PROTOCOL

PEIpro®
DNA transfection kit for virus production

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 viruses, proteins, 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. When required, you may switch to our PEIpro®-HQ and PEIpro®-GMP reagents using the same protocol for continuous performance with higher quality grades.

1. Transfection protocol for virus production in suspension cells

PEIpro® is perfectly suited for DNA transfection of cells grown in suspension in shaker flasks, spinners, cell culture bags or bioreactors in serum-free media. PEIpro® is compatible with the use of antibiotics in the cell culture medium.

1.1 Cell seeding

On the day of cell seeding, adjust cell density according to your process to reach the exponential growth phase with a viable cell density (VCD) around 2 – 2.5 x 10^6 cells/mL at the time of transfection.

Cell seeding must be adjusted according to the culture vessel you are using:

- Shake flask: we recommend seeding 1 x 10^6 cells/mL to ensure that cells reach the optimal VCD after 24 hours.
- Other (*e.g.* culture bags, bioreactors, etc.): the day of transfection will depend on the growth time needed for your cells to reach a VCD of 2 – 2.5 x 10^6 cells/mL.

On the day of transfection, *VCD must be determined to adjust the DNA amount used for transfection, especially when using a high cell density system.*

1.2 Preparation of the complexes

The following protocol is given for the transfection of plasmids into suspension cells for virus production. For co-transfection of multiple plasmids, the suitable plasmid ratio depends on the virus produced, 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 mL of culture. The different complexation parameters are described in Table 1. For each parameter, we recommend a specific condition that may be further optimized according to your process (Table 2).

Table 1. Complexation parameters for the transfection of suspension cells.

Parameter	Recommended condition	Range of optimization
DNA amount (per 10^6 cells)	1 µg DNA	0.5 µg – 2 µg
Ratio (µg DNA : µL PEIpro®)	1:1	1:1 – 1:3
Complexation volume (% total culture volume)	10%	5% - 10%
Complexation time	15 min	10 min – 20 min
Complexation medium (without supplements)	DMEM high glucose	Own culture medium, DMEM low glucose, Opti-MEM, Freestyle™ F17, BalanCD™ HEK-293

- *The complexation medium should contain neither Pluronic® F-68/Poloxamer 188/Kolliphor® P188 nor antibiotics.*
- *To ensure a good transfection efficiency as well as reproducibility between experiments, the incubation time is critical and should not exceed 30 minutes.*
- *We recommend using polypropylene tubes to prepare DNA/PEIpro® complexes and avoid polycarbonate ones.*

Table 2. Recommended DNA/PEIpro® ratios for various HEK-293 serum-free media.

Growth medium	Starting DNA : PEIpro® ratio
FreeStyle™ 293	1:1 – 1:2
BalanCD™ HEK-293	1:1 – 1:2
Pro293™	1:2 – 1:4
FreeStyle™ F17	1:2
Expi293™	1:1 - 1:2
HyClone™ HyCell™ TransFx™-H	1:2

