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PGbioFect 3000plus Transfection Protocol for 293 Suspension Cells

Product Overview

PGbioFect 3000plus is a cationic liposome transfection reagent designed for DNA transfection into eukaryotic cells, especially optimized for transfecting 293 suspension cells in serum-free media. It facilitates efficient gene delivery and expression research.

Product Composition and Storage

01. Product Composition

Includes PGbioFect™ 3000plus transfection reagent and T3000 auxiliary reagent, available in multiple packaging specifications.

02. Storage Conditions

Store at 4°C. Do not freeze to maintain reagent activity.

Experimental Materials

01. Experimental Materials

- High-purity plasmid DNA (free of phenol, sodium chloride, and other contaminants; extraction using specific kits like HiPure Plasmid Kits is recommended)
- 293 suspension cells (e.g., 293-F cells)
- 293 expression medium
- Opti-MEM® reduced-serum medium
- 125-mL polycarbonate vented-cap conical shake flask
- Vortex mixer
- Cell counter
- Trypan blue stain
- CO_2 incubator (37°C, controlled humidity, 8% CO_2)
- Temperature-controlled shaker (125 rpm)

02. Pre-Experimental Preparations

- Warm 293 expression medium and Opti-MEM® reduced-serum medium to room temperature before the experiment.
- Ensure all operations are performed in a sterile environment.

Transfection Procedures

01. Cell Preparation

◆ Cell Culture and Expansion

- Expand 293 suspension cells according to their doubling time to maintain logarithmic growth. For 293-F cells, passage at a density of 6–7 × 10^5 cells/mL.

◆ Cell Counting and Viability Testing

- Take a small amount of cell suspension, use Trypan blue exclusion method with a cell counter or hemocytometer to count cells and assess viability, ensuring viability >90%.
- If cell clumps exist, vortex vigorously for 10–45 seconds to form a single-cell suspension.

◆ Cell Seeding

- Calculate the required cell suspension volume to have 3 × 10^7 cells in a 30 mL transfection system. Inoculate cells into a 125 mL conical shake flask, adding pre-warmed 293 expression medium to 28 mL.

02. Preparation of Transfection Complex (for 30 mL Transfection System)

◆ DNA Dilution

- In a sterile centrifuge tube, dilute 30 µg of plasmid DNA with Opti-MEM reduced-serum medium to a total volume of 1 mL, mix gently.

◆ Dilution of PGbioFect 3000plus and T3000 Reagent

- In another sterile centrifuge tube, add 60 µL of PGbioFect 3000plus reagent and an appropriate amount of T3000 auxiliary reagent (according to product-recommended ratio) to Opti-MEM reduced-serum medium, dilute to a total volume of 1 mL, mix gently, and incubate at room temperature for 5 minutes. Incubation exceeding 5 minutes may reduce transfection activity.

◆ Mixing and Incubation

- After 5 minutes, add the diluted DNA solution to the solution containing PGbioFect™ 3000plus and T3000 reagent to a total volume of 2 mL, mix gently, and incubate at room temperature for 20–30 minutes to allow full formation of the DNA-transfection reagent complex.

03. Transfection and Culture

◆ Transfection Operation

- Add the prepared 2 mL transfection complex dropwise to the shake flask containing cells, shake gently to ensure full contact between the complex and cells. The total volume in the flask is now 30 mL, with a final cell density of approximately 1 × 10^6 viable cells/mL.

◆ Control Setup

- Set up a negative control group by adding 2 mL of Opti-MEM reduced-serum medium to the corresponding shake flask instead of the transfection complex.

◆ Cell Culture

- Incubate the shake flask in a temperature-controlled shaker at 37°C, 8% CO_2, and 125 rpm.

04. Result Detection

◆ Protein Expression Detection

- 2–7 days after transfection, select detection methods based on the characteristics of the recombinant protein. If the protein is secreted into the medium, collect the medium for detection; if expressed intracellularly, collect cells for analysis using techniques such as Western Blot or ELISA.

◆ Expression Optimization

- Due to differences in recombinant protein properties, expression levels vary. Perform time-course experiments by harvesting cells or medium at different time points after transfection to optimize protein expression conditions.

Notes

01. Maintain strict aseptic technique throughout the experiment to prevent microbial contamination that may lead to experimental failure.
02. Ensure the quality of plasmid DNA, as contaminants can damage cells, interfere with complex formation, and reduce transfection efficiency.
03. Handle reagents gently during dilution and mixing to avoid bubble formation, which affects transfection efficiency.
04. Follow recommended transfection conditions strictly. If adjusting the system, perform pre-experiments to determine optimal parameters first.
05. This product is for research use only, not for diagnostic or clinical treatment purposes.

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