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# jetPEI™ *in vitro* DNA Transfection Protocol

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## Company Information

### Technical Assistance and Scientific Advice

Contact the friendly Polyplus technical support *via*:  
The Polyplus website: [www.polyplus-transfection.com](http://www.polyplus-transfection.com)  
Email: [support@polyplus-transfection.com](mailto:support@polyplus-transfection.com)  
Phone: + 33 (0)3 90 40 61 87

### 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.

#### **POLYPLUS-TRANSFECTION SA**

Bioparc  
Boulevard Sébastien Brant  
BP 90018  
67401 ILLKIRCH cedex  
FRANCE  
Phone : +33 3 90 40 61 80  
Fax: + 33 3 90 40 61 81  
[info@polyplus-transfection.com](mailto:info@polyplus-transfection.com)  
[www.polyplus-transfection.com](http://www.polyplus-transfection.com)

#### **POLYPLUS-TRANSFECTION Inc.**

1251 Ave of the Americas, 34<sup>th</sup> fl.  
NEW YORK, NY 10020  
USA  
Phone: +1 508 315 9629

## Product information

### Description

jetPEI™ is a powerful reagent that ensures effective and reproducible DNA and oligonucleotide transfection into mammalian cells with low toxicity. jetPEI™ is mainly composed of a linear polyethylenimine manufactured at Polyplus-transfection. It has been shown to provide superior *in vitro* transfection when compared to other cationic lipids and polymers. Over 400 publications using jetPEI™ can be found in the Product Citation Database. In addition a Cell Transfection Database gives transfection conditions over 400 cell lines and primary cells. Both databases are available on the Polyplus website, [www.polyplus-transfection.com](http://www.polyplus-transfection.com), under the Products/Technical Resources heading.

### Ordering information

Cat #	Reagent	Buffer	Number of transfections
101-01N	0.1 ml	5 ml 150 mM NaCl	50 transfections in 24-well plates
101-10	1 ml	-	500 transfections in 24-well plates
101-10N	1 ml	50 ml 150 mM NaCl	
101-40	4 x 1 ml	-	2000 transfections in 24-well plates
101-40N	4 x 1 ml	4 x 50 ml 150 mM NaCl	
101B-010	10 ml	-	5000 transfections in 24-well plates
101B-010N	10 ml	2 x 250 ml 150 mM NaCl	

### 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 in 101B-010N or can be purchased separately (50ml: cat # 702-50, 250 ml: cat # 702-250).

### Content

1 ml of jetPEI™ transfection reagent is sufficient to perform ca. 250 to 500 transfections in 24-well plates or 100 to 200 transfections in 60-mm dishes.

### Reagent use and Limitations

For research use only. Not for use in humans.

## 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<sup>9</sup> RLU (relative light unit)/mg of protein. The value for each batch is indicated on the Certificate of Analysis.

## 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.

## Mechanism of transfection using jetPEI™

jetPEI™ 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™. 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.

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

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

# 1. Transient transfection protocol for adherent cells (Forward)

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.

## 1.1 Cell seeding

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 <sup>2</sup> )	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 <sup>2</sup>	400 000 - 800 000	28	5 - 10
10 cm/flask 75 cm <sup>2</sup>	1 000 000 - 2 000 000	78.5	10 - 15
14 cm/flask 175 cm <sup>2</sup>	2 × 10 <sup>6</sup> - 5 × 10 <sup>6</sup>	153 - 175	20 - 30

