Calcium Phosphate Cell Transfection Kit

Cat. No.: C1509487 | Pack size: 200T | Storage: Store at 2-8°C; store at -20°C

Overview

There are many methods for introducing foreign genes into eukaryotic cells, such as calcium phosphate transfection, DEAE-dextran transfection, lipofection, electroporation, microinjection, etc. The Calcium Phosphate Cell Transfection Kit is improved based on the traditional calcium phosphate transfection method, with higher transfection efficiency and lower cytotoxicity. It can be used for calcium phosphate-mediated cell transfection for both transient expression and stable cell line selection. HEK293 is one of the most suitable cell lines for calcium phosphate transfection. Under optimized conditions, transfection efficiency can reach above 85%, and typically ranges from 40% to 50%. Other common cell lines (such as HeLa, CHO, etc.) are also compatible but with slightly lower efficiency than 293 cells.

This kit is suitable for transfection of most adherent cells and some suspension cells. The recommended DNA concentration is generally 10-50 μg. Cell lines such as HeLa and BALB can be incubated for 16 h with precipitate; cell lines such as CHO, DUKX, and BⅡ can be subjected to heat shock with glycerol or DMSO to improve transfection efficiency. This kit is for research use only and not for clinical diagnosis or other applications.

Contents & Storage

Cat. No.	Component	Size	Storage
C1509487A	Calcium Chloride Solution	20 mL	2-8°C.
C1509487B	BBS Solution	20 mL	-20°C.

Materials Provided by User

Trypsin digestion solution, complete culture medium, PBS, sterile water.

Protocol (For Reference Only)

(I) Transfection of Adherent Cells

1. 24 h before transfection: Digest cultured cells with trypsin, seed an appropriate number of log-phase cells into a new culture vessel. Transfection can be performed when cell confluency reaches 70-80%. All procedures below are calculated for a 6-well plate. Adjust volumes proportionally for other culture vessels.

2. 2-4 h before adding DNA: Add 2 mL of antibiotic-free complete medium and incubate at 37℃ in a 5% CO_2 incubator.
3. Add 2-6 μg DNA (volume ≤20 μL) to 100 μL Calcium Chloride Solution, mix well to form DNA-$CaCl_2$ solution.
4. Take 100 μL BBS Solution. While gently pipetting the BBS Solution, dropwise add the DNA-$CaCl_2$ solution (slow operation, about 1-2 min).
5. Incubate at room temperature for 20-30 min to form DNA-$CaCl_2$-BBS solution. Very fine precipitate may appear.
6. Take the mixture (including fine precipitate at the bottom) and add evenly to the cells in the 6-well plate. Mix gently by shaking.
7. Incubate at 37℃ in a 5% CO_2 incubator for 4-16 h. For cell lines such as CHO and DUKX, shock treatment can greatly improve efficiency: After 4-6 h incubation, replace medium with 2 mL complete medium containing 10% glycerol or 20% DMSO, incubate at room temperature for 3 min, then add 5 mL PBS and mix gently.
8. Remove the medium, wash cells twice with PBS, add 2 mL complete medium and continue culture. Transgene expression is usually visible after 24 h.

(II) Transfection of Suspension Cells

1. Collect suspension cells by low-speed centrifugation, wash once with PBS.
2. Add 2-6 μg DNA (volume ≤20 μL) to 100 μL Calcium Chloride Solution, mix well to form DNA-$CaCl_2$ solution.
3. Take 100 μL BBS Solution. While gently pipetting the BBS Solution, dropwise add the DNA-$CaCl_2$ solution (slow operation, about 1-2 min).
4. Incubate at room temperature for 20-30 min to form DNA-$CaCl_2$-BBS solution. Very fine precipitate may appear.
5. Resuspend every 1×10^6 cells with 100 μL DNA-$CaCl_2$-BBS solution, incubate at room temperature for 20-30 min.
6. Add 2 mL antibiotic-free complete medium per well of a 6-well plate. Add the DNA-$CaCl_2$-BBS mixture evenly and mix gently.
7. Incubate at 37℃ in a 5% CO_2 incubator for 4-16 h. Remove medium, wash cells twice with PBS, add 2 mL complete medium and continue culture. Transgene expression is usually visible after 24 h.

Precautions

1. Perform aseptic operations to avoid contamination. DNA should be free of protein and phenol.
2. Shock treatment can greatly improve transfection efficiency in some cell lines, but prolonged glycerol exposure may cause cell death.
3. 12-24 h after transfection, adding sodium butyrate to a final concentration of 10 mmol/L can increase viral titer.
4. The pH of BBS Solution directly affects transfection efficiency. Avoid prolonged exposure to air to prevent acidification by CO_2.

5. Use the reagent promptly after opening to avoid affecting experimental results.
6. For your safety and health, wear a lab coat and disposable gloves during operation.

Storage and Shipping

Attribute	Value
Storage Conditions	Store at 2-8℃, Store at -20℃
Shipped In	Ice chest + Ice pads
Stability And Storage	Each component has a shelf life of 1 year under corresponding storage conditions.

Contact & Global Offices

Whether you have a technical question, need help with a quotation, or want to inquire about an order, our regional teams are ready to assist. Please contact the office for your region; for general inquiries, the North American office is the corporate primary.

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Limitations & Disclaimer

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This product is not a CE-marked in vitro diagnostic device under IVDR (EU) 2017/746 and is not an FDA-cleared device under 21 CFR. Use is restricted to verified businesses, institutions, and qualified professionals.

Where any kit component is classified as hazardous under CLP (EC) 1272/2008 or OSHA HCS (29 CFR 1910.1200), the product Safety Data Sheet (SDS) takes precedence over this document for handling, storage, transportation, disposal, and emergency procedures.

Performance depends on sample type, sample condition, handling, and operator technique. Users are responsible for validating the product for their specific application.

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