FectoCHO™ Expression system

DNA transfection kit for protein production

PROTOCOL

DESCRIPTION

FectoCHO™ Expression system is a three-component kit that includes FectoPRO® transfection reagent, FectoPRO® Booster and FectoCHO™ CD Expression Medium. FectoCHO™ Expression system is a complete and easy solution specifically designed for enhanced Transient Gene Expression using low DNA amounts, in all types of suspension CHO cells, such as ExpiCHO-S™, CHO-S, and CHO-K1 cells. FectoCHO™ CD Expression Medium is a chemically defined animal component free medium that allows fast and easy cell adaptation. It has been specifically developed to work in synergy with FectoPRO® to provide amazing protein and antibody production yields. This kit is perfectly suited for small to large scale production of recombinant proteins using transient gene expression as no feeding strategy is needed. It outperforms all commercially available CHO expression systems, in plug-and-play system thus easy-to-use.

1. THAWING AND CRYOPRESERVATION OF CELLS

1.1 ADAPTATION TO FECTOCHO™ CD EXPRESSION MEDIUM

- Cryopreserved Cell Stock

When bringing suspension CHO cells out of cryopreservation, use FectoCHO™ CD Expression Medium supplemented with 8 mM L-Glutamine and 0.1 % Poloxamer 188 to dilute cells immediately post-thaw. Please note that these components are not included in the kit. We recommend thawing 1 x 10^7 cells in 30 mL of FectoCHO™ CD Expression Medium. Incubate cells in a shake flask at an appropriate rpm at 37°C, 8% CO_2. Monitor cell growth and viability daily. When viability reaches > 95% and the cells are doubling every ≤ 24 hours, the cells are fully adapted.

- Ongoing Culture

If cells are currently being cultured in a different medium, subculture cells directly in 100% FectoCHO™ CD Expression Medium. Incubate cells in a shake flask at an appropriate rpm at 37°C, 8% CO_2. Monitor cell growth and viability daily; when viability reaches > 95% and the cells are doubling every ≤ 24 hours, the cells are fully adapted.

1.2 MAINTENANCE OF SUSPENSION CHO AND EXPICHO-S™ CELLS

- ExpiCHO-S™cells

Cells should be subcultured to a density of 0.5 x 10^6 or 0.3 x 10^6 cells/mL, respectively during 3 and 4 days. Cells should grow higher than 4 – 6 x 10^6 cells/mL.

- CHO cells

Cells should be subcultured to a density of 0.5 x 10^6 or 0.3 x 10^6 cells/mL, respectively during 2 and 3 days. Cells should not grow to a density higher than 1 x 10^7 cells/mL or lower than 2 - 2.5 x 10^6 cells/mL.

1.3 CRYOPRESERVATION OF CHO AND EXPICHO-S™ CELLS

ExpiCHO-S™ and other CHO cells can be frozen directly in FectoCHO™ CD Expression Medium supplemented with 8 mM L-Glutamine and 0.1% Poloxamer 188. Before freezing, make sure that cells attain a viability >95%. Centrifuge and resuspend cells at a density of 1 x 10^7 viable cells/mL with 90% ice cold FectoCHO™ CD Expression Medium and 10% DMSO. Freeze the cells with a freezing rate of 1°C per minute, transfer frozen vials to liquid nitrogen for long-term storage.

