7040 Virginia Manor Road
Beltsville, MD 20705, USA
Web: www.abpbio.com;
Email: info@abpbio.com

FectCHO™ Transfection Reagent

Table 1. Product Package and Storage

Cat No.	Product Name	Amount	Storage
FP316	FectCHO™ Transfection Reagent	1 mL	4°C: one year

Introduction

FectCHO™ Transfection Reagent is a biodegradable polymer based transfection reagent that forms a complex with DNA, and transports the complex into a variety of adherent and suspension cell lines. A remarkable feature of the reagent is the rapid and complete degradation of polymer after transfection complex endocytosis, leading to much less cytotoxicity.

Feature

- Superior transfection efficiency for CHO cells.
- Does not require removal of serum or culture medium.
- Does not require washing or changing of medium after transfection.
- Low cytotoxicity.

Protocols

Important Notes:

1. For optimal transfection efficiency, dilute FectCHO™ Transfection Reagent and plasmid DNA in serum-free DMEM prior to the formation of transfection complex.
2. Make sure your plasmid DNA is in high quality and clean and sterile without contamination of phenol and salt.

Recommended Conditions for Transfection:

To transfect suspension CHO cells in their standard cuture medium, use the following optimized transfection conditions. To perform transfection experiments in a larger volume, simply scale up the volume of reagents accordingly.

1. Final transfection volume: 32 mL.
2. Number of cells to transfect: 3×10^7 cells at final cell density of 1×10^6 cells/mL cultured in standard culture medium. Make sure that the cells are healthy and greater than 90% viable before transfection.
3. Amount of plasmid DNA: ~25 μg.
4. Amount of FectCHO™ Reagent: ~60 μL. Lock the ratio of FectCHO™ Reagent/DNA at 2.4:1.

Procedures for Transfecting Suspension CHO Cells:

Follow the procedure below to transfect suspension CHO cells in a 30 ml volume. If you wish to transfect the suspension cells in a larger volume, scale up the transfection condistions in proportion to the culture volume.

1. The day before transfection, determine the numbers of the cells and grow suspension CHO cells so that at the day of transfection (roughly 24 hours after) the cell density reaches 3×10^7 cells in total 30 mL standard culture medium.
2. At the day of transfection, count cell viability and adjust cell density at 1×10^6 per mL in total 30 mL (total 3×10^7 cells) standard culture medium. Place the shaker flask containing cells in a 37°C incubator on an orbital shaker.

   Important: For best results, make sure to have a single-cell suspension. It may be necessary to vortex the cells vigorously for 10–30 seconds to break down cell clumps. The viability of cells must be >90%.

   For each transfection, prepare lipid-DNA complexes as follows:
3. Dilute 25 μg of plasmid DNA in serum free DMEM to a total volume of 1 mL. Vortex to mix.
4. Dilute 60 μL of FectCHO™ Reagent in serum free DMEM to a total volume of 1 mL. Vortex to mix.

Note: Never use Opti-MEM to diliute plasmid DNA and FectCHO™ Reagent because trace of serum from Opti-MEM may interfere formation of lipid-DNA complex.

5. Add diluted FectCHO™ reagent to the diluted DNA right away at all once to obtain total volume of 2 mL transfection mix. Vortex to mix.
6. Incubate for 10 minutes at room temperature to allow the formation of lipid-DNA complex.

   Important: Never leave the lipid-DNA complex longer than 20 minutes at RT before addition to suspension CHO cells.
7. Add the 2 mL of lipid-DNA complex to each shaker flask containing 30-mL suspension CHO cells.
8. Incubate the cells in a 37°C incubator with a humidified atmosphere of 8% CO_2 in air on an orbital shaker rotating at 125 rpm.
9. Harvest cells or media (if recombinant protein is secreted) at around 48 hours post-transfection and assay for recombinant protein expression.