# PEIpro®

# *in vitro* DNA transfection reagent

# PROTOCOL

## DESCRIPTION

Polyplus-transfection® supplies a ready-to-use chemically defined and optimized linear polyethylenimine for DNA transfection. PEIpro® is a 1 mg/ml solution of fully characterized linear PEI guaranteed free of components of animal origin. This reagent is dedicated for medium to large scale bioproduction of recombinant proteins, viruses and antibodies. PEIpro® complies with biomanufacturing guidelines for raw material and is supplied with appropriate quality controls: product characterization, transfection efficiency and microbiology tests. This proprietary PEIpro® guarantees reliable, safe and reproducible batch to batch protein and virus production.

# 1.TRANSFECTION PROTOCOL FOR SUSPENSION CELLS

PEIpro® is perfectly suited for DNA transfection of cells grown in suspension in shaker flasks, spinners, roller bottles, cell culture bags or bioreactors in serum-free media. PEIpro®-mediated transfection can also be performed in the presence of antibiotics.

## 1.1 PREPARATION OF THE CELLS

**The day before transfection**, prepare a cell suspension at 1 x $10^6$ cells / ml to obtain cells at log phase the following day.

On the day of transfection, cell density does not need to be readjusted.

## 1.2 PREPARATION OF THE COMPLEXES AND TRANSFECTION

**Table 1. Recommended DNA amount and PEIpro® volume for transfection of cells grown in suspension according to the volume of culture.**

| Volume of culture medium | Amount of DNA | Volume of PEIpro® for HEK-293 and derivatives | Volume of PEIpro® for CHO and derivatives | Volume of serum-free medium for both DNA and PEIpro® |
|---|---|---|---|---|
| 30 ml | 30 µg | 30 to 120 µl | 30 to 180 µl | 1.5 ml |
| 100 ml | 100 µg | 100 to 400 µl | 100 to 600 µl | 5 ml |
| 1 L | 1 mg | 1 to 4 ml | 1 to 6 ml | 25 ml |

The amount of PEIpro® may be optimized from 1 to 4 µl per µg of DNA for HEK-293 and derivatives and from 1 to 6 µl per µg of DNA for CHO and derivatives depending on the medium used. The amount of plasmid DNA may be adjusted from 0.5 to 2 µg per ml (or 0.5 to 2 mg per Liter) of cell culture.

***NOTE:*** Some serum-free media are not permissive to transfection. Please ensure that the medium you are using is permissive to transfection and suitable for high transfection efficiency. Feel free to contact Polyplus technical support online for tips and advice:
http://www.polyplus-transfection.com/others/contact-us/.

**The following protocol is given for transfection of HEK-293 in 1 Liter of cell culture medium**. For CHO cells and other volumes of culture see Table 1.

1. Dilute 1 mg of DNA in serum-free medium (without Pluronic® F-68 nor antibiotics) to a final volume of 25 ml. Vortex gently.
2. Vortex PEIpro® reagent for 5 sec and spin down before use.
3. Dilute 1 to 4 ml of PEIpro® in serum-free medium (without Pluronic® F-68 nor antibiotics) to a final volume of 25 ml. Vortex gently.
4. Add the 25 ml PEIpro® solution to the 25 ml DNA solution all at once.
5. Vortex the solution immediately.
6. Incubate for 15 minutes at room temperature.
7. Add the 50 ml PEIpro®/DNA mix to the cells.
8. Harvest protein or virus when required.

**Table 2. Recommended DNA/PEIpro® ratios for various serum-free media**

| Growth medium | Starting DNA : PEIpro® ratio |
|---|---|
| FreeStyle™ 293 | 1:1 – 1:2 |
| Pro293™ | 1:2 – 1:4 |
| FreeStyle™ F17 | 1:2 |
| Expi293™ | 1:2 |
| HyClone™ HyCell™ TransFx™-H | 1:2 |
| FreeStyle™ CHO | 1:2 |
| CHO-S-SFM II | 1:2 |
| CD-FortiCHO™ | 1:2 |
| Power-CHO™1 | 1:4 – 1:6 |
| Pro-CHO™4 | 1:2 – 1:4 |

# 2.TRANSFECTION PROTOCOL FOR ADHERENT CELLS

PEIpro® is ideal for virus production in adherent cells. For cotransfection of multiple plasmids, the total DNA amount per plate should not exceed the maximal DNA amount indicated in Table 5. The ratio to use for each plasmid depends on the size of the plasmids, the plasmid constructs and the desired expression level of each plasmid. Please adjust the ratios according to your application. Each plasmid should represent at least 10% of the total DNA amount per well/plate.