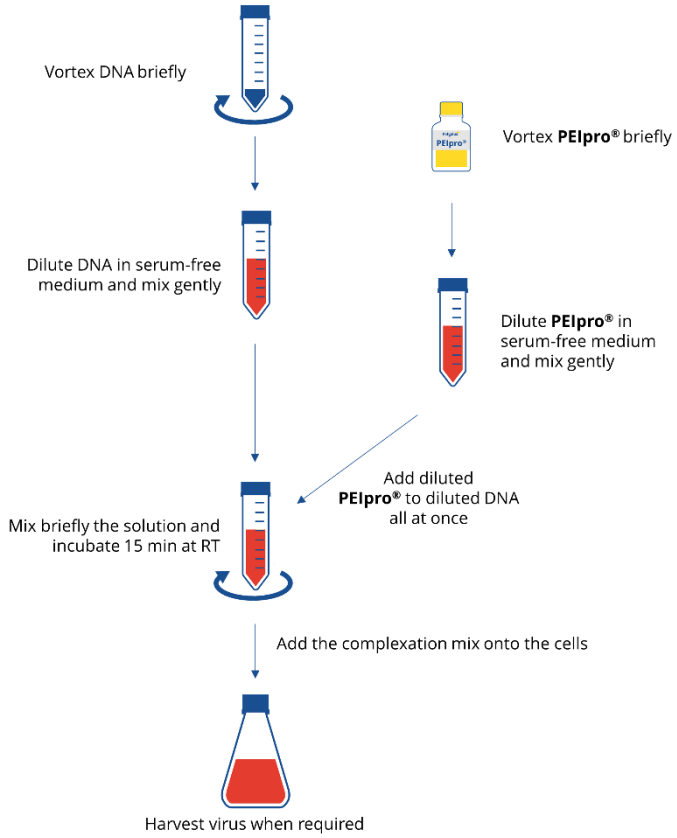
Some serum-free media are not permissive to transfection. Please ensure that the medium you are using is permissive to transfection and suitable for high transfection efficiency. Feel free to contact Polyplus scientific support online for tips and advice: support@polyplus-transfection.com.

1.3 Transfection

Transfection parameters (*i.e.* DNA amount and PEIpro® volume) have to be adjusted according to the cell density reached at the time of transfection.

The following protocol is given for transfection of HEK-293 cells in 1 liter of cell culture medium according to the recommended conditions in Table 1.

1. On the day of transfection, measure the cell density and determine transfection parameters (DNA amount and PEIpro® volume per million cells) according to Table 1 & 2. For this example, **we assume a VCD of 2 x 10^6 cells / mL.**
2. Dilute 2 mg of DNA in serum-free medium (without supplements) to a final volume of 50 mL. Vortex gently.
3. Vortex PEIpro® reagent briefly.
4. Dilute 2 mL of PEIpro® in serum-free medium (without supplements) to a final volume of 50 mL. Vortex gently.
5. Add the 50 mL PEIpro® solution **onto** the 50 mL DNA solution all at once.
6. Mix immediately the solution, either by briefly vortexing it or inverting the tube few times.
7. Incubate the complexes at rest and room temperature for 15 minutes.
8. Add the 100 mL PEIpro®/DNA mix to the cells.
9. Incubate cells at appropriate temperature, shaking and CO2 levels (*e.g.* 37°C, 130 rpm, 8%) and harvest virus when required.



2. Transfection protocol for virus production in adherent cells

PEIpro® is ideal for virus production in adherent cells grown in plates, flasks, roller-bottles, cell-factories, and bioreactors. PEIpro® is compatible with the use of serum and antibiotics in the cell culture medium. For co-transfection of multiple plasmids, the suitable plasmid ratio depends on the virus produced, 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 vessel.

2.1 Cell seeding

Cell seeding must be adjusted according to your cell culture vessel and process. For optimal transfection conditions with PEIpro®, we recommend seeding the cells the day before transfection to reach 50-80% confluent cells on the day of transfection.

The cell density is crucial to determine transfection conditions. Thus, we recommend dedicating a separate well or plate to measure cell density at the time of transfection.

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	50 000 ± 25 000 cells/cm²

The optimal seeding is dependent on cell subclone (doubling time), culture medium and time of harvest of the virus of interest.

2.2 Preparation of the complexes and transfection

We recommend measuring the VCD at the time of transfection to determine optimized transfection conditions for each transfection. Calculating the amount of DNA per million of cells will improve inter-assay reproducibility and the scale up of your process. Table 4 shows the starting conditions as well as a suggested range of optimization.

Table 4. Complexation parameters for the transfection of adherent cells.

Parameter	Recommended condition	Range of optimization
DNA amount (per 10^6 cells)	1.5 µg DNA	1 µg – 2 µg
Ratio (µg DNA : µL PEIpro®)	1:1	1:1 – 1:3
Complexation volume (% total culture volume)	10%	5% - 10%
Complexation time	15 min	10 min – 20 min
Complexation medium (without supplements)	DMEM high glucose	Own culture medium, Opti-MEM, DMEM low glucose, etc.

