



Dec 12, 2020

Transfection with PEI

JCPrice¹¹BYU*In Development*

This protocol is published without a DOI.

Chemistry 586 Advanced Biochemical Methods

JCPrice
BYU

PROTOCOL CITATION

JCPrice 2020. Transfection with PEI . [protocols.io](https://protocols.io/view/transfection-with-peி-bqnpmdn)
<https://protocols.io/view/transfection-with-peி-bqnpmdn>

LICENSE

This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Dec 11, 2020

LAST MODIFIED

Dec 12, 2020

PROTOCOL INTEGER ID

45487

MATERIALS TEXT

Polyethylenimine (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:

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

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 $\mu\text{g}/\mu\text{L}$) 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 $\mu\text{g}/\mu\text{l}$) = 9 μg PEI to 3 μg DNA
 - b. You will need 200 μL serum-free DMEM + 3 μg DNA+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 $\mu\text{g}/\mu\text{l}$) = 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.