FectoPRO®

in vitro DNA transfection reagent

PROTOCOL

DESCRIPTION

FectoPRO® transfection kit is specifically designed for enhanced Transient Gene Expression using low DNA amounts, in suspension CHO and HEK-293 cells as well as their derivatives in various serum-free media. FectoPRO® and FectoPRO® Booster are guaranteed free of components of animal origin. This kit is perfectly suited for small to large scale Bioproduction of recombinant proteins and antibodies. FectoPRO® transfection kit provides excellent protein and antibody production yields, while ensuring reproducible results.

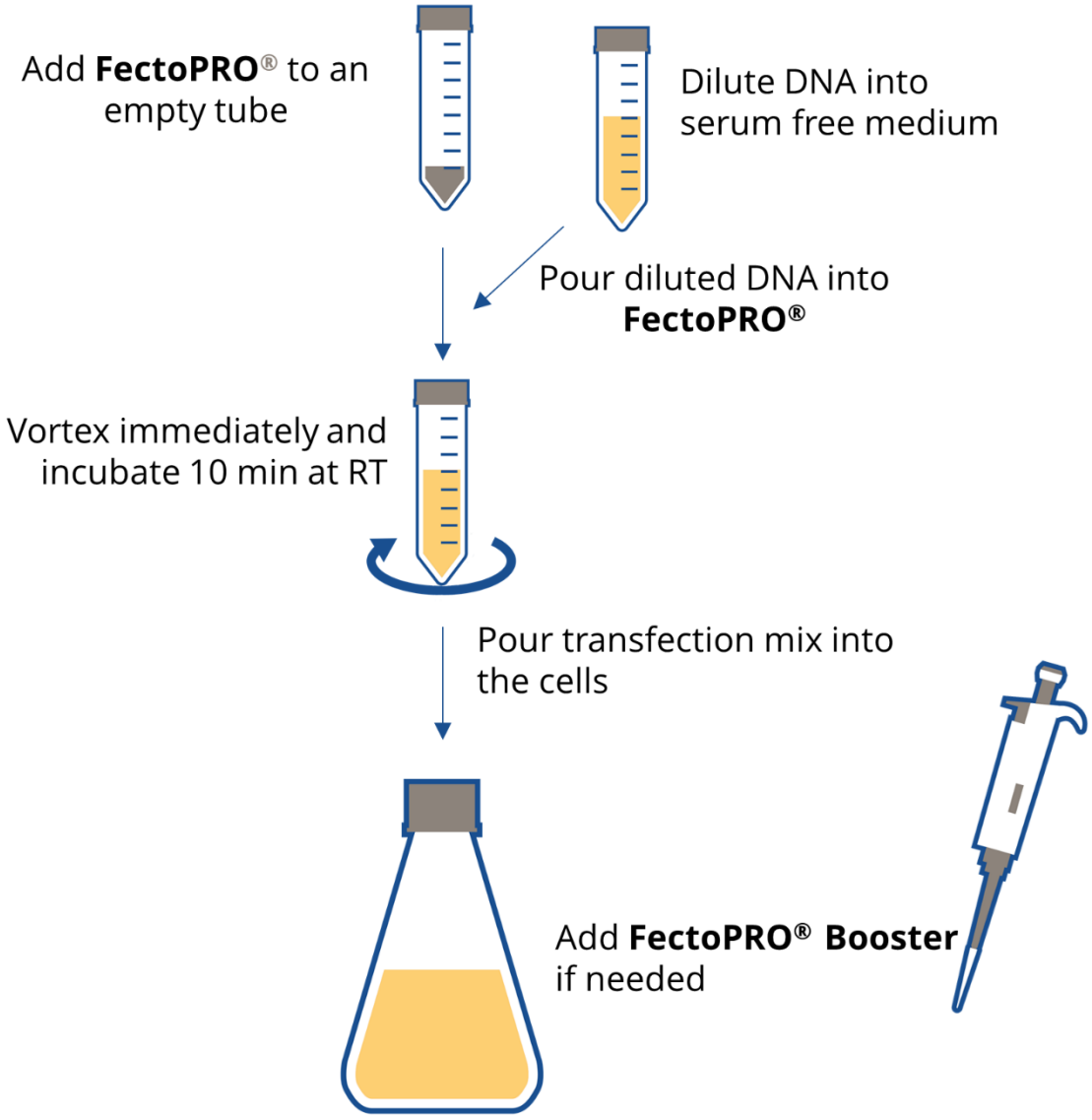
1 TRANSIENT TRANSFECTION PROTOCOL

FectoPRO® kit is perfectly suited for DNA transfection of CHO and HEK-293 cells grown in suspension under agitation in serum-free media in deep-well plates, filter top tubes, shaker flasks, spinners, cell culture bags or bioreactors.

1.1 PREPARATION OF THE CELLS

The day before transfection, prepare a cell suspension at 1 x 10^6 cells / mL by centrifuging the cells and resuspending them in fresh, pre-warmed serum-free medium. On the day of transfection, cell density does not need to be readjusted.

1.2 TRANSFECTION PROTOCOL



1.3 TRANSFECTION OF CHO CELLS AND DERIVATIVES

1.3.1 STANDARD CONDITIONS

As a starting point, we suggest testing conditions A and B indicated in the Table 1. We recommend using FectoCHO™ CD Expression Medium (Ref# 716-XX). Please find specific conditions for various CHO media in Table 2, including the FectoCHO™ CD Expression Medium.

We recommend preparing the complexes in DMEM high glucose and the transfection mix should represent 10 % of the final volume of cell culture. Other complexation media can be used such as the serum-free medium used to grow the cells or OptiMEM®.

Table 1. Starting conditions for transfection of suspension CHO cells (conditions **per mL** of culture medium).

	Amount of DNA	Volume of FectoPRO® reagent	DNA to FectoPRO® ratio (µg / µL)	Volume of serum-free medium for complexes preparation	Volume of FectoPRO® Booster*
