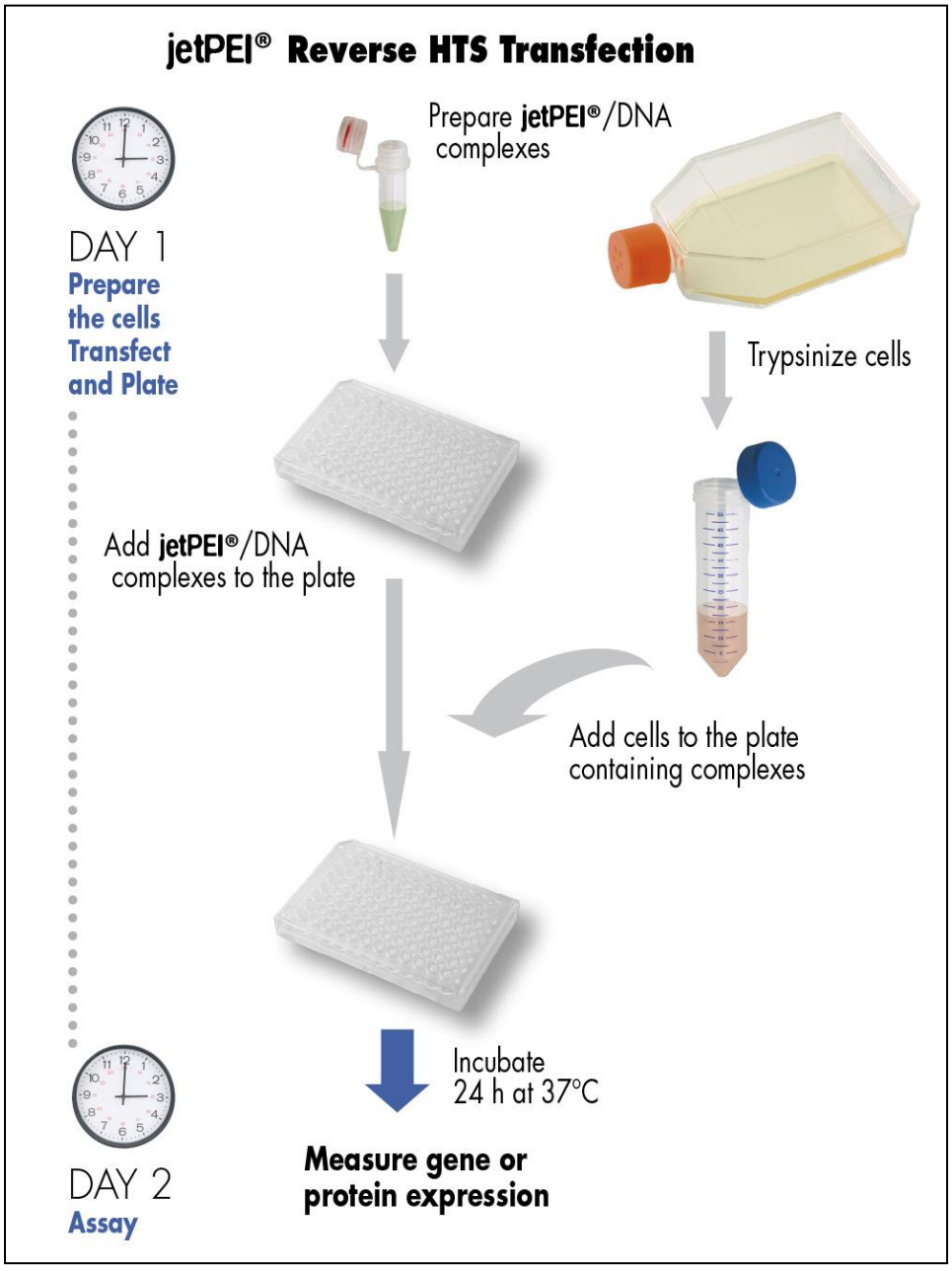
4. Vortex the solution immediately.
5. Incubate for 15 minutes at room temperature.
6. Add 50 µl jetPEI®/DNA complexes per well in the pre-coated plate.

4.3 PREPARATION OF THE CELLS AND TRANSFECTION

1. Trypsinize the cells to be transfected as usual.
2. Wash once with serum-containing medium.
3. Prepare a cell suspension at 50 000 to 100 000 cells/ml in culture medium containing serum.
4. Distribute 200 µl of the cell suspension per well in order to obtain 10 000 - 20 000 cells per well.
5. Return the plates to the cell culture incubator.
6. Perform reporter gene assay 24 to 48 h following transfection.

Table 5. Recommended number of cells for different plate formats.