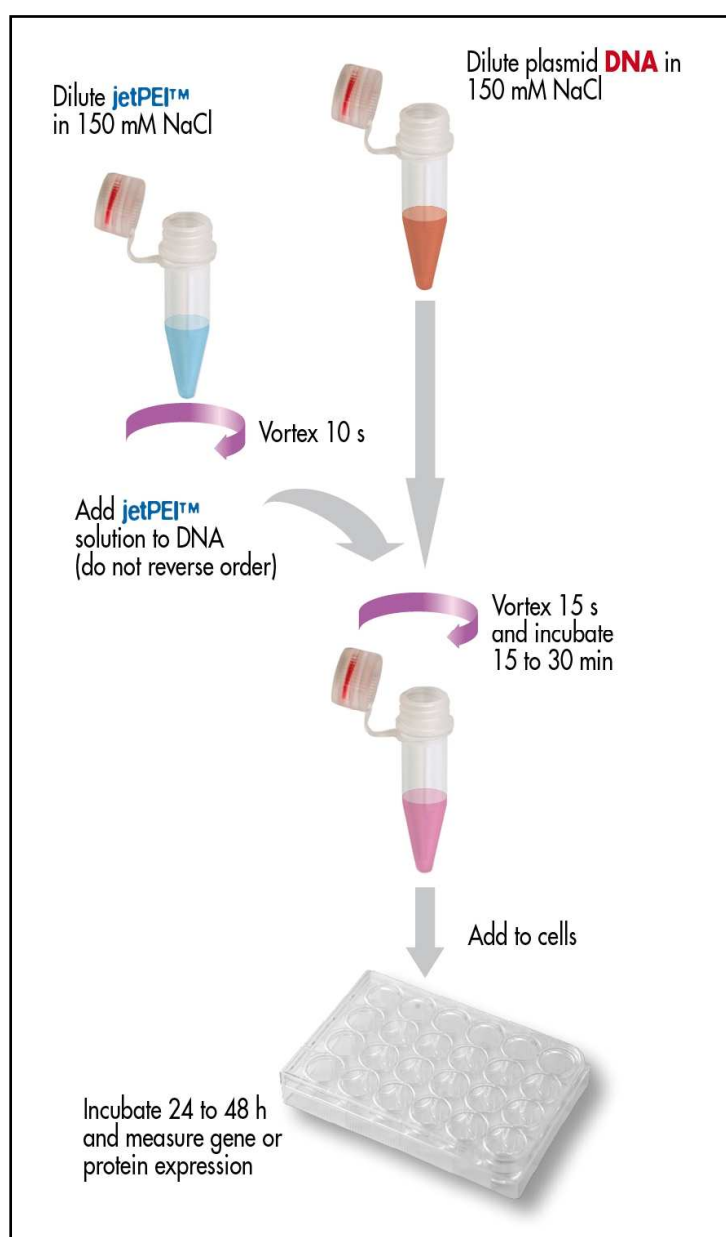
\* **Note:** Transfection efficiency may be increased by reducing the volume. In this case, add the same volume of fresh serum-containing medium 2 h post transfection.

## 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 a majority of adherent cell lines are given in the forward protocol described below. Check Table 3 for specific conditions optimised for A549, BHK-21, CaCo-2, CHO, HEK 293 and NIH/3T3 cells or check the extensive online 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™ 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 following transfection.

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

We recommend using 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.

**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 <sup>2</sup>	5	10	250	500
10 cm/flask 75 cm <sup>2</sup>	10 - 20	20 - 40	250	500
14 cm/flask 175 cm <sup>2</sup>	20 - 30	40 - 60	500	1000

We have optimized transfection conditions for specific cell lines. Please use conditions in Table 3 for A549, BHK-21, CaCo-2, CHO, HEK 293 and NIH/3T3 cells. Other conditions for specific cells are listed in the online Cell transfection database.

**Table 3. Optimal transfection conditions for adherent cell lines.**

Cell line	Amount of DNA (µg)	Volume of jetPEI™ reagent (µl)	Volume of transfection medium in a 24-well plate (ml)
A549	1	2	0.5
BHK-21	1	2	0.5
CaCo-2	1	2	0.5
CHO	1	1.2	1
HEK 293	1	2	0.5
NIH/3T3	1	2	0.5

### 1.3 Factors affecting transfection efficiency

- jetPEI™ is **not** affected by the presence of serum during transfection. Therefore, jetPEI™/DNA complexes can be added directly to cells in serum-containing medium.
- Transfection efficiency can usually be improved by using smaller volumes of medium (see Table 1) and/or by centrifugation of the culture plate (5 min at 180 g at room temperature) following addition of the jetPEI™/DNA complexes to the cells, both increase complex adherence to the cells.
- If cytotoxicity is observed, we recommend changing medium after 4 hours.

## 2. Transient transfection protocol for cells grown in suspension (Forward)

### 2.1 Cell seeding

For optimal transfection conditions with jetPEI™ seed the appropriate number of cells according to the culture vessel used (Table 4) and transfect right away.

