

Enfector™ Transfection Reagent

For the transient and stable transfection of animal cells

Cat. No. 18668-05 **Size: 1.5ml**
Store at +4°C

Description

Engreen Biosystem Co,Ltd. is a professional R&D manufacturer of transfection reagents. Enfector™ is a nano-polymer transfection reagent, synthesized by Engreen Biosystem Co. Ltd.

Enfector™ is a patent-pending, animal-origin free formulation for the transfection of DNA into animal cells providing the following advantages:

- Highest transfection efficiency with low toxicity in many cell types and formats.
- Enfector™ complexes can be added directly to cells in culture medium, in the presence or absence of antibiotics and serum.
- It is not necessary to remove complexes or change/add medium after transfection until next necessary medium change.

Important Guidelines for Transfection

- Antibiotics DO NOT affect transfection efficiency or product toxicity during the Enfector™ transfection procedure.
- It is NOT necessary to change the medium after transfection 4-6 hours, medium can be replaced after 18 hours.
- Due to the low toxicity of Enfector™, cells can be **40-70% confluent** at the time of transfection. This can offer cells more culture time (e.g. 48-72 hours) after transfection, and obtain sufficient biological effects.
- Optimizing transfection. To obtain the highest transfection efficiency and lowest non-specific effects, optimize transfection conditions by varying the ratio between DNA amount (µg) and Enfector™(µl) from 1:0.4 to 1:2.

Transfection Procedure

Use the following procedure to transfect mammalian cells in a 35-mm culture dish. For other formats, see **Scaling Up or Down Transfections**. All amounts and volumes are given on a per well basis.

1. Preparation of cells for transfection.

Adherent cells: One day before the transfection experiment, trypsinize, adjust the cell concentration, and plate the cells in the chosen cell-culture vessel. For most cell types, plating $0.8\text{--}3 \times 10^5$ cells in a 35-mm culture dish in 2 ml of medium (or a 6-well plate) overnight will achieve the desired density of 40–70% confluency. If using culture plates of a different size, adjust the starting volume of Enfactor™ Reagent and the starting mass of DNA in proportion to the relative surface area (Table 1).

Suspension cells:

Use freshly passaged cells at a concentration of $4 \times 10^4/\text{ml}$ to $1 \times 10^6/\text{ml}$ (2 ml in a 35-mm culture dish or 6-well plate). Determine the cell number based on your needs and the cell type to be transfected.

2. Preparation of Enfactor™ Reagent:DNA complex and transfection of cells

- a. Dilute DNA in 250 μl of serum-free medium (e.g. Opti-MEM® I Medium, PBS or 150mM NaCl). Mix gently.
 - b. Mix Enfactor™ gently before use, then dilute the appropriate amount in 250 μl of serum-free medium (e.g. Opti-MEM® I Medium, PBS or 150mM NaCl). Incubate for 5 minutes at room temperature.
 - c. After 5 minutes incubation, combine the diluted DNA with diluted Enfactor™ (total volume = 500 μl). Mix gently and incubate for 30 minutes at room temperature. Note: Complexes are stable for 6 hours at room temperature.
3. Add the 500 μl of complexes to each well containing cells and medium in the presence or absence of antibiotics and serum. Mix gently by rocking the plate back and forth.
 4. Incubate cells at 37°C in a CO₂ incubator for 18-48 hours prior to testing for transgene expression. It is not necessary to change the medium after transfection 4-6 hours, medium can be replaced after 18 hours.
 5. For stable cell lines: Passage cells at a 1:10 (or higher dilution) into fresh growth medium 24 hours after transfection. Add selective medium (if desired) on the following day. For suspension cells: 4 hours post-transfection, add PMA and/or PHA (if desired) to enhance CMV promoter activity and increase gene expression.

Scaling Up or Down Transfections

To transfect cells in different tissue culture formats, vary the amounts of Enfactor™, DNA, cells, and medium in proportion to the relative surface area, as shown in the table 1. With automated and high-throughput systems, a complexing volume of 50 μl is recommended for transfections in 96-well plates. Note: You may perform rapid 96-well plate transfections by plating cells directly into the transfection mixture. Prepare complexes in the plate and directly add cells at twice as the cell density as in the basic protocol in a 100 μl volume. Cells will adhere as usual in the presence of complexes.

Table 1 Reagents amount for different incubation containers

Culture vessel	Surface Area per well (cm ²)	Ratio of Surface Area to 24-well	Vol. of plating medium	DNA (μg) in media vol. (μl)	Enfector™ (μl) in media vol. (μl)
96-well	0.3	0.2	0.1ml	0.2μg in 25 μl	0.5μl in 25 μl
24-well	1.9	1	0.5ml	0.8μg in 50 μl	2μl in 50 μl
12-well	3.8	2	1ml	1.6μg in 100 μl	4μl in 100 μl
6-well/35-mm	10	5	2ml	4.0μg in 250 μl	10μl in 250 μl
60 mm/T25 flask	21	10	50ml	8.0μg in 500 μl	20μl in 500 μl
100 mm/T75 flask	58	30	15ml	24.0μg in 1.5 ml	60μl in 1.5 ml

Storage and stability

- Enfector™ Reagent is shipped at room temperature.
- Enfector™ Transfection Reagent is stabilized for extended storage at +2 to +8°C through the expiration date printed on the label (one year from the date of manufacture) when very tightly closed.

Quality control

- Functional analysis
Three microliters of Enfector™ Transfection Reagent is combined with 1 μg of reporter-gene vector DNA, and used to transfect Hela cells (in a monolayer [50–70% confluent]) in the presence of 10% fetal bovine serum (FBS). Following transfection, the percentage of transfected cells is analyzed. Typically, 50–80% of Hela cells express reporter-gene protein.
- Cytotoxicity analysis
Hela cells that are continuously exposed to Enfector™ Reagent for 26 hours, with or without DNA, in the presence of serum, and without a change of medium, are >90% viable by flow cytometric analysis based on propidium-iodide staining.

Related products

Entranster™-H: Transfect DNA into HEK293、Hela and CHO cells.

Entranster™-R: Transfect siRNA into animal cells.

Entranster™-in vivo: In vivo transfection.

Notice to Purchaser

Purchaser represents and warrants that Enfector™ Transfection Reagent will be used only for research purposes. Transfected cells, materials produced and any data derived from the use of Enfector™ Transfection Reagent, should be used only for the internal research of Purchaser whether Purchaser is a “for-profit” or a “non-profit” organization. Under no circumstances may Enfector™ Transfection Reagent be used by Purchaser or any third party for a commercial purpose unless Purchaser has negotiated a license for commercial use with Engreen Biosystem (contact information: License@Engreen.com.cn). For purposes of the foregoing sentence,

“commercial purpose” shall mean use of EnfectoTMr Transfection Reagent for profit or commercial gain. By using EnfectoTMr Transfection Reagent, Purchaser agrees to be bound by the above terms. If Purchaser wishes not to be bound by these terms, Purchaser agrees to return the EnfectoTMr Transfection Reagent to Engreen Biosystem for a full refund.

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