Condition A	0.5 µg	1.0 µL	1 : 2	0.1 mL	0.5 µL
Condition B	0.8 µg	1.6 µL	1 : 2	0.1 mL	-

* Addition of the FectoPRO® Booster is optional and is specifically recommended for enhanced Transient Gene Expression when using low amounts of DNA.

- *The complexation medium should contain neither Pluronic® F-68/Poloxamer 188/Kolliphor® P188 nor antibiotics.*
- *We recommend using polypropylene tubes to prepare DNA/FectoPRO® complexes and avoid polycarbonate ones.*

For transfection of CHO derivatives such as CHO-K1 you may need to optimize the DNA and reagent amounts. Feel free to contact Scientific Support for more details on condition to use at: support@polyplus-transfection.com or call us at +33 3 90 40 61 87.

The following protocol is given for transfection of CHO cells grown in 100 mL of FectoCHO™ CD Expression Medium, using condition A (Table 1). Condition B should be tested using the same approach.

1. *The day before transfection,* prepare 90 mL of cell suspension at 1 x 10^6 cells/mL. Incubate cells overnight at appropriate temperature, shaking and CO_2 levels (e.g. 37 °C, 125 rpm, 8 %).
2. *On the day of transfection*, vortex FectoPRO® reagent for 5 sec and spin down before adding 100 µL of FectoPRO® to an empty 50 mL tube (we recommend using polypropylene tubes).
3. In a second 50 mL tube, dilute 50 µg of DNA in serum-free medium (the medium should contain neither Pluronic® F-68/Poloxamer 188/Kolliphor® P188 nor antibiotics) to a final volume of 10 mL. Homogenize gently.

4. Pour the diluted DNA into the pure FectoPRO® reagent all at once. Homogenize the solution immediately and incubate for 10 minutes at room temperature.
5. Pour the FectoPRO®/DNA transfection mix onto the cells, homogenize the culture.
6. If FectoPRO® Booster is to be added, add 50 µL (final concentration 0.5 µL/mL) directly to the cell culture 0 to 4 hours post-transfection, homogenize.
7. Incubate cells at appropriate temperature, shaking and CO_2 levels (e.g. 37 °C, 125 rpm, 8 %) and harvest protein or antibody when required.