**Table 4. 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	$2 \times 10^4 - 5 \times 10^4$	0.2	0.2 - 0.4	0.4 - 0.8	10	20
48-well	$5 \times 10^4 - 10^5$	0.5	0.5 - 1	1 - 2	25	50
24-well	$10^5 - 2 \times 10^5$	0.5 - 1	1 - 2	2 - 4	50	100
12-well	$2 \times 10^5 - 5 \times 10^5$	1 - 2	2 - 4	4 - 8	50	100
6-well/ 35 mm	$5 \times 10^5 - 1 \times 10^6$	2 - 4	6 - 12	8 - 24	100	200
6 cm/ flask 25 cm <sup>2</sup>	$1 \times 10^6 - 2 \times 10^6$	5 - 10	10 - 20	20 - 40	250	500
10 cm/ flask 75 cm <sup>2</sup>	$3 \times 10^6 - 6 \times 10^6$	10 - 15	30 - 60	60 - 120	500	1000

We recommend using the lower amounts of DNA as starting conditions and 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.

### 2.2 Preparation of the complexes and transfection

The optimal conditions of transfection for most cell lines in suspension are given below. For Daudi, K562 and Molt 4 cells, check Table 5. For other cell lines, check the online Cell Transfection database.

**The following protocol is given for transfection 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.

We have optimized transfection conditions for specific cell lines. For Daudi, K562 and Molt 4 cells, please use the conditions below (Table 5). For others, check out the online Cell Transfection database

**Table 5. Optimal transfection conditions for some specific suspension cell lines in a 24-well plate.**

Cell line	Amount of DNA (µg)	Volume of jetPEI™ reagent (µl)	Volume of transfection medium in a 24-well plate (ml)
Daudi	2	<b>6.4</b>	<b>0.5</b>
K 562	2	<b>4</b>	<b>0.5</b>
Molt 4	2	<b>6.4</b>	<b>0.5</b>

## 3. Reverse transfection protocol

The following protocol has been developed for reproducible and efficient reverse transfection in 96-well plates for high throughput screening (HTS). For reverse transfection in 384-well plates and other formats, refer to Tables 6 and 7. For forward protocol, refer to Section 1. The reverse transfection protocol is time saving compared to the forward protocol.

**Table 6. Recommended conditions per well 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	5 000
96-well	0.2	0.6 - 0.8	25	2 × 10 <sup>4</sup>
48-well	0.5	1.6 - 2	25	4 × 10 <sup>4</sup>
24-well	1	3 - 4	50	8 × 10 <sup>4</sup>

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 using the lower volumes of jetPEI™ and testing two ratios of jetPEI™/DNA:

- 3 µl of jetPEI™ per 1 µg of DNA
- 4 µl of jetPEI™ per 1 µg of DNA

### 3.1 Preparation 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<sup>2</sup>). 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.

## 3.2 Preparation of the complexes

The conditions are given per well for **96-well** plates. Refer to Tables 6 and 7 for other culture formats.

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™ solution **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 the 50 µl jetPEI™/DNA complexes per well in the pre-coated plate.