- *The complexation medium should contain neither Pluronic® F-68/Poloxamer 188/Kolliphor® P188 nor antibiotics.*
- *To ensure a good transfection efficiency as well as reproducibility between experiments, the incubation time is critical and should not exceed 30 minutes.*
- *We recommend using polypropylene tubes to prepare DNA/PEIpro® complexes and avoid polycarbonate ones.*

Table 5. Recommended DNA/PEIpro® ratios for various HEK-293 adherent media.

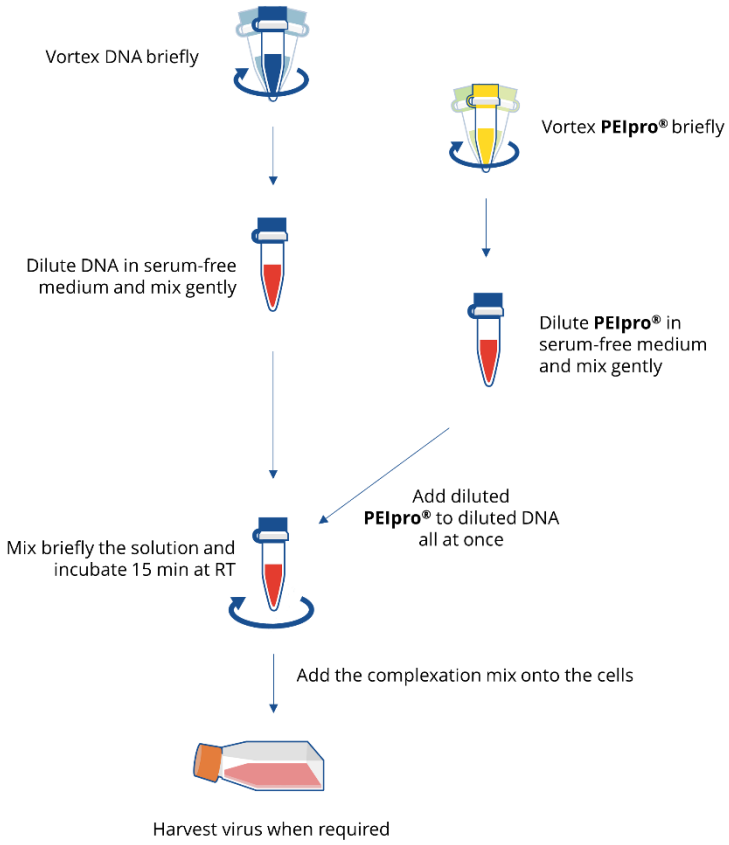
Growth medium	Starting DNA : PEIpro® ratio
DMEM	1:1 – 1:2

2.3 Transfection

Transfection parameters (*i.e.* DNA amount and PEIpro® volume) have to be adjusted according to the cell density reached at the time of transfection.

Standard protocol for the transfection of HEK-293 in a flask 75 cm²:

1. On the day of transfection, measure the cell density and determine transfection parameters (DNA amount and PEIpro® volume per million cells) according to Table 4. For this example, ***we assume a VCD of 75 000 cells / cm².***
2. Dilute 8.5 µg of DNA in serum-free medium to a final volume of 500 µL. Vortex gently.
3. Vortex PEIpro® reagent briefly.
4. Dilute 8.5 µL of PEIpro® in serum-free medium to a final volume of 500 µL. Vortex gently.
5. Add the 500 µL PEIpro® solution onto the 500 µL DNA solution all at once.
6. Mix immediately the solution, either by briefly vortexing the solution or inverting the tube 3-4 times.
7. Incubate the complexes at rest and room temperature for 15 minutes.
8. Add the 1 mL PEIpro®/DNA mix dropwise to the cells in 10 mL of medium and homogenize by gently swirling the plate.
9. Return the plates to the cell culture incubator.
10. Incubate cells at appropriate temperature and CO2 levels (*e.g.* 37°C, 5%) and harvest virus when required.



