GeneCopoeia™
Expressway to Discovery

# Protocol • EndoFectin™ CHO Transfection Reagent • Catalog Nos. EFC1002-01/02

## Description

EndoFectin™ CHO Transfection Reagent is a proprietary cationic polymer formulation optimized for efficient and simple delivery of nucleic acids into CHO cells. EndoFectin CHO provides the following advantages:

- **Superior transfection efficiency**
- **High expression levels of recombinant proteins**
- **Compatibility with serum-containing media**
- **Low cytotoxicity**
- **Easy to use**

## Contents and storage

Each vial contains 1 ml of sterile-filtered EndoFectin CHO reagent.

EndoFectin CHO is shipped at ambient temperature. Store the reagent at 4–8°C with the cap tightly closed. The reagent is stable for at least 12 months when stored at 4–8°C.

## Quality control

Every batch of EndoFectin CHO was tested by transfecting 70-80% confluent CHO cells with an eGFP-expressing plasmid (GeneCopoeia Catalog Number EX-EGFP-Lv01). About 80 % of cells expressed eGFP 24 hours post-transfection.

## Before you start

### Quality of plasmid

It is critical to use plasmid DNA of the highest quality. Determine the DNA concentration by reading the absorption at 260 nm. DNA purity is measured by using the 260 nm / 280 nm ratio (the ratio should be in the range of 1.8 to 2.0). Check the plasmid integrity by agarose gel electrophoresis.

### Condition of cells

Use only healthy CHO cells that are well maintained and regularly passaged. Make sure the culture is free from bacteria, fungi, or *Mycoplasma* contamination.

## Protocol for transient transfection

### 1. Plate cells

On the day before transfection, trypsinize and count the cells. Adjust the cell concentration and plate the cells in a cell-culture vessel with a total volume as suggested in Table 1. The number of cells plated should be determined so that they are 70–80% confluent at the time of transfection. The recommended growth medium for CHO is F-12K Medium supplemented with 10% fetal bovine serum.

### 2. Prepare DNA/EndoFectin CHO complex

DNA, EndoFectin CHO reagent, and diluents should be acclimated to room temperature prior to the following steps.

Dilute the required amount of DNA with Opti-MEM® I (Invitrogen). Refer to Table 1 for suggested volumes. Dilute EndoFectin CHO reagent also with Opti-MEM® I [1]. Use 3.0 µl of EndoFectin CHO reagent per 1 µg of DNA[2].

Add the diluted EndoFectin CHO reagent drop-wise to the DNA solution while gently vortexing the DNA-containing tubes. (**Note**: Do not reverse the adding sequence.) For larger volumes use round-bottom polypropylene tubes such as Falcon® 5-ml or 14-ml tubes (BD Catalog No. 352053/352059).

Incubate the mixture for 10–25 minutes at room temperature to allow the DNA-EndoFectin CHO complex to form.

**Table 1. Suggested starting conditions for transfecting CHO cells**

| Culture vessel | Surface area ($cm^2$) | Medium volume | Total amount of DNA per well | DNA dilution volume | Ratio of EndoFectin (μl) to DNA (μg) | EndoFectin dilution volume |
|---|---|---|---|---|---|---|
| 96-well plate (one well) | 0.3 | 100 μl | 10 – 60 ng | 10 – 20 μl | 3:1 | 10 – 20 μl |
| 24-well plate (one well) | 1.9 | 0.5 ml | 50 – 400 ng | 25 – 50 μl | 3:1 | 25 – 50 μl |
| 12-well plate (one well) | 4.0 | 1.0 ml | 0.2 – 1 μg | 25 – 100 μl | 3:1 | 25 – 100 μl |
| 6-well plate (one well) | 9.3 | 2.0 ml | 0.4 – 2 μg | 50 – 200 μl | 3:1 | 50 – 200 μl |
| 35-mm dish | 7.5 | 2.0 ml | 0.4 – 2 μg | 50 – 200 μl | 3:1 | 50 – 200 μl |
| 6-cm dish | 21.0 | 5.0 ml | 0.8 – 4.5 μg | 0.2 – 0.5 ml | 3:1 | 0.2 – 0.5 ml |
| 10-cm dish | 49.0 | 10 ml | 2 – 10 μg | 0.5 – 1 ml | 3:1 | 0.5 – 1 ml |

**3. Transfect cells**

Add the DNA-EndoFectin CHO complex directly to the culture vessels while gently swirling the vessels.

For transfection in the absence of serum, replace the normal growth medium with serum-free F-12K medium; then add the DNA-EndoFectin CHO complex. Add ½ volume of F-12K containing 30% serum 3 hours after transfection.

**4. Incubate cells and analyze results**

Incubate the cells in a $CO_2$ incubator at 37°C until they are ready for assay. Expression of the transgene can be detected in as little as 7 hours after transfection. Determine your own optimal assay time.

## Protocol for stable transfection

The above procedure is also suitable for stable transfection.

About 24 hours after transfection, passage the cells at a 10-fold or higher dilution into fresh growth medium. Incubate the cells overnight in a $CO_2$ incubator at 37°C. On the following day, add the appropriate selection drug for the transfected resistance gene.

Allow drug-resistant colonies to form for 1–2 weeks. Replace the growth medium containing the selection drug as often as necessary during this period.

## Special notes

1. The DNA-EndoFectin CHO complex must be formed in the absence of proteins even though the complex is able to transfect cells in the presence of proteins such as 10% serum. Opti-MEM I is recommended for optimal transfection efficiency. Sterile 150 mM NaCl may be used if Opti-MEM I is not available, but the transfection efficiency will be compromised.

2. The ratio of 3.0 μl of EndoFectin CHO reagent per 1 μg of DNA is efficient for transfection. Users may optimize the ratios by testing 1 to 4 μl of EndoFectin CHO reagent per 1 μg of DNA.

 