Transfection with PEI

This protocol describes a simple PEI-based transfection method, that is effective for a range of epithelial cell types, including epidermal keratinocytes.

Protocol

1. Cells should be seeded a day prior to transfection in 6 well plates at a density of 300,000 cells per well.
2. Add 2 µg DNA To 50 µL of protein-free basal medium (containing no growth factors, serum, antibiotics or other proteins). Mix by pipetting up-and-down
3. Add 15 µg of PEI (i.e. 15 µL from 1 mg/mL solution). Mix by pipetting up-and-down
4. Incubate for 8 min at room temp.
5. Add 450 µl complete CnT medium supplemented with 10% FCS
6. Remove medium from 6-well plate, and was cells twice with PBS
7. Apply the 500 uL DNA/PEI mixture to the cells.
8. Incubate for 2 hours at 37°C, with occasional rocking
9. Wash cells with PBS, and add 2 mL of complete CnT-Medium containing 10% FCS
10. Incubate overnight (Note: some cell death may occur during this period)
11. Wash cells 3 x with PBS, change medium to complete CnT medium (without serum)

Transfection efficiency for mouse keratinocytes is ~25 %

Preparation of PEI stock solution

Dissolve PEI powder to a concentration of 1 mg/ml in water which has been heated to 80°C.

Allow solution to cool to room temp.

Adjust pH to 7.0 with 5 M HCl

Filter sterilize

Freeze aliquots at –80°C

Materials

PEI: Polyethylenimine, linear, MW-25,000. Use for transfection may be subject to claims in European Patent 0770140 (and corresponding patents in other regions) owned by Polyplus Transfection.

In case of further questions, please email out scientists directly: scientist@cellntec.com