2. TRANSIENT TRANSFECTION PROTOCOL

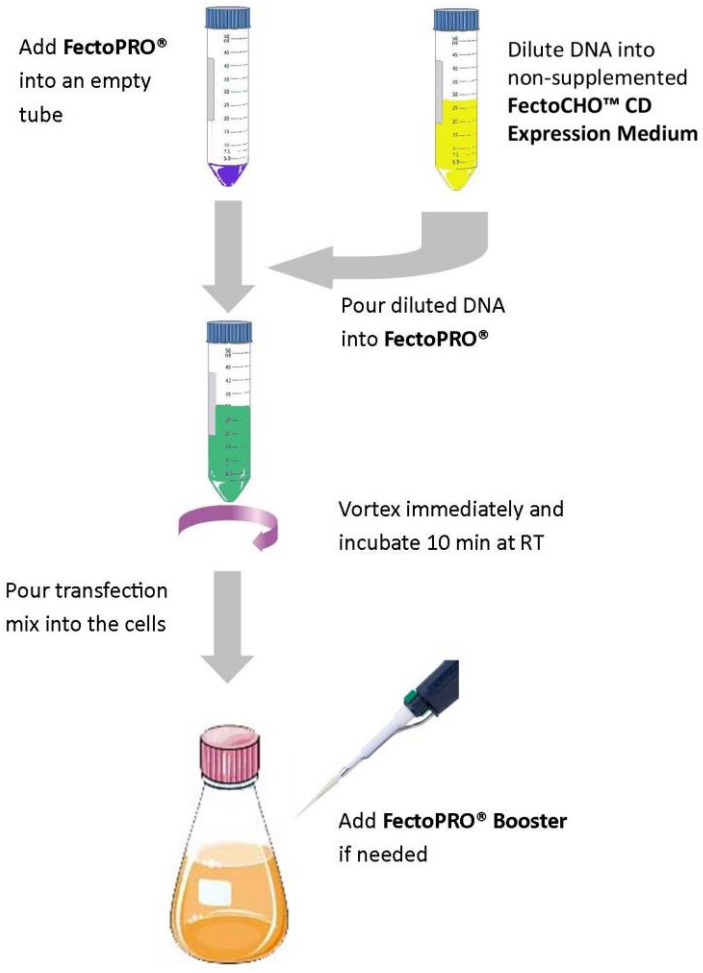
FectoCHO™ Expression system is perfectly suited for DNA transfection of all types of suspension CHO cells, such as ExpiCHO-S™, CHO-S, CHO-K1 cells grown in suspension under agitation in deep-well plates, filter top tubes, shake flasks, spinners, cell culture bags or bioreactors.

2.1 TRANSIENT TRANSFECTION OF EXPICHO-S™ CELLS

2.1.1 PREPARATION OF EXPICHO-S™ CELLS

The day of transfection, prepare a cell suspension at 6 x 10^6 cells/mL by centrifuging the cells and resuspending them in fresh, pre-warmed FectoCHO™ CD Expression Medium supplemented with 8 mM L-Glutamine and 0.1% Poloxamer 188.

2.1.2 TRANSFECTION OVERVIEW



2.1.3 TRANSFECTION OF EXPICHO-S™ CELLS

Standard conditions

As a starting point, we recommend testing a condition optimized for ExpiCHO-S™ cells, as indicated in Table 1. The transfection mix should be prepared in non-supplemented FectoCHO™ CD Expression Medium and should represent 10% of the final volume of cell culture.

Table 1. Starting conditions for transfection of ExpiCHO-S™ cells (conditions **per mL** of culture medium).

	Amount of DNA	Volume of FectoPRO® reagent	DNA to FectoPRO® ratio (µg / µL)	Volume of non-supplemented FectoCHO™ CD Expression Medium
Standard condition	0.8 µg	1.6 µL	1 : 2	0.1 mL

The following protocol is given for transfection of ExpiCHO-S™ cells grown in 30 mL of FectoCHO™ CD Expression Medium, using standard condition (Table 1).

1. *The day of transfection,* prepare 27 mL of cell suspension at 6 x 10^6 cells/mL in supplemented FectoCHO™ CD Expression Medium.
2. Vortex FectoPRO® reagent for 5 seconds before adding 48 µL of FectoPRO® to an empty 15 mL tube.
3. In a second 15 mL tube, dilute 24 µg of DNA in **non-supplemented FectoCHO™ CD Expression Medium** (the medium should contain neither Poloxamer 188 nor antibiotics) to a final volume of 3 mL. Homogenize gently.
4. Pour the diluted DNA into the pure FectoPRO® reagent all at once. Vortex 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. Incubate cells at appropriate temperature, shaking and CO_2 levels (e.g. 37°C, 125 rpm, 8%) and harvest protein or antibody when required.