The conditions below have been given for the use of FectoPRO® in various commercially available CHO culture media.

Table 2. Starting conditions for transfection of suspension CHO cells in various serum-free media (conditions **per mL** of culture medium).

Growth medium	Amount of DNA	Volume of FectoPRO® reagent	DNA to FectoPRO® ratio (µg / µL)	Volume of FectoPRO® Booster
FectoCHO™ CD Expression Medium*	0.5 µg	1 µL	1 : 2	0.5 µL
	0.8 µg	1.6 µL	1 : 2	-
FreeStyle™ CHO, HyClone™ HyCell™ TransFx™-C, CD-FortiCHO™, CD-CHO™	0.5 µg	1 µL	1 : 2	0.5 µL
	0.8 µg	1.2 µL	1 : 1.5	-
FreeStyle™ F17	0.5 µg	1.25 µL	1 : 2.5	0.5 µL
	0.8 µg	1.2 µL	1 : 1.5	-
CHO-S-SFM II	0.5 µg	1.5 µL	1 : 3	0.5 µL
	0.8 µg	2 µL	1 : 2.5	-
Pro-CHO™4	1 µg	1.5 µL	1 : 1.5	0.25 µL
	1.5 µg	1.5 µL	1 : 1	0.25 µL

* FectoCHO™ CD Expression Medium is a chemically defined medium that allows a fast and easy cell adaptation. It is included in the FectoCHO™ Expression System (more information can be found online: https://www.polyplus-transfection.com/products/fectocho/) or it can be purchased separately.

Addition of the FectoPRO® Booster is optional and is specifically recommended for enhanced Transient Gene Expression when using low amounts of DNA.

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.

1.3.2 FECTOPRO® TRANSFECTION IN THE EXPICHO™ SYSTEM.

As a starting point, we suggest testing conditions indicated in the table 3.

Table 3. Starting conditions for transfection of ExpiCHO™-S cells grown in ExpiCHO™ medium (conditions **per mL** of culture medium).

Growth medium	Amount of DNA	Volume of FectoPRO® reagent	DNA to FectoPRO® ratio (µg / µL)	Volume of FectoPRO® Booster
ExpiCHO™	0.9 µg	2.7 µL	1 : 3	-

- *The complexation medium should contain neither Pluronic® F-68/Poloxamer 188/Kolliphor® P188 nor antibiotics.*
- *We recommend using polypropylene tubes to prepare DNA/PEIpro® complexes and avoid polycarbonate ones.*

The following protocol is given for transfection of ExpiCHO™ cells grown in 25 mL of ExpiCHO™ Expression Medium.

1. *Three to four days before transfection,* prepare 30 mL of cell suspension at 0.3 x 10^6 cells/mL. Incubate cells at appropriate temperature, shaking and CO_2 levels (e.g. 37°C, 125 rpm, 8 %).
2. *On the day of transfection*, dilute the cells at the density of **6 x 10^6 cells/mL** with fresh medium to a final volume of 25 mL.
3. Dilute 25 µg of DNA in Opti-MEM™ I Reduced Serum Medium to a final volume of 3 mL. Homogenize gently.
4. In a second 50 mL tube, add 75 µL of FectoPRO®.
5. Pour the diluted DNA into the pure FectoPRO® reagent all at once. Homogenize the solution immediately and incubate for 10 minutes at room temperature.
6. Pour the FectoPRO®/DNA transfection mix onto the cells, homogenize the culture.
7. Incubate cells at appropriate temperature, shaking and CO_2 levels (e.g. 37°C, 125 rpm, 8 %) and harvest protein or antibody when required.

1.3.3 OPTIMIZATION GUIDELINES

In the optimization phase, the amounts of plasmid DNA and FectoPRO® reagent may be adjusted as follows: for low DNA amounts, we recommend using a higher DNA to FectoPRO® ratio, whereas for higher amounts of DNA, lower DNA to FectoPRO® ratio can be used.

For more details see Troubleshooting section.

1.4 TRANSFECTION IN HEK-293 CELLS AND DERIVATIVES

1.4.1 STANDARD CONDITIONS

As a starting point, we recommend testing conditions C and D indicated in the Table 4. Please find further optimization for specific HEK-293 media in Table 5.

