PROTOCOL

jetPEI®
in vitro DNA transfection reagent

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 1500 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 1000 cell lines and primary cells.

1. Transfection protocol

1.1. Transfection protocol for adherent cells (forward)

1.1.1. 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.

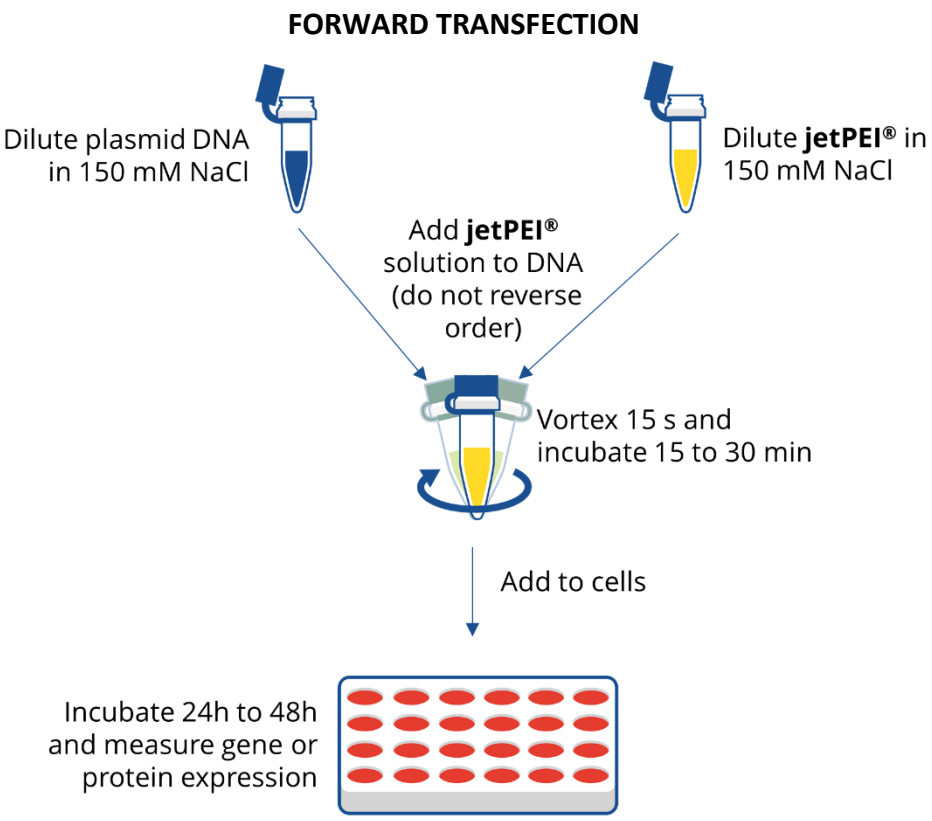
Culture vessel	Number of adherent cells to seed	Surface area per well (cm^2)	Volume of medium per well or plate (mL)
384-well	5 000 - 10 000	0.056	0.05 - 0.1
96-well	10 000 - 17 000	0.3	0.1 - 0.2
48-well	25 000 - 50 000	1	0.25 - 0.5
24-well	50 000 - 100 000	1.9	0.5 - 1
12-well	80 000 - 200 000	3.8	1 - 2
6-well / 35 mm	200 000 - 400 000	9.4	2 - 4
60 mm / flask 25 cm^2	400 000 - 800 000	28	5 - 10
100 mm / 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

1.1.2. 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 Polyplus-transfection® Database for

optimized conditions for various cell lines (http://www.polyplus-transfection.com/resources/cell-transfection-database/).



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. Vortex jetPEI® reagent for 5 sec and spin down before use.
3. 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.
4. Add the 50 µL jetPEI® solution into the 50 µL DNA solution all at once. Please note that mixing the solutions in the reverse order may reduce transfection efficiency.
5. Vortex the solution immediately and spin down briefly.
6. Incubate for 15 to 30 minutes at room temperature.
7. 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.
8. Return the plates to the cell culture incubator.
9. Perform reporter gene assay 24 to 48 h after transfection.

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

Table 2. Preparation of complexes 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	10	20
96-well	0.25	0.5	25	50
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

1.2 Transient transfection protocol for cells growing in suspension (forward)

1.2.1 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	25	50
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 cm^2	1×10^6 - 2×10^6	5 - 10	10 - 20	20 - 40	250	500
10 cm/flask 75 cm^2	3×10^6 - 6×10^6	10 - 15	30 - 60	60 - 120	500	1000

1.2.2. 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 Polyplus-transfection® database.