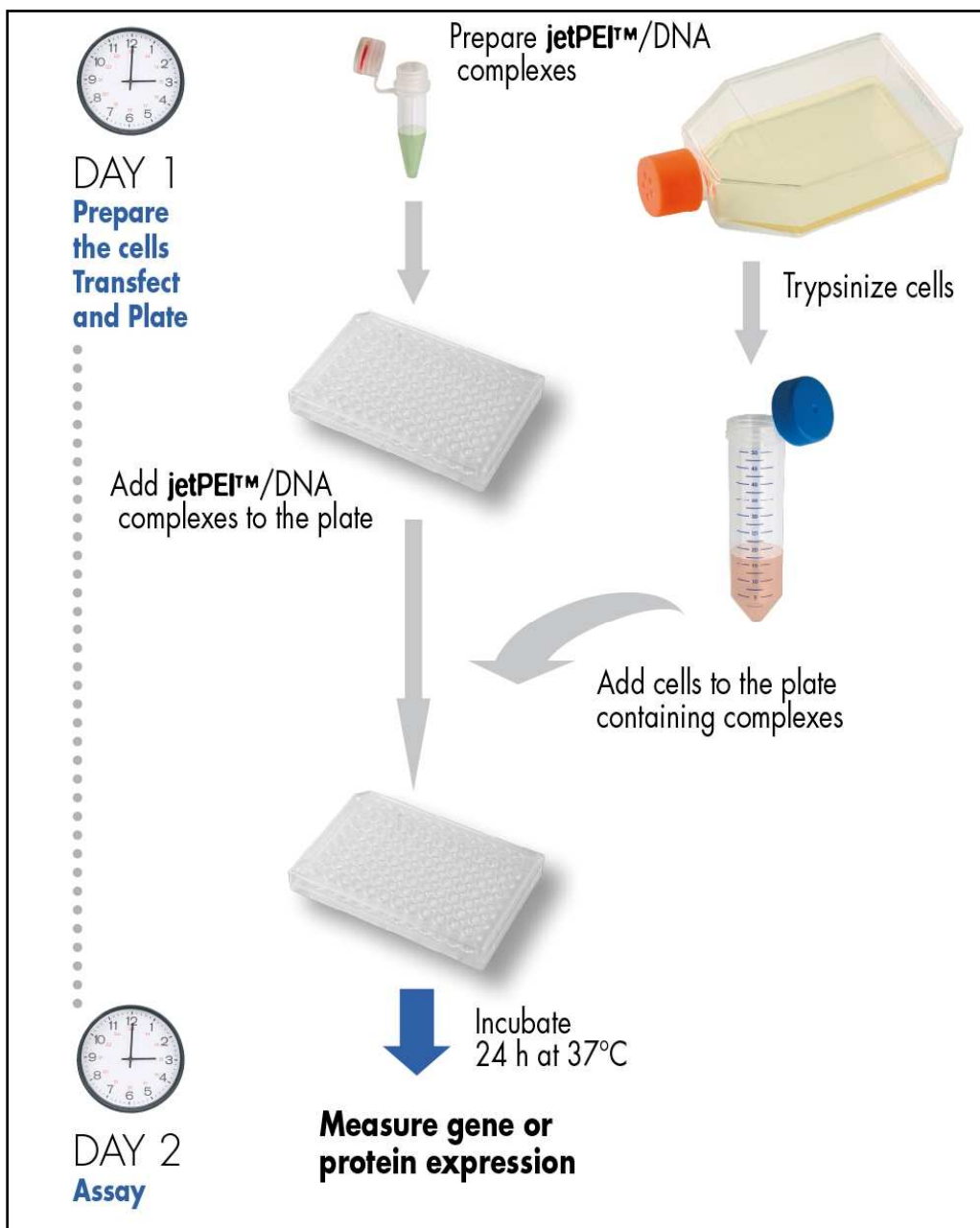
## 3.3 Preparation of the cells and transfection

1. Trypsinize the cells to be transfected as usual.
2. Wash once with serum-containing medium.
3. Prepare a cell suspension at 135 000 cells/ml in culture medium containing serum.
4. Distribute 150 µl of the cell suspension per well in order to obtain 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 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	5 000 ± 500	50	20 ml (100 000 cells/ml)
96-well	20 000 ± 2 500	150	15 ml (135 000 cells/ml)
48-well	40 000 ± 10 000	400	20 ml (100 000 cells/ml)
24-well	80 000 ± 25 000	500	12.5 ml (160 000 cells/ml)

### REVERSE TRANSFECTION



## 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 HTS applications.

The following protocol is given for transfection in 24-well plates. For other culture formats, please refer to Tables 8 and 9.

**Table 8. 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	5 000 ± 500	125	0.05 - 0.1	0.1 - 0.2	5	10
96-well	20 000 ± 2 500	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
24-well	80 000 ± 25 000	500	0.5 - 1	1 - 2	50	100