Culture format	Number of cells added per well	Volume of cells per well (µl)	Minimal volume of cells per plate
384-well	2 500 – 5 000	50	20 ml (50 000 - 100 000 cells/ml)
96-well	10 000 – 20 000	200	20 ml (50 000 - 100 000 cells/ml)


jetPEI® Reverse HTS Transfection
DAY 1
Prepare the cells Transfect and Plate
Prepare jetPEI®/DNA complexes
Trypsinize cells
Add jetPEI®/DNA complexes to the plate
Add cells to the plate containing complexes
Incubate 24 h at 37°C
DAY 2
Assay
Measure gene or protein expression

5. BATCH PROTOCOL (TRYPSINIZATION AND TRANSFECTION ON THE SAME DAY)

This protocol is optimized to carry out splitting and transfection of cells on the same day. Immediately after trypsinization, the cells are transfected using jetPEI® while still in suspension. This protocol can be performed in the presence of serum. Pre-coating of wells with collagen or fibronectin is recommended to ensure even cell spreading. This protocol is ideal for some HTS applications such as drug screening.

The following protocol is given for transfection in 96-well plates. For other culture formats, please refer to Tables 6 and 7.

Table 6. Recommended number of cells, volume of medium and amount of DNA needed for transfection, relative to the cell culture vessel.

Culture vessel	Number of cells to seed	Volume of medium per well (µl)	Amount of DNA (µg)	Volume of jetPEI® reagent (µl)	Volume of NaCl solution for both DNA and jetPEI® (µl)	Total volume of complexes added per well (µl)
384-well	2 500 ± 5 000	50	0.05 - 0.1	0.1- 0.2	5	10
96-well	10 000 ± 20 000	200	0.1 - 0.2	0.2 – 0.4	10	20
48-well	40 000 ± 10 000	400	0.25 - 0.5	0.5 – 1	25	50

We recommend starting with the lower amounts of DNA and 2 µl of jetPEI® per µg of DNA, then adjust as required.

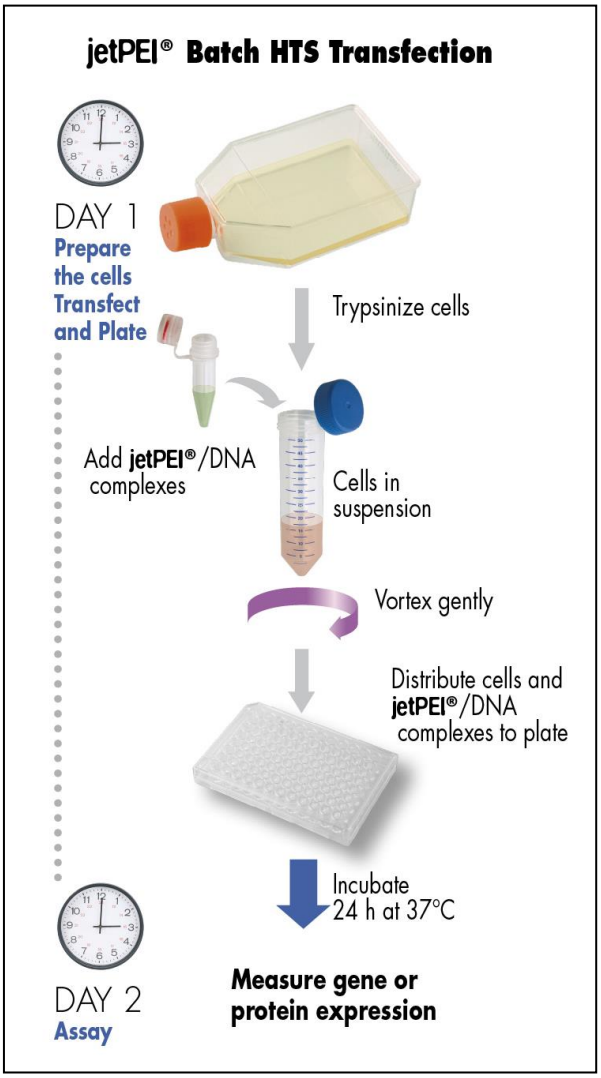
5.1 PRECOATING OF THE PLATES

In order to facilitate adhesion of the cells to the plate, we recommend pre-coating the wells with fibronectin (2-5 µg/cm^2). For this purpose, prepare a solution of fibronectin at 20 µg/ml, add to wells to cover the bottom. Leave for 30 min, aspirate and leave to dry.

5.2 PREPARATION OF THE COMPLEXES

The following protocol is given for transfection in 96-well plates. For other culture formats, please refer to Tables 6 and 7.

1. Per well, dilute 0.2 µg of DNA in 150 mM NaCl to a final volume of 10 µl. Vortex gently and spin down briefly.
2. Per well, dilute 0.4 µl of jetPEI® reagent in 150 mM NaCl to a final volume of 10 µl. Vortex gently and spin down briefly.
3. Add the 10 µl jetPEI® solution **to** the 10 µl DNA solution all at once. Please note mixing the solutions in the reverse order may reduce transfection efficiency.
4. Vortex the solution immediately and spin down briefly.
5. Incubate for 15 minutes at room temperature.