<u>Transfection procedure in a 24-well plate:</u>

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. Vortex jetPEI® reagent for 5 sec and spin down before use.
3. Per well, dilute 4 µL of jetPEI® in 150 mM NaCl to a final volume of 50 µL. Vortex gently and spin down briefly.
4. Add the 50 µL jetPEI® solution into the 50 µL DNA solution all at once. Please note that mixing the solutions in the reverse order may reduce transfection efficiency.
5. Vortex the solution immediately and spin down briefly.
6. Incubate for 15 to 30 minutes at room temperature.
7. 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.
8. Return the plates to the cell culture incubator.
9. Perform reporter gene assay 24 to 48 h following transfection.

1.3 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 timesaving 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® reagent (µL)	Volume of NaCl solution for both DNA and jetPEI® (µL)	Total volume of complexes added per well (µL)	Number of cells
384-well	0.1	0.3 - 0.4	10	20	2 500 - 5 000
96-well	0.2	0.6 - 0.8	25	50	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

1.3.1 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/cm2). 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.

1.3.2 Preparation of the complexes (per well)

1. Vortex jetPEI® reagent for 5 sec and spin down before use.
2. Dilute 0.6 µL jetPEI® in 150 mM NaCl to a final volume of 25 µL. Vortex briefly.
3. Dilute 200 ng of DNA in 150 mM NaCl to a final volume of 25 µL. Vortex briefly.
4. Add the 25 µL of jetPEI® solution into the 25 µL of DNA all at once.
   Note: mixing the solutions in the reverse order may reduce transfection efficiency.
5. Vortex the solution immediately.
6. Incubate for 15 minutes at room temperature.
7. Add 50 µL jetPEI®/DNA complexes per well in the pre-coated plate.

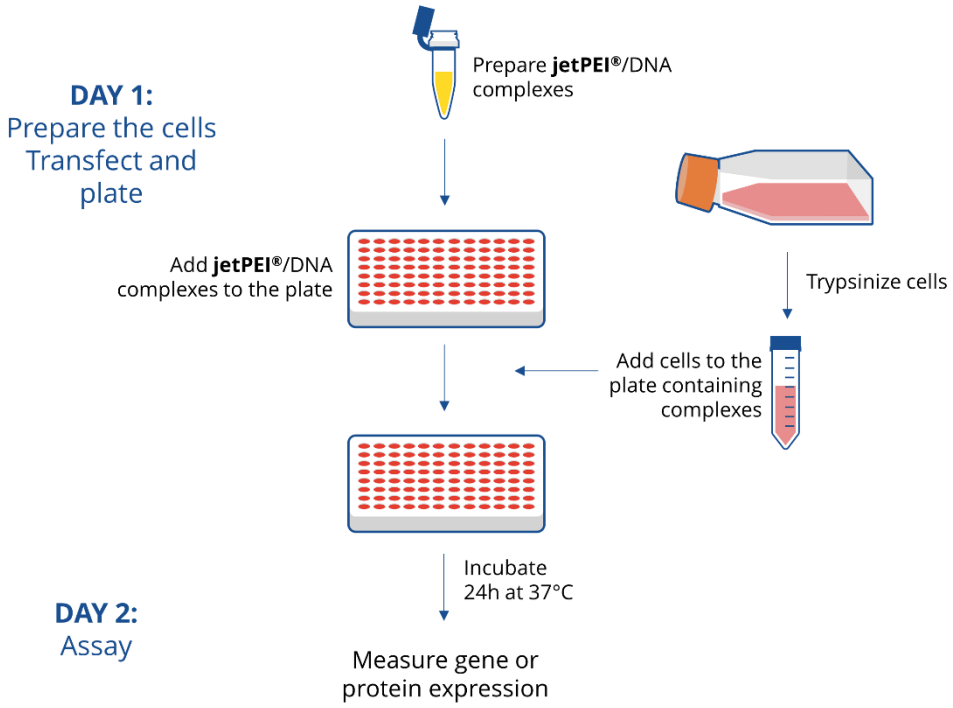
1.3.3 Preparation of the cells and transfection

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)

REVERSE HTS TRANSFECTION



1.4 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	10	20
96-well	10 000 ± 20 000	200	0.1 - 0.2	0.2 – 0.4	25	50
48-well	40 000 ± 10 000	400	0.25 - 0.5	0.5 – 1	25	50

We recommend starting with the lowest amount of DNA and 2 µL of jetPEI® per µg of DNA, then adjust as required.

