GeneCopoeia™
Expressway to Discovery

Protocol • EndoFectin™ Plus Transfection Reagent • Catalog Nos. EFP1003-01/02

Efficient transfection of nucleic acids into mammalian and insect cells

Description

EndoFectin™ Plus Transfection Reagent is a proprietary cationic polymer formulation that forms a complex with nucleic acids and transports the complex into animal cells. EndoFectin Plus has been proven to work in a wide range of commonly used cell lines including HEK-293, HEK293T, Hep G2, HeLa, CHO-K1, COS-1, COS-7, NIH/3T3, and Sf9. It is optimized for efficient and simple delivery of nucleic acids even in the presence of serum. EndoFectin Plus 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 one 1 ml of sterile-filtered EndoFectin Plus reagent.

EndoFectin Plus 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 lot of EndoFectin Plus is tested by transfecting subconfluent HEK-293 cells with an eGFP-expressing plasmid (GeneCopoeia Catalog Number EX-EGFP-Lv01). Over 95% of cells expressed eGFP 16 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). If possible, check the plasmid integrity by agarose gel electrophoresis.

Condition of cells

Always use high-quality cells that are well maintained and routinely authenticated which includes testing for bacteria, fungi, or Mycoplasma contamination. If the cells are from a recent liquid nitrogen stock, passage the cells at least 2 times before transfection.

Protocol for transient transfection

1. Plate cells[1]

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 in each well should be determined to be 70–80% confluent at the time of transfection[2].

2. Prepare DNA/EndoFectin Plus complex

DNA, EndoFectin Plus 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) or other appropriate protein-free media. Refer to Table 1 for suggested volumes. Dilute EndoFectin Plus reagent with the same protein-free diluents[3]. Use 3.0 µl of EndoFectin Plus reagent per 1 µg of DNA[4].

Add the diluted EndoFectin Plus reagent drop-wise to the DNA solution while gently vortexing the DNA-containing tubes. (Note: Do not reverse the addition sequence.) Use round-bottom polypropylene tubes such as Falcon® 5-ml or 14-ml tubes (BD) for larger volumes (Catalog No. 352053 and 352059).

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

Table 1. Suggested starting conditions for transfection of adherent 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 – 200 ng	10 – 20 µl	3:1	10 – 20 µl
24-well plate (one well)	1.9	0.5 ml	0.1 – 1.25 µg	25 – 50 µl	3:1	25 – 50 µl
12-well plate (one well)	4.0	1.0 ml	0.2 – 2.5 µg	25 – 100 µl	3:1	25 – 100 µl
6-well plate (one well)	9.3	2.0 ml	0.4 – 6 µg	50 – 200 µl	3:1	50 – 200 µl
35-mm dish	7.5	2.0 ml	0.3 – 5 µg	50 – 200 µl	3:1	50 – 200 µl
6-cm dish	21.0	5.0 ml	1 – 12 µg	0.2 – 0.5 ml	3:1	0.2 – 0.5 ml
10-cm dish	49.0	10 ml	2.5 – 30 µg	0.5 – 1 ml	3:1	0.5 – 1 ml

3. **Transfect cells**

Add the DNA-EndoFectin Plus complex directly to each well and gently swirl the plates/dishes.

For transfection in the absence of serum, remove the normal growth medium and replace with serum-free medium, then add the DNA-EndoFectin Plus complex. Add ½ volume of the growth medium 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 assaying. 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. For some types of cells such as HEK-293, HEK293T, NIH/3T3, and COS cells, plating the cells 2 days before transfection leads to significantly higher expression levels of transfected genes. If choosing to plate the cells 2 days before transfection, reduce the plating density accordingly so that the cells still reach 70–80% confluence at the time of transfection.

2. For cells sensitive to contact-inhibition, lower plating densities should be used.

3. The DNA-EndoFectin Plus 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. Other protein-free media should be tested for compatibility with EndoFectin Plus reagent.

4. The ratio of 3.0 µl of EndoFectin Plus reagent per 1 µg of DNA is efficient for transfecting most cell types. Users may optimize the ratios by testing 1 to 4 µl of EndoFectin Plus reagent per 1 µg of DNA.