3. Transfection protocol for protein production in suspension CHO cells

3.1 Cell seeding

We recommend following the cell seeding strategy mentioned in section 1.1.

3.2 Preparation of the complexes and transfection

The following protocol is given for the transfection of DNA coding for a protein of interest into CHO cells grown in suspension.

Table 5. Complexation parameters for the transfection of suspension cells.

Parameter	Recommended condition	Range of optimization
DNA amount (per 10^6 cells)	1 µg DNA	0.5 µg – 2 µg
Ratio (µg DNA : µL PEIpro®)	1:2	1:1 – 1:6
Complexation volume (% total culture volume)	10%	5% - 10%
Complexation time	15 min	10 min – 20 min
Complexation medium (without supplements)	Opti-MEM	Own culture medium, DMEM, etc.

- *The complexation medium should contain neither Pluronic® F-68/Poloxamer 188/Kolliphor® P188 nor antibiotics.*
- *To ensure a good transfection efficiency as well as reproducibility between experiments, the incubation time is critical and should not exceed 30 minutes.*
- *We recommend using polypropylene tubes to prepare DNA/PEIpro® complexes and avoid polycarbonate ones.*

3.3 Transfection

Transfection parameters (*i.e.* DNA amount and PEIpro® volume) have to be adjusted according to the cell density reached at the time of transfection.

Standard protocol for the transfection of CHO cells in 30 mL of cell culture medium:

1. On the day of transfection, measure the cell density and determine transfection parameters (DNA amount and PEIpro® volume per million cells) according to Table 5 & 6. For this example, ***we assume a VCD of 2 x 10^6 cells / mL.***
2. Dilute 60 µg of DNA in serum-free medium (without supplements) to a final volume of 1.5 mL. Vortex gently.
3. Vortex PEIpro® reagent briefly.
4. Dilute 120 µL of PEIpro® in serum-free medium (without supplements) to a final volume of 1.5 mL. Vortex gently.
5. Add the 1.5 mL PEIpro® solution onto the 1.5 mL DNA solution all at once.

6. Mix immediately the solution, either by briefly vortexing the solution or inverting the tube 3-4 times.
7. Incubate the complexes at rest and room temperature for 15 minutes.
8. Add the 3 mL PEIpro®/DNA mix to the cells.
9. Incubate cells at appropriate temperature and CO2 levels (*e.g.* 37°C, 130 rpm, 8%) and harvest protein when required.

