GeneCopoeia™
Expressway to Discovery

Protocol • EndoFectin™ Max Transfection Reagent • Catalog Nos. EFM1004-01/02

For transfection of nucleic acids into mammalian cells.

Description

EndoFectin™ Max is a proprietary cationic lipid formulation that complexes with nucleic acids and facilitates their delivery into a wide range of commonly used mammalian cell lines and primary cells.

Contents and storage

Each vial contains one 1 ml of sterile EndoFectin Max.

EndoFectin Max is shipped on ice packs. 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. Do not freeze EndoFectin Max reagent.

Quality control

Each lot of EndoFectin Max was tested by transfecting subconfluent HEK-293 cells with an eGFP expression plasmid (GeneCopoeia Catalog No. EX-EGFP-Lv01). Over 95% 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). If possible, check the plasmid integrity by agarose gel electrophoresis.

Condition of cells

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

Protocol for transient transfection of adherent cells:

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 cells must be maintained in antibiotic-free growth medium. The number of cells plated in each well should be determined so that they are about 80% confluent at the time of transfection[2].

2. Prepare DNA/EndoFectin Max complex

Dilute the required amount of DNA with Opti-MEM® I (Invitrogen) or other appropriate protein-free media. Refer to table 1 for suggested volumes. Mix EndoFectin Max reagent gently and thoroughly by inverting the tube 5–7 times before use. Dilute EndoFectin Max reagent with the same protein-free diluents[3]. Use 2.0 to 4.0 µl of EndoFectin Max reagent per 1 µg of DNA[4].

Add the diluted EndoFectin Max reagent to the DNA solution. Mix the solution gently by tapping the tubes. Incubate the mixture for 25 minutes at room temperature to allow the DNA-EndoFectin Max complex to form.

3. Transfect cells

Add the DNA-EndoFectin Max 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 Max complex. Add ½ volume of the growth medium containing 30% serum 3 hours after transfection.

Table 1. Suggested starting conditions for transfection of adherent cells.

Culture vessel	Surface area (cm^2)	Volume of medium	Total amount of DNA per well	DNA dilution volume	Ratio of EndoFectin (㎖) to DNA (㎎)	EndoFectin dilution volume
96-well plate (one well)	0.3	100 ㎖	5 – 80 ng	10 – 20 µl	2:1–4:1	10 – 20 µl
24-well plate (one well)	1.9	0.5 ml	0.04 – 0.5 µg	25 – 50 µl	2:1–4:1	25 – 50 µl
12-well plate (one well)	4.0	1.0 ml	0.08 – 1 µg	25 – 100 µl	2:1–4:1	25 – 100 µl
6-well plate (one well)	9.3	2.0 ml	0.2 – 2.5 µg	50 – 200 µl	2:1–4:1	50 – 200 µl
35-mm dish	7.5	2.0 ml	0.2 – 2.2 µg	50 – 200 µl	2:1–4:1	50 – 200 µl
6-cm dish	21.0	5.0 ml	0.4 – 5 µg	0.2 – 0.5 ml	2:1–4:1	0.2 – 0.5 ml
10-cm dish	49.0	10 ml	1 – 13 µg	0.5 – 1 ml	2:1–4:1	0.5 – 1 ml

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 transient transfection of suspension cells:

Right before transfection, spin down log phase growth suspension cells and re-suspend the cells with antibiotic-free growth medium so that the cell density is 5 –10×10^5 cells/ml. Use 0.1–1.0 µg of DNA for each ml of cells. The ratio of EndoFection Max to DNA is similar to that for adherent cells.

Protocol for stable transfection

The above procedures are 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 adherent 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 Max 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 Max reagent.

4. The ratio of 3.0 µl of EndoFectin Max 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 Max reagent per 1 µg of DNA.