FectoPRO™

DNA transfection reagent for Bioproduction

PROTOCOL

DESCRIPTION

FectoPRO™ transfection kit is specifically designed for enhanced Transient Gene Expression using low DNA amount, 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 largely outperforms other transfection reagents commonly used in Bioproduction processes and guarantees excellent protein and antibody production yields, while ensuring reproducible results.

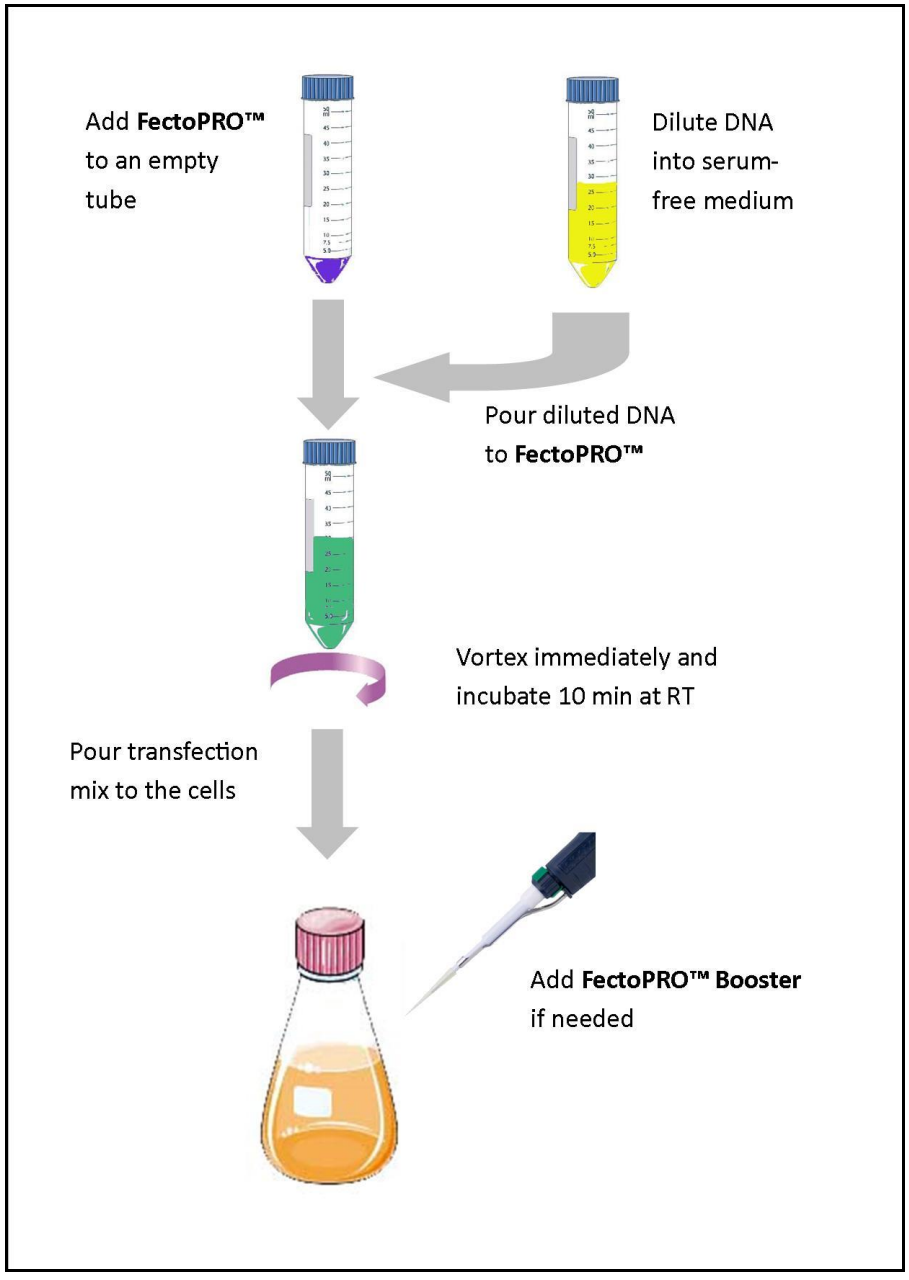
1. TRANSIENT TRANSFECTION PROTOCOL

FectoPRO™ kit is perfectly suited for DNA transfection of HEK-293 and CHO cells grown in suspension under agitation in serum-free media in deep-well plates, agitated 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 OVERVIEW



1.3 TRANSFECTION IN CHO CELLS AND DERIVATIVES

As starting conditions, we recommend testing conditions A and B, as indicated in Table 1.

