Protocol ● EndoFectin™ Expi293 Transfection Reagent ● Catalog No. EF007

For efficient transfection of nucleic acids into the mammalian cell line Expi293F™

Description

EndoFectin™ Expi293 Transfection Reagent is a proprietary lipid-based formulation that forms a complex with nucleic acids and transports the complex into animal cells. EndoFectin™ Expi293 has been proven to work in the suspension cell line Expi293F™, the most commonly used suspension HEK293 cell line for transient expression to achieve high cell density. It is optimized for efficient and simple delivery of nucleic acids even in the presence of serum. EndoFectin™ Expi293 provides the following advantages:
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- Superior transfection efficiency of Expi293F™ cells compared with Expifectamine™ 293
- Low cytotoxicity
- Does not require removal of serum or culture medium.
- Does not require washing or changing of medium after transfection

Contents and storage

Each vial contains 1 ml of sterile EndoFectin™ Expi293 transfection reagent, which is sufficient for transfection of 350 mL of cultured Expi293F suspension cells..

EndoFectin™ Epi293 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™ Expi293 is tested by transfecting Expi293F cells with an eGFP-expressing plasmid (GeneCopoeia Catalog Number EX-EGFP-M02). Over 95% of cells expressed eGFP 16 hours post-transfection.

Before you start

Quality of plasmid

It is critical to use endotoxin-free 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

For transfection of Expi293F cells. If you are using 293 cells other than Expi293F™, optimize the transfection conditions by varying the amount of EndoFectin™ Expi293 transfection reagent (e.g., 40, 50, 60, 80, 100 µL used with 30 µg plasmid DNA for transfection of 30 ml cell culture).

Notes before transfection:

- Use 30 mL Expi293F™ cells in a 125 ml Erlenmeyer flask (you can scale the transfection volume up or down proportionally).
- Final transfection volume: 30 mL
- Number of cells to transfect: 7.5×10^7 cells (final cell density of 2.5×10^6 cells/mL) with >95% viability

- Amount of plasmid DNA: 30 µg
- Amount of EndoFectin™ Expi293 reagent: 80 µL
- Calculate the number of cells that you will need for your transfections and expand the cells accordingly.
- You may keep the cells in Expi293™ Expression medium (Invitrogen Cat. no. A14351) during transfection. Do not add antibiotics to media during transfection because it may decrease transfection efficiency.
- Plasmid DNA for transfection into eukaryotic cells must be clean, sterile, and free from phenol and sodium chloride. We suggest using endotoxin free DNA
- Gently mix the EndoFectin™ Expi293 transfection reagent by pipetting it up and down before use.

Transfecting Expi293F™ cells

1. The day before transfection, determine the number of cells needed for your experiment. For each 30-mL transfection, you need 7.5×10^7 cells in 25.5 mL of Expi293™ Expression medium.
2. To transfect cells on the following day, seed the cells at a density of 2.0×10^6 viable cells/mL and incubate at 37°C in a humidified atmosphere of 8% CO_2 in air on an orbital shaker rotating at 125 rpm
3. On the day of transfection, determine number and viability of the cells using an automated cell counter or the trypan blue dye exclusion method. To proceed with transfection, the viability of cells must be over >90%.
4. Calculate the volume of cell suspension containing 7.5×10^7 cells for each 30-mL transfection
5. Add the appropriate volume of cell suspension to each sterile, disposable125-mL Erlenmeyer shaker flask and bring up the volume to 25.5 mL by adding fresh, pre-warmed Expi293™ Expression Medium for each 30-mL transfection. There will be 7.5×10^7 cells in 25.5 ml volume in the flask. Return the cells to the incubator.
6. For 25.5 ml cells, prepare lipid-DNA complexes as follows:
    a. Dilute 30 µg of plasmid DNA in Opti-MEM® I Reduced Serum medium (Cat. no. 31985-062) to a total volume of 1.5 mL. Mix gently.
    b. Dilute 80 µL of EndoFectin™ Expi293 reagent in Opti-MEM® I medium to a total volume of 1.5 mL. Mix gently and incubate for 5 minutes at room temperature (longer incubation times may result in decreased activity).
    c. After the 5 minute incubation, add the diluted DNA to the diluted EndoFectin™ Expi293 reagent to obtain a total volume of 3 mL. Mix gently.
    d. Incubate DNA-EndoFectin™ Expi293 reagent mixture for 20–30 minutes at room temperature to allow the DNA-EndoFectin™ Expi293 reagent complexes to form.
7. After the DNA-EndoFectin™ Expi293 reagent complex incubation is complete, add 3 mL of DNA-EndoFectin™ Expi293 reagent complex to each shaker flask from Step 5. To the negative control flask, add 3 mL of Opti-MEM® I medium instead of DNA-EndoFectin™ Expi293 reagent complex. Each flask should contain a total volume of 28.5 mL.
8. Incubate the cells in a 37°C incubator with a humidified atmosphere of 8% CO2 in air on an orbital shaker rotating at 125 rpm.
9. Cells or media (if recombinant protein is secreted) may be harvested beginning at approximately 48 hours post-transfection and assayed for recombinant protein expression. The amount of time required for optimal protein expression depends on the nature of your recombinant protein which could be up to 5 days after transfection..