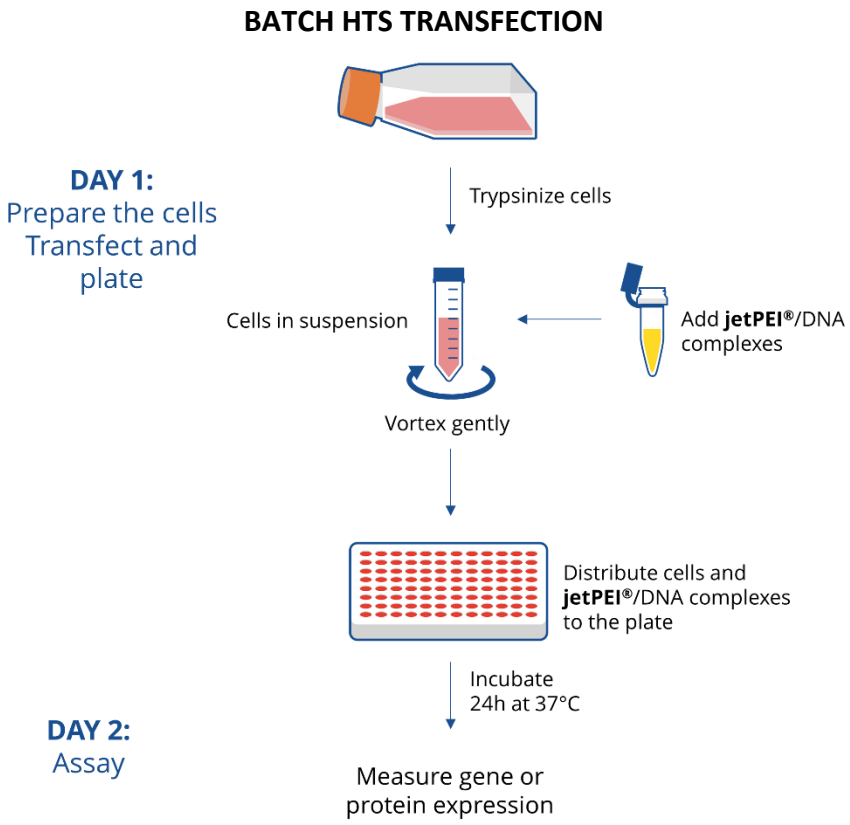
1.4.1 Precoating of the plates

In order to facilitate adhesion of the cells to the plate, we recommend pre-coating the wells with fibronectin (2-5 µg/cm2). 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.

1.4.2 Preparation of the complexes

The following protocol is given for transfection <u>in 96-well plates</u>. 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 25 µL. Vortex gently and spin down briefly.
2. Vortex jetPEI® reagent for 5 sec and spin down before use.
3. Per well, dilute 0.4 µL of jetPEI® reagent in 150 mM NaCl to a final volume of 25 µL. Vortex gently and spin down briefly.
4. Add the 25 µL jetPEI® solution to the 25 µL DNA solution all at once. Please note mixing the solutions in the reverse order may reduce transfection efficiency.
5. Vortex the solution immediately and spin down briefly.
6. Incubate for 15 minutes at room temperature.



1.4.3 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)

1.5. 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.

2. Troubleshooting

Observations	Actions
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®.

3. Product information

3.1. Ordering information

Part N°	jetPEI® Reagent	Buffer
101000053	1 mL	50 mL 150 mM NaCl
101000020	4 x 1 mL	4 x 50 mL 150 mM NaCl

3.2. Additional reagent

A 150 mM NaCl sterile solution is required to dilute jetPEI® and DNA. This solution is provided with Part numbers 101000053 & 101000020 or can be purchased separately (50 mL: part # 201000002).

3.3. Content

1 mL of jetPEI® transfection reagent is sufficient to perform ca. 5 000 to 10 000 transfections in 96-well plates or 2 000 to 4 000 transfections in 24-well plates.

3.4. Reagent use and limitations

For research use only. Not for use in humans.

3.5. 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.

Certificates of Analysis are available online in your Customer Area: https://myaccount.polyplus-transfection.com/wp-login.php

3.6. Formulation and storage

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

jetPEI® 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.

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.

3.7. Trademarks

Polyplus-transfection® and jetPEI® are registered trademarks of Polyplus-transfection S.A.

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

3.8. Contact information

Do you have any technical question regarding your product?

- Website: www.polyplus-transfection.com
- Email: support@polyplus-transfection.com
- Phone: +33 3 90 40 61 87

Contact the friendly Scientific Support team which is composed of highly educated scientists, PhDs and Engineers, with extensive hands-on experience in cell culture and transfection. The Scientific Support is dedicated to help our customers reach their goals by proposing different services such as: protocol optimization, personalized transfection conditions, tailored protocols, etc.

For any administrative question, feel free to contact our administration sales team:

- Reception Phone: +33 3 90 40 61 80
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Please note that the Polyplus-transfection® support is available by phone from 9:00 am to 5:00 pm CEST.