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 1. Starting conditions for transfection of CHO suspension 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 A	0.5 µg	1.0 µl	1 : 2	0.1 ml	0.5 µl
Condition B	0.8 µg	1.2 µl	1 : 1.5	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 following protocol is given for transfection of CHO cells in 100 ml of cell culture 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*, add 100 µl of FectoPRO™ to an empty 50 ml tube.
3. In a second tube, dilute 50 µg of DNA in serum-free medium (the medium should contain neither Pluronic® F-68 nor antibiotics) to a final volume of 10 ml. Vortex gently.
4. Pour the diluted DNA to the pure FectoPRO™ reagent all at once.
5. Vortex the solution immediately.
6. Incubate for 10 minutes at room temperature.
7. Pour the FectoPRO™/DNA transfection mix to the cells, homogenize the culture.
8. If FectoPRO™ Booster is to be added, add 50 µl directly to the cell culture 0 to 4 hours post-transfection, homogenize.
9. Incubate cells at appropriate temperature, shaking and CO_2 levels (e.g. 37°C, 125 rpm, 8%) and harvest protein or antibody when required.

In an 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.

The volume of FectoPRO™ Booster to be added should be optimized depending on the medium and the cell system used.

Table 2. Optimization conditions for transfection of suspension CHO cells and derivatives (conditions **per ml** of culture medium)

Amount of DNA	DNA to FectoPRO™ ratio (µg / µl)	Volume of FectoPRO™ Booster
0.5 ± 0.1 µg	1 : 2	0.25 – 0.75 µl
0.8 ± 0.2 µg	1 : 1.5	0 – 0.75 µl

NOTE: Some serum-free media may inhibit transfection. Please ensure that the medium you are using allows for efficient transfection. Feel free to contact Polyplus-transfection technical support online for tips and advices: support@polyplus-transfection.com.

1.4 TRANSFECTION IN HEK-293 CELLS AND DERIVATIVES

As starting conditions, we recommend testing conditions C and D, as indicated in Table 3.

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 3. 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	0.45 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 following protocol is given for transfection of HEK293 cells 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*, add 75 µl of FectoPRO™ to an empty 50 ml tube.
3. In a second tube, dilute 50 µg of DNA in serum-free medium (the medium should contain neither Pluronic® F-68 nor antibiotics) to a final volume of 10 ml. Vortex gently.
4. Pour the diluted DNA to the pure FectoPRO™ reagent all at once.
5. Vortex the solution immediately.
6. Incubate for 10 minutes at room temperature.
7. Pour the 10 ml FectoPRO™/DNA transfection mix to the cells, homogenize the culture.
8. If FectoPRO™ Booster is to be added, add 45 µl directly to the cell culture 0 to 4 hours post-transfection, homogenize.
9. Incubate cells at appropriate temperature, shaking and CO_2 levels (e.g. 37°C, 125 rpm, 8%) and harvest protein or antibody when required.

In an 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.

The volume of FectoPRO™ Booster to be added should be optimized depending on the medium and the cell system used.

Table 4. Optimization conditions for transfection of suspension HEK293 cells and derivatives (conditions **per ml** of culture medium)

Amount of DNA	DNA to FectoPRO™ ratio (µg/µl)	Volume of FectoPRO™ Booster
0.5 ± 0.1 µg	1 : 1.5	0.3 – 0.6 µl
0.8 ± 0.2 µg	1 : 1	0 – 0.6 µl

NOTE: Some serum-free media may inhibit transfection. Please ensure that the medium you are using allows for efficient transfection. Feel free to contact Polyplus-transfection technical support online for tips and advices: support@polyplus-transfection.com.

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	**Troubleshooting** (Contact us for tips and advices: support@polyplus-transfection.com)
Low protein yields / Low transfection efficiency	✚ Optimize the FectoPRO™ volume up to 2 µl per µg of DNA. ✚ Optimize the amount of plasmid DNA up to 1 µg/ml of cell culture. ✚ Optimize the volume of FectoPRO™ Booster from 0.25 to 1 µl per ml of cell culture. ✚ 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®, instead of serum-free medium. ✚ Ensure that the medium used allows for high transfection efficiency. ✚ Optimize the cell seeding from 0.5 to 1 x 10^6 cells/ml the day before transfection ✚ 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…) ✚ CHO cultures can be placed at lower temperature (32°C), 4 to 24 hours after adding the transfection mix to improve productivity.
Cellular toxicity	✚ Decrease the FectoPRO™ amount down to 1.25 µl per µg of DNA for CHO cells and down to 1 µl per µg of DNA for HEK-293 cells. ✚ Decrease the amount of plasmid DNA down to 0.4 µg per ml cell culture. ✚ 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.

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 (0)3 90 40 61 87

4. PRODUCT INFORMATION

4.1 ORDERING INFORMATION

Cat. #	FectoPRO™ Reagent	FectoPRO™ Booster
116-001	1 ml	1 ml
116-010	10 ml	10 ml
116-100	10 x 10 ml	10 x 10 ml
704-10	-	10 ml

4.2 CONTENT

FectoPRO™ transfection reagent is provided with FectoPRO™ Booster.

1 ml of FectoPRO™ transfection reagent is sufficient to transfect 1 L of cell culture.

4.3 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 are shipped at room temperature but 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.

4.4 REAGENT USE AND LIMITATIONS

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

4.5 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.