The transfection mix should be prepared in the same serum-free medium as the one used to grow the cells and should represent 10% of the final volume of cell culture.

Table 4. Starting conditions for transfection of suspension HEK-293 cells and derivatives (conditions **per mL** of culture medium)

	Amount of DNA	Volume of FectoPRO® reagent	DNA to FectoPRO® ratio (µg / µL)	Volume of serum-free medium for complexes preparation	Volume of FectoPRO® Booster*
Condition C	0.5 µg	0.75 µL	1 : 1.5	0.1 mL	0.5 µL
Condition D	0.8 µg	0.8 µL	1 : 1	0.1 mL	-

* Addition of the FectoPRO® Booster is optional and is specifically recommended for enhanced Transient Gene Expression when using low amounts of DNA.

- *The complexation medium should contain neither Pluronic® F-68/Poloxamer 188/Kolliphor® P188 nor antibiotics.*
- *We recommend using polypropylene tubes to prepare DNA/FectoPRO® complexes and avoid polycarbonate ones.*

The following protocol is given for transfection of HEK-293 cells grown in 100 mL of cell culture medium according to condition C (Table 3). Condition D should be tested using the same approach.

1. *The day before transfection*, prepare 90 mL of cell suspension at 1 x 10^6 cells/mL. Incubate cells overnight at appropriate temperature, shaking and CO_2 levels (e.g. 37 °C, 125 rpm, 8 %).
2. *On the day of transfection*, vortex FectoPRO® reagent for 5 sec and spin down before adding 75 µL of FectoPRO® to an empty 50 mL tube (we recommend using polypropylene tubes).
3. In a second 50 mL tube, dilute 50 µg of DNA in serum-free medium (the medium should contain neither Pluronic® F-68/Poloxamer 188/Kolliphor® P188 nor antibiotics) to a final volume of 10 mL. Vortex gently.
4. Pour the diluted DNA to the pure FectoPRO® reagent all at once. Homogenize the solution immediately and incubate for 10 minutes at room temperature.

5. Pour the 10 mL FectoPRO®/DNA transfection mix to the cells, homogenize the culture.
6. If FectoPRO® Booster is to be added, add 50 µL directly to the cell culture 0 to 4 hours post-transfection, homogenize.
7. Incubate cells at appropriate temperature, shaking and CO_2 levels (e.g. 37 °C, 125 rpm, 8 %) and harvest protein or antibody when required.

The conditions below have been given for the use of FectoPRO® in various commercially available HEK-293 culture media.

Table 5. Starting conditions for transfection of suspension HEK-293 cells and derivatives in various serum-free media (conditions **per mL** of culture medium).

Growth medium	Amount of DNA	Volume of FectoPRO® reagent	DNA to FectoPRO® ratio (µg / µL)	Volume of FectoPRO® Booster*
FreeStyle™ 293, HyClone™ HyCell™ TransFx™-H, FreeStyle™ F17	0.5 µg	0.75 µL	1 : 1.5	0.5 µL
	0.8 µg	0.8 µL	1 : 1	-
Pro293™	1 µg	1 µL	1 : 1	-
	1 µg	1.5 µL	1 : 1.5	-
Expi293™	0.5 µg	1 µL	1 : 2	-
	0.8 µg	1.2 µL	1 : 1.5	-

* Addition of the FectoPRO® Booster is optional and is specifically recommended for enhanced Transient Gene Expression when using low amounts of DNA.

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.

1.4.2 OPTIMIZATION GUIDELINES

In the optimization phase, the amounts of plasmid DNA and FectoPRO® reagent may be adjusted as follows: for low DNA amounts, we recommend using a higher DNA to FectoPRO® ratio, whereas for higher amounts of DNA, lower DNA to FectoPRO® ratio can be used.

For more details see Troubleshooting section.

2 STABLE TRANSFECTION PROTOCOL

FectoPRO® is suitable for stable DNA transfection.

