Dec 12, 2020

Transfection with PEI

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In Development This protocol is published without a DOI.

Chemistry 586 Advanced Biochemical Methods

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PROTOCOL CITATION

JCPrice 2020. Transfection with PEI . **protocols.io**
https://protocols.io/view/transfection-with-pei-bqnpmvdn

LICENSE

CREATED

Dec 11, 2020

LAST MODIFIED

Dec 12, 2020

PROTOCOL INTEGER ID

45487

MATERIALS TEXT

Polyethyleneimine (PEI, linear MW 25,000) in 1 milligram per milliliter solution
serum-free media appropriate for your cells
Purified endotoxin-free plasmid DNA ~100 micrograms
Complete media appropriate for your cells
Sterile Eppendorf tubes and cell culture dishes

SAFETY WARNINGS

PEI penetrates cells and is a significant irritant to skin and mucus membranes

DISCLAIMER:

BEFORE STARTING

Prepare a clean space in the biosafety cabinet with all the reagents and a empty waste container and warm media

1 Cells should be 50-70% confluency and have a healthy morphology

2 In a clean biosafety cabinet, remove serum media from cells (DO NOT TRYPSINIZE!)

3 Add 1.5 mL of serum-free cell culture media (DMEM or similar)

4 In a sterile tube dilute total plasmid DNA (µg) in serum-free DMEM. **6 well plate:** 200 µl + 3 µg of total DNA per well; **24 well plate**: 30 µl + 0.5 µg of total DNA per well Add PEI (1 µg/µL) to the diluted DNA. Mix immediately by vortexing or pipetting. The volume of PEI used is based on a 3:1 ratio of PEI (µg):total DNA (µg). This ratio can be optimized to maximize transfection efficiency.
   a. 6-well plate: 9 µl of PEI(1 µg/µl) = 9 µg PEI to 3 µg DNA
   b. You will need 200 µL serum-free DMEM + 3 µgDNA+9 µg PEI for each well of a 6-well plate you plan to transfect.
   c. 24 well plate: 1.5 µl of PEI(1 µg/µl) = 1.5 µg PEI to 0.5 µg DNA
   d. You will need 30 µl serum-free DMEM + 0.5 µg DNA + 1.5 µg PEI for each well of a 24-well plate.

5 For the negative control, add 9 µg PEI to 200 µL serum-free media - no plasmid DNA.

6 Incubate 15 minutes at r.t.

7 Add DNA/PEI mixture to cells using the volume per well described above

8 Allow cells to incubate with DNA/PEI mixture for 4 hours in the 37°C cell culture incubator.

9 After 4 hours, remove serum-free medium with DNA/PEI mixture and replace with 2-3 mL complete serum media.

10 Incubate cells for 24-48 hours, at 37°C to allow for plasmid expression and protein translation

11 Fix or harvest for downstream analysis.