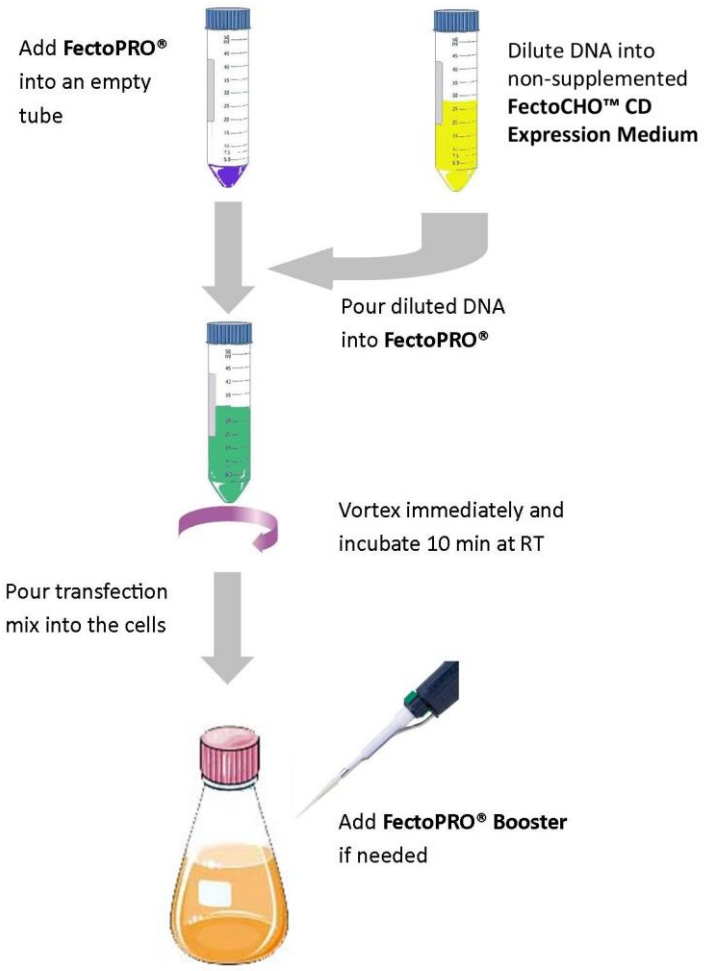
Contact our Scientific and Technical Support for tailored conditions at: support@polyplus-transfection.com or call us at +33 3 90 40 61 87.

2.2 TRANSIENT TRANSFECTION OF CHO CELLS

2.2.1 PREPARATION OF CHO 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 FectoCHO™ CD Expression Medium supplemented with 8 mM L-Glutamine and 0.1% Poloxamer 188. On the day of transfection, cell density does not need to be readjusted.

2.2.2 TRANSFECTION OVERVIEW



2.2.3 TRANSFECTION OF CHO CELLS

Standard conditions

As a starting point, we recommend testing a condition optimized for suspension CHO cells, as indicated in Table 2. A second condition allows lower DNA amount and requires addition of FectoPRO® Booster.

The transfection mix should be prepared in **non-supplemented FectoCHO™ CD Expression Medium** and should represent 10% of the final cell culture volume.

Table 2. Starting conditions for transfection of suspension CHO cells (conditions **per mL** of culture medium). The standard condition corresponds to a starting point, with DNA amount varying between 0.5 and 1.5 µg per mL of cell culture.

	Amount of DNA	Volume of FectoPRO® reagent	DNA to FectoPRO® ratio (µg / µL)	Volume of non-supplemented FectoCHO™ CD Expression Medium	Volume of FectoPRO® Booster*
Standard condition	0.8 µg	1.6 µL	1 : 2	0.1 mL	-
Low DNA condition	0.5 µg	1 µL	1 : 2	0.1 mL	0.5 µL

* 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 grown in 30 mL of FectoCHO™ CD Expression Medium, using standard condition (Table 2).

1. *The day before transfection,* prepare 27 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 seconds before adding 48 µL of FectoPRO® to an empty 15 mL tube.

3. In a second 15 mL tube, dilute 24 µg of DNA in non-supplemented FectoCHO™ CD Expression Medium (the medium should contain neither Poloxamer 188 nor antibiotics) to a final volume of 3 mL. Homogenize gently.

4. Pour the diluted DNA into the pure FectoPRO® reagent all at once. Vortex 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. Incubate cells at appropriate temperature, shaking and CO_2 levels (e.g. 37°C, appropriate rpm, 8%) and harvest protein or antibody when required.