## 2.1 CELL SEEDING

In this protocol, adherent cells are split and seeded the day before transfection. The transfection complexes are added subsequently to the cells the following day.

For optimal transfection conditions with PEIpro®, we recommend using 50-80% confluent cells on the day of transfection.

PEIpro® is compatible with the use of antibiotics, therefore you may use antibiotic containing medium during the entire experiment.

**Table 3. Recommended number of cells to seed the day before transfection.**

| Culture vessel | Number of adherent cells to seed the day before transfection |
|---|---|
| 10 cm/T-75/T-175/multilayer cell factories | 25 000 – 50 000 cells/cm² |
| HYPER*Flask*®/HYPER*Stack*® | 15 000 – 30 000 cells/cm² |

## 2.2 PREPARATION OF THE COMPLEXES AND TRANSFECTION

We recommend using 1 µl of PEIpro® per µg of DNA as starting conditions, however the amount of PEIpro® may be adjusted from 1 to 2 µl per µg of DNA depending on the cell line and the medium used.

**Table 4. Recommended DNA amounts for different cell culture formats.**

| Culture vessel | Amount of DNA |
|---|---|
| 10 cm/T-75/T-175/multilayer cell factories | 0.10 – 0.20 µg/cm² |
| HYPER*Flask*®/HYPER*Stack*® | 0.35 – 0.58 µg/cm² |

**Table 5. Complexes preparation for transfection in different cell culture formats.**

| Culture vessel | Amount of DNA | Volume of PEIpro® reagent for the 1:1 ratio | Volume of serum free medium for both DNA and PEIpro® | Total volume of complexes added per well |
|---|---|---|---|---|
| 10 cm/T-75 (75 $cm^2$) | 7.5 – 15 µg | 7.5 – 15 µl | 250 µl | 500 µl |
| 14 cm (153 $cm^2$) | 15 – 30 µg | 15 – 30 µl | 500 µl | 1 ml |
| T-175 (175 $cm^2$) | 17.5 – 35 µg | 17.5 – 35 µl | 1 ml | 2 ml |
| HYPER*Flask*® (1720 $cm^2$) | 0.6 – 1 mg | 0.6 – 1 ml | 40 ml | 80 ml |
| HYPER*Stack*® (6000 $cm^2$) | 2.1 – 3.5 mg | 2.1 – 3.5 ml | 60 ml | 120 ml |
| 10-layer cell factory (6360 $cm^2$) | 0.64 – 1.3 mg | 0.64 – 1.3 ml | 50 ml | 100 ml |

The following protocol is a standard protocol for transfection of HEK-293T in a 10 cm plate; for transfection of other cell lines or in other culture formats, please refer to Tables 3, 4 and 5.

The classical transfection guidelines are given in the protocol described below. Optimization such as decreasing the DNA amount may be required.

**Transfection in a 10 cm plate**:

1. Dilute 15 µg of DNA in serum-free medium to a final volume of 250 µl. Vortex gently.
2. Vortex PEIpro® reagent for 5 sec and spin down before use.
3. Dilute 15 to 30 µl of PEIpro® in serum-free medium to a final volume of 250 µl. Vortex gently.
4. Add the 250 µl PEIpro® solution **to** the 250 µl DNA solution all at once.
5. Vortex the solution immediately.
6. Incubate for 15 minutes at room temperature.
7. Add the 500 µl PEIpro®/DNA mix drop-wise to the cells in 10 ml of medium and homogenize by gently swirling the plate.
8. Return the plates to the cell culture incubator.
9. Harvest protein or virus when required.

***NOTE:*** Some serum-free media are not permissive to transfection. Please ensure that the medium you are using is permissive to transfection and suitable for high transfection efficiency. Feel free to contact Polyplus technical support online for tips and advice: http://www.polyplus-transfection.com/others/contact-us/.