1. If needed, linearize plasmid DNA construct encoding antibiotic resistance.
2. Perform transfection as described in the standard protocol in Sections 1.3. or 1.4.
3. Start antibiotic selection 24 – 48 h after transfection.
4. Maintain antibiotic selection as long as required.
5. Check for integration of the plasmid DNA or stable expression of your protein of expression.
6. Harvest protein or antibody when required.

3 TROUBLESHOOTING

Observations	Actions
Low protein yields / Low transfection efficiency	• Optimize the FectoPRO® volume depending on the medium used up to 3 µL per µg of DNA (see Tables 2 and 4). • Optimize the amount of plasmid DNA up to 1.5 µg/mL of cell culture. • Optimize the volume of FectoPRO® Booster from 0.25 to 1 µL per mL of cell culture. • Ensure that the medium used allows high transfection efficiency. For CHO cells, we recommend using FectoCHO™ CD Expression Medium (Ref# 716-XX). • Optimize the volume of serum-free medium for complexes preparation from 5 to 25°% of the final volume. • Prepare the transfection mix in a medium optimized for transfection, such as OptiMEM® or DMEM, instead of serum-free medium. • Optimize the cell seeding from 0.5 to 1 x 10^6 cells/mL the day before transfection. • CHO cultures can be placed at lower temperature (32 °C), 4 to 24 hours after adding the transfection mix to improve productivity. • Use high-quality plasmid preparation, free of proteins, RNA ($OD_{260/280}$ > 1.8) and endotoxins. • Use a positive control such as a plasmid encoding for a common reporter gene (Control Antibody, GFP, Luciferase, etc…).
Cellular toxicity	• Decrease the amount of plasmid DNA down to 0.4 µg per mL cell culture. • Decrease the FectoPRO® amount (down to 1 µL/µg DNA). • Optimize the volume of the FectoPRO® Booster or do not use it. • Prepare the transfection mix in a higher volume of serum-free medium, up to 25 % of the final volume. • Dilute the cell culture up to 2 folds, 4 to 24 hours after transfection. • Add fresh medium or feeder nutrients post-transfection. • Make sure that the plasmid preparation is endotoxin-free. • CHO cultures can be placed at lower temperature (32 °C), 4 to 24 hours after adding the transfection mix to improve cell viability.

4 PRODUCT INFORMATION

4.1 ORDERING INFORMATION

Ref. N°	FectoPRO® Reagent	Booster
116-001	1 mL	1 mL
116-010	10 mL	10 mL
116-100	100 mL	100 mL

4.2 CONTENT

FectoPRO® transfection kit contains FectoPRO® reagent and FectoPRO® Booster. 1 mL of FectoPRO® transfection reagent is sufficient to transfect approximately 1 L of cell culture.

4.3 REAGENT USE AND LIMITATIONS

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

4.4 QUALITY CONTROL

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.

All lots of FectoPRO® kit are tested during and after manufacturing to guarantee accurate chemical composition and to ensure constant quality and lot-to-lot reproducibility. FectoPRO® kit efficacy is evaluated in a DNA transfection experiment on suspension CHO cells, followed by quantification of protein production.

4.5 FORMULATION AND STORAGE

- Volume: each vial/bottle contains the specified volume ± 3 %.
- FectoPRO® and FectoPRO® Booster are chemically-defined and guaranteed free of components of animal origin.
- FectoPRO® and FectoPRO® Booster should be stored at 4°C upon arrival to ensure long term stability. FectoPRO® and FectoPRO® booster, as guaranteed and indicated in the Certificate of Analysis, are stable for at least one year when stored appropriately.

4.6 TRADEMARKS

Polyplus-transfection® and FectoPRO® are registered trademarks of Polyplus-transfection® S.A.
Pluronic and Kolliphor are registered trademarks of BASF. OptiMEM, FreeStyle, Expi293, CD-CHO and CD-FortiCHO are trademarks of Life Technologies Corporation. Pro293 and Pro-CHO are trademarks of Lonza Group. HyClone, HyCell and TransFx are trademarks of GE Healthcare.

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

4.7 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® S.A	Polyplus-transfection® Inc.
Bioparc 850 Bd S. Brant 67400 Illkirch France	1251 Ave of the Americas 34th fl. New-York - NY 10020 United States

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