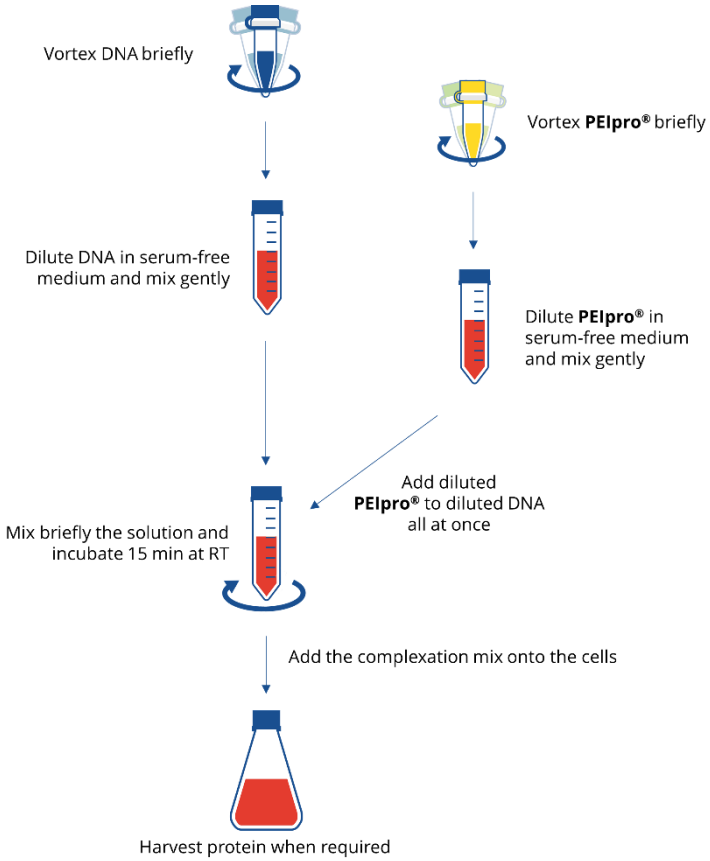


Table 6. Recommended DNA/PEIpro® ratios for various CHO serum-free media.

Growth medium	Starting DNA : PEIpro® ratio
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

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 scientific support online for tips and advice: support@polyplus-transfection.com.

4. Troubleshooting

Observations	Actions
Low viral titer or low protein yields	• Optimize the PEIpro® to DNA ratio. • Optimize the amount of plasmid DNA per million cells. • Optimize the ratio between the different plasmids used. • If using serum-free medium, ensure that the medium is permissive to transfection. • Use a positive control such as a plasmid encoding for a common reporter gene (Luciferase, GFP, control antibody, etc…). • For adherent cells grown in suspension, adapt the cells to grow in suspension in serum-free medium for several days before performing transfection. • Use high-quality plasmid preparation, free of proteins and RNA ($OD_{260/280}$ > 1.8). • For adherent cells, ensure that cells are at 50-80% confluency at the time of transfection.
Cellular toxicity	• Optimize the DNA to PEIpro® ratio by decreasing the PEIpro® volume. • Check the DNA concentration and decrease the amount of plasmid DNA used, keeping the DNA to PEIpro® ratio constant. • For adherent cells, change medium 4 to 6 hours after transfection. • 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. • For suspension cells, dilute the cell culture up to 2 folds, 4 hours after transfection. • Check the toxicity of the expressed virus/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.
Scale-up concerns	• Contact us for tips and advice: support@polyplus-transfection.com

5. Product information

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

5.1 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® should be stored at 4°C upon arrival to ensure long term stability.
- Stability: This product will stay within its specifications for at least one year 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.

5.2 Reagent use and limitations

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

5.3 Protocol suitable for PEIpro®, PEIpro®-HQ and PEIpro®-GMP reagents

This protocol is suitable for all different quality grades of the PEIpro® product range: PEIpro®, PEIpro®-HQ and PEIpro®-GMP.

5.4 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. Detailed results of Quality Controls (QCs) are displayed on PEIpro® Certificate of Analysis are available online in your Customer Area: https://myaccount.polyplus-transfection.com/wp-login.php.

For PEIpro®-HQ and PEIpro®-GMP, please refer to the provided documentation.

5.5 Ordering information

Part N°	Reagent	Average volume of transfection
101000017	1.5 mL	Transfection of 0.75 Liter of cell culture
101000033	10 mL	Transfection of 5 Liters of cell culture
101000026	100 mL	Transfection of 50 Liters of cell culture

5.6 Trademarks

Polyplus-transfection and PEIpro are registered trademarks of Polyplus-transfection. HYPERFlask and HYPERStack are registered trademarks of Corning Incorporated, Corning, NY. Pluronic is a registered trademark of BASF. FreeStyle, Expi293, and CD-FortiCHO are trademarks of Life Technologies Corporation. Pro293, Power-CHO and Pro-CHO are trademarks of Lonza Group. HyClone, HyCell and TransFx are trademarks of GE Healthcare.

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

5.7 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
- Fax: +33 3 90 40 61 81
- Addresses:

Polyplus-transfection®		
Vectura 75, rue Marguerite Perey 67400 Illkirch France	1251 Ave of the Americas 34th fl. New-York - NY 10020 United States	Room 1506, Tower B, Sunyoung Center No. 28 Xuanhua Road Changning District, Shanghai China

Please note that the Polyplus-transfection® support is available by phone from 9:00 am to 5:00 pm CEST.