# 3. TROUBLESHOOTING

| Observations | Troubleshooting |
|---|---|
| Low transfection efficiency | ▪ Optimize the PEIpro® to DNA ratio starting from 1 µl PEIpro®/µg DNA up to 6 µl PEIpro®/µg DNA for cells grown in suspensions and from 1 µl PEIpro®/µg DNA up to 2 µl PEIpro®/µg DNA for cells grown adherently.<br>▪ Optimize the amount of plasmid DNA.<br>▪ If using serum-free medium, ensure that the medium is permissive to transfection. Contact us online for tips and advice: http://www.polyplus-transfection.com/others/contact-us/<br>▪ Use a positive control such as a plasmid encoding for a common reporter gene (Luciferase, GFP, control Antibody, etc…)<br>▪ For adherent cells grown in suspension, adapt the cells to growth in suspension in serum-free medium for several days before performing transfection.<br>▪ Use high-quality plasmid preparation, free of proteins and RNA ($OD_{260/280}$ > 1.8).<br>▪ For adherent cells, ensure that the cells are at 50-80% confluency at the time of transfection. |
| Cellular toxicity | ▪ Decrease the PEIpro®/DNA ratio.<br>▪ Check the DNA concentration and decrease the amount of plasmid DNA used, keeping the PEIpro®/DNA ratio constant.<br>▪ For adherent cells, change medium 4 to 6 hours after transfection.<br>▪ For suspension cells, the day before transfection, prepare the cell suspension at 1 x $10^6$ cells / ml by centrifuging the cells and resuspending them in fresh, pre-warmed serum-free medium<br>▪ For suspension cells, dilute the cell culture up to 2 folds, 4 hours after transfection.<br>▪ Check 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.<br>▪ Make sure that the plasmid preparation is endotoxin-free. |

## TECHNICAL ASSISTANCE AND SCIENTIFIC ADVICE

**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

## MECHANISM OF TRANSFECTION USING POLYETHYLENIMINE

Polyethylenimine compacts DNA into positively charged particles - called complexes - capable of interacting with anionic proteoglycans at the cell surface and thus of entering the cell by endocytosis. The DNA is released from the endosomes into the cytoplasm by a "proton sponge" mechanism[1,2], thereby allowing nuclear transport for subsequent transcription.


1. *Boussif O., F. Lezoualc'h, M. A. Zanta, M. D. Mergny, D. Scherman, B. Demeneix and J. P. Behr (1995) 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*

2. *Boussif O., M. A. Zanta and J. P. Behr (1996) Optimized Galenics Improve in-Vitro Gene-Transfer with Cationic Molecules Up to 1000-Fold. Gene Therapy 3, 1074-1080*


# 4. PRODUCT INFORMATION

1 Liter of PEIpro® transfection reagent is sufficient to transfect on average 500 L of cell culture.

## FORMULATION AND STORAGE

- Content: 1 mg/ml linear polyethylenimine.
- Volume: each vial/bottle contains the specified volume ± 3%.
- PEIpro® is chemically-defined.
- PEIpro® is guaranteed free of components of animal origin.
- PEIpro® is shipped at room temperature but should be stored at 4°C upon arrival to ensure long term stability.
- Stability: This product will stay within its specifications for at least two years when stored appropriately as indicated in the Certificate of Analysis.

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.

## REAGENT USE AND LIMITATIONS

For bioproduction and research use only. Not intended for animal or human diagnostic or therapeutic use.

## QUALITY CONTROL

All lots of PEIpro® are tested during and after manufacturing to guarantee accurate chemical composition and to ensure constant quality and lot-to-lot reproducibility. PEIpro® potency is evaluated in a DNA transfection experiment of HEK-293 cells.

The provided Certificate of Analysis displays detailed results of the following lot release Quality Controls (QCs):

| Quality Control | Method |
|---|---|
| **Product activity** | Transfection of suspension HEK-293 cells with pFUSE-mIgG3-Fc2 plasmid DNA |
| **Viability assay** | Trypan blue |
| **Sterility test** | Current EP 2.6.1 / USP <71> |
| **Bacterial endotoxins assay (LAL)** | Current EP 2.6.14 / USP <85> |
| **Mycoplasma detection test** | Cross detection by PCR and indirect epifluorescence microscopy |
| **Identity** | $^{1}$H-NMR analysis |

## ORDERING INFORMATION

| Reference Number | Reagent | Average number of transfections |
|---|---|---|
| **115-010** | 10 ml | Transfection of 5 x 1 Liter of cell culture |
| **115-100** | 100 ml | Transfection of 50 x 1 Liter of cell culture |
| **115-400** | 4 x 100 ml | Transfection of 200 x 1 Liter of cell culture |
| **115-01K** | 10 x 100 ml | Transfection of 500 x 1 Liter of cell culture |

## TRADEMARKS



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