5.3 PREPARATION OF THE CELLS AND TRANSFECTION

1. Trypsinize the cells to be transfected according to standard protocol.
2. Wash the cells once with serum-containing medium and count the cells. Prepare a cell suspension at 50 000 - 100 000 cell/ml. Seed 200 µl per well in a sterile tube (10 000 - 20 000 cells).
3. Add 20 µl of jetPEI®/DNA mix to each tube and immediately gently vortex or invert the tube several times.
4. Transfer the cells + jetPEI®/DNA complexes solution into a well/plate (preferably pre-coated with collagen or fibronectin).
5. Return the plates to the cell culture incubator.
6. Perform reporter gene assay 24 to 48 h following transfection.

Table 7. Recommended number of cells for different plate formats.

Culture format	Number of cells added per well	Volume of cells per well (µl)	Minimal volume of cells per plate
384-well	2 500 - 5 000	50	20 ml (50 000 – 100 000 cells/ml)
96-well	10 000 – 20 000	200	20 ml (50 000- 100 000 cells/ml)
48-well	40 000 ± 10 000	400	20 ml (100 000 cells/ml)

6. STABLE TRANSFECTION

For stable transfection, perform transfection in 6-well plates, 60 mm or 10 cm dishes.

1. If needed, linearize plasmid DNA construct encoding for antibiotic selection.
2. Perform transfection as described in the standard protocol in Section 1.2.
3. Start antibiotic selection 24 – 48 h after transfection.
4. Maintain antibiotic selection as long as required, usually until cells are confluent again.
5. Check for integration of the plasmid DNA.

7. TROUBLESHOOTING

Observations	Troubleshooting
Low transfection efficiency	• Perform transfection in the presence of serum • Ensure that adherent cells are 50-70% confluent the day of transfection. • Optimize the amount of plasmid DNA. • Decrease the volume of culture medium per well. • Gently centrifuge the culture plates for 5 min at 180g after adding jetPEI®/DNA complexes to the cells, if the cells can withstand it. • Optimize the jetPEI® to DNA ratio starting from 1 µl jetPEI®/µg DNA to 4 µl jetPEI®/µg DNA. • Use high-quality plasmid preparation, free of proteins and RNA ($OD_{260/280}$ > 1.8). • Preferably use a DNA preparation at a concentration of 0.3 to 1 µg/µl. • Use a plasmid containing a common reporter gene such as Luciferase as positive control.
Cellular toxicity	• Decrease the amount of plasmid DNA used in the transfection assay, keeping the jetPEI®/DNA ratio constant. • Check the DNA concentration and ensure that jetPEI®/DNA ratio is no more than 2 µl of jetPEI® for 1 µg of DNA. • Change medium 4h after transfection or even after 2h if needed. • Verify the toxicity of the expressed protein. 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. • As control, we recommend using an empty plasmid complexed with jetPEI®.

8. PRODUCT INFORMATION

8.1 ORDERING INFORMATION

Cat #	Reagent	Buffer	Number of transfections
101-01N	0.1 ml	5 ml 150 mM NaCl	250 transfections in 96-well plates
101-10N	1 ml	50 ml 150 mM NaCl	2500 transfections in 96-well plates
101-40N	4 x 1 ml	4 x 50 ml 150 mM NaCl	10000 transfections in 96-well plates
101B-010N	10 ml	2 x 250 ml 150 mM NaCl	25000 transfections in 96-well plates

8.2 ADDITIONAL REAGENT

A 150 mM NaCl sterile solution is required to dilute jetPEI® and DNA. This solution is provided with catalog numbers 101-01N, 101-10N, 101-40N & 101B-010N or can be purchased separately (50 ml: cat # 702-50, 250 ml: cat # 702-250).

8.3 CONTENT

1 ml of jetPEI® transfection reagent is sufficient to perform ca. 5000 to 10000 transfections in 96-well plates or 2000 to 4000 transfections in 24-well plates.

8.4 REAGENT USE AND LIMITATIONS

For research use only. Not for use in humans.

8.5 QUALITY CONTROL

Every batch of jetPEI® is tested by DNA transfection of HeLa cells. Transfection with a firefly Luciferase gene under the control of CMV promoter gives at least 10^9 RLU (relative light unit)/mg of protein. The value for each batch is indicated on the Certificate of Analysis.

8.6 FORMULATION AND STORAGE

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

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

jetPEI® is chemically-defined and guaranteed free of animal origin products.

8.7 TRADEMARKS

jetPEI and Polyplus-transfection are registered trademarks of Polyplus-transfection.

8.8 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 3 90 40 61 87