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

### 4.1 Precoating of the plates

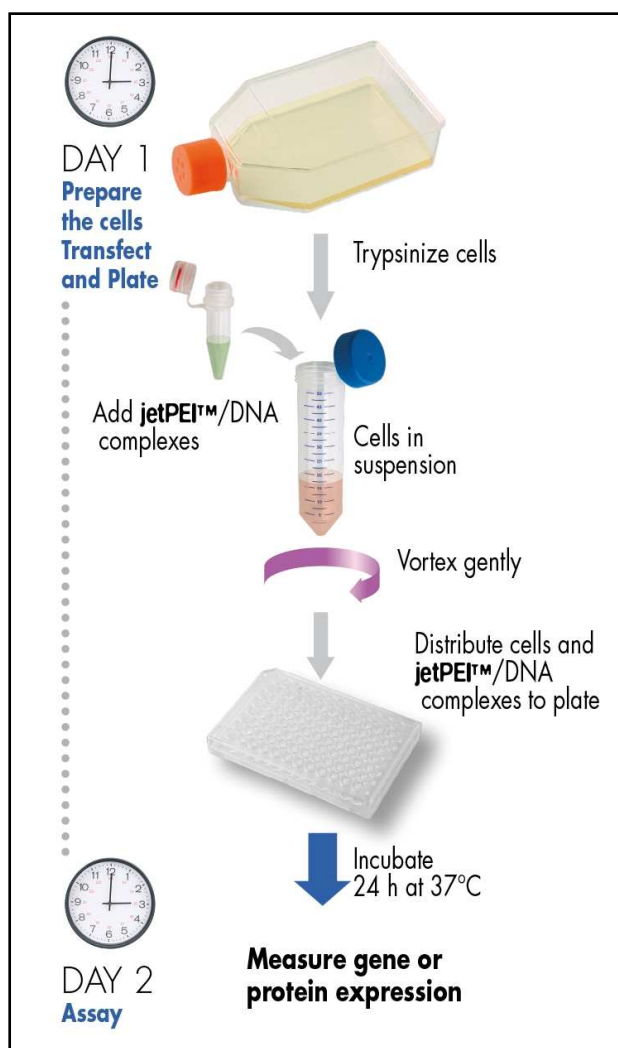
In order to facilitate adhesion of the cells to the plate, we recommend pre-coating the wells with fibronectin (2-5 µg/cm<sup>2</sup>). For this purpose, prepare a solution of fibronectin at 20 µg/ml, add to wells to cover the bottom. Leave for 30 min, aspirate and leave to dry.

## 4.2 Preparation of the complexes

The following protocol is given for transfection in **24-well plates**. For other culture formats, please refer to Tables 8 and 9.

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™ solution 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 mixing the solutions in the reverse order reduces transfection efficiency.
4. Vortex the solution immediately and spin down briefly.
5. Incubate for 15 minutes at room temperature.

### BATCH TRANSFECTION



### 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 of 200 000 cell/ml. Seed 500 µl per well in a sterile tube (100 000 cells).
3. Add 100 µl of jetPEI™/DNA mix to each tube and immediately gently vortex.
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 9. 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	5 000 ± 500	50	20 ml (100 000 cells/ml)
96-well	20 000 ± 2 500	150	15 ml (135 000 cells/ml)
48-well	40 000 ± 10 000	400	20 ml (100 000 cells/ml)
24-well	80 000 ± 25 000	500	12.5 ml (160 000 cells/ml)

## 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.

## 6. Large scale transfection

For large scale transfection in bioreactors for 1, 10 and 100 L of culture or more, our scientific specialists will be pleased to give you suitable guidelines adapted to your cells and culture medium. Qualified PEI is available for biomanufacturing.

## Contact our Technical Assistance and Scientific Advice Service:

Contact the friendly Polyplus technical support *via*:

The Polyplus website: [www.polyplus-transfection.com](http://www.polyplus-transfection.com)

Email: [support@polyplus-transfection.com](mailto:support@polyplus-transfection.com)

Phone: + 33 (0) 3 90 40 61 87

## Troubleshooting

Observations	Troubleshooting
Low transfection efficiency	<ul style="list-style-type: none"> <li>• Optimize the amount of plasmid DNA.</li> <li>• Use high-quality plasmid preparation, free of proteins and RNA (OD<sub>260/280</sub> &gt; 1.8).</li> <li>• Ensure that adherent cells are 50-70% confluent the day of transfection.</li> <li>• Optimize the jetPEI™ to DNA ratio starting from 1 µl jetPEI™/µg DNA to 4 µl jetPEI™/µg DNA.</li> <li>• Use a plasmid containing a common reporter gene such as Luciferase as positive control.</li> <li>• Decrease the volume of culture medium.</li> <li>• Gently centrifuge the culture plates for 5 min at 180g after adding jetPEI™/DNA complexes to the cells, if the cells can withstand it.</li> <li>• Preferably use a DNA preparation at a concentration of 0.3 to 1 µg/µl.</li> </ul>
Cellular toxicity	<ul style="list-style-type: none"> <li>• Decrease the amount of plasmid DNA used in the transfection assay, keeping the jetPEI™/DNA ratio constant.</li> <li>• Check the DNA concentration and ensure that jetPEI™/DNA ratio is no more than 2 µl of jetPEI™ for 1 µg of DNA.</li> <li>• Reduce the incubation time of the jetPEI™/DNA complexes with the cells to 2 to 4 hours and change medium after this time.</li> <li>• 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.</li> <li>• Make sure that the plasmid preparation is endotoxin-free.</li> </ul>

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## Notes