Low DNA condition should be tested using the same approach. If FectoPRO® Booster is to be added, add 15 µL (final concentration 0.5 µL/mL) directly to the cell culture 0 to 4 hours post-transfection, homogenize.

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3. OPTIMIZATION GUIDELINES

Observations	**Guidelines** (Contact us for tips and advices: support@polyplus-transfection.com)
Cells not properly adapted to FectoCHO™ CD Expression Medium	✦ Monitor cell density and viability and make sure that viability is above 95% when starting adaptation. ✦ Adapt cells to FectoCHO™ CD Expression Medium gradually. At each passage, increase FectoCHO™ CD Expression Medium proportion by 25% and monitor density and viability daily. When viability reaches more than 95%, passage cells and increase FectoCHO™ CD Expression Medium proportion by another 25%. Repeat these steps until 100% FectoCHO™ CD Expression Medium is reached. For a more detailed protocol, contact our tech support at support@polyplus-transfection.com.
Low protein yields / Low transfection efficiency	✦ Optimize cell seeding: • CHO cells from 0.5 to 1 x 10^6 cells/mL the day before transfection. • ExpiCHO-S™ cells from 4 to 6 x 10^6 cells/mL the day of transfection. ✦ CHO and ExpiCHO-S™ 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. ✦ Make sure to use non-supplemented FectoCHO™ CD Expression Medium for complex formation (the medium should contain neither Poloxamer 188 nor antibiotics). ✦ Optimize the amount of plasmid DNA up to 1.5 µg/mL of cell culture. ✦ Optimize the FectoPRO® volume up to 3 µL per µg of DNA. ✦ Optimize the volume of FectoPRO® Booster from 0.25 to 1 µL per mL of cell culture. ✦ Optimize the volume of FectoCHO™ CD Expression Medium for complexes preparation from 5 to 25% of the final volume. ✦ 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 of cell culture. ✦ Decrease the FectoPRO® amount down to 1 µL per µg of DNA. ✦ Optimize the volume of the FectoPRO® Booster or do not use it. ✦ Prepare the transfection mix in a higher volume of FectoCHO™ CD Expression Medium, up to 25% of the final volume. ✦ Add fresh medium or feeder nutrients post-transfection. ✦ Make sure that the plasmid preparation is endotoxin-free. ✦ CHO and ExpiCHO-S™ 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 3 90 40 61 87

4. PRODUCT INFORMATION

4.1 ORDERING INFORMATION

Ref #	FectoCHO™ CD Expression Medium	FectoPRO® Transfection Reagent	FectoPRO® Booster
716-01LKIT	1 L	1 mL	1 mL
716-06LKIT	6 x 1 L	5 x 1 mL	5 x 1 mL
716-01L	1 L	-	-
716-06L	6 x 1 L	-	-
116-001	-	1 mL	1 mL
116-010	-	10 mL	10 mL
116-040	-	4 x 10 mL	4 x 10 mL
704-10	-	-	10 mL

Poloxamer 188 and L-glutamine are not included in FectoCHO™ Expression system and must be purchased separately.

4.2 CONTENT

FectoCHO™ Expression system contains FectoCHO™ CD Expression Medium, FectoPRO® reagent and FectoPRO® Booster. 1 mL of FectoPRO® transfection reagent is sufficient to transfect approx. 1 L of cell culture.

4.3 FORMULATION AND STORAGE

- Volume: each vial/bottle contains the specified volume ± 3%.
- FectoCHO™ CD Expression Medium, FectoPRO® and FectoPRO® Booster are chemically-defined and guaranteed free of components of animal origin.
- FectoCHO™ CD Expression Medium is filtered with a 0.1 µm diameter Sterile filter.
- FectoCHO™ CD Expression Medium, FectoPRO® and FectoPRO® Booster are shipped at room temperature but should be stored at 5 ± 3°C upon arrival to ensure long term stability. As guaranteed and indicated in the Certificate of Analysis, FectoPRO® and FectoPRO® booster are stable for at least one year when stored appropriately, and FectoCHO™ CD Expression Medium is stable for 1 year.

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.

Each component of FectoCHO™ Expression system is produced following formulation, test procedures and released after a validation of all defined specifications. For more information, please refer to the dedicated certificate of analysis.

TRADEMARKS