



HOST-PATHOGEN
INTERACTIONS
LAB
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**TITLE: PEI TRANSFECTION
FOR HELA CELLS:
Date: 15/07/2021
Name: Ipsita Nandi**

Reagents:

- DMEM high glucose liquid media [Biological industries, #01-055-1A]
- Gln (amino acid- glutamine): 1%
- 1 mg/ml PEI solution, linear, **MW 25000** (Polyscience, #23966)

Method:

1. From a healthy 80% confluent 9cm plate, split (in the morning) 1:2 into 9 cm plates.
2. Next day: choose best looking plate (should be ~60% confluent) for transfection.
3. In a 15 cm poly propylene tube prepare the following transfection solution:
 - I. 520 ul warm high glucose DMEM+1% Gln
 - II. 5 µg DNA/10 cm plate (OD ratio 260/280 greater than 1.8)
 - III. 30 ul PEI (1 mg/ml in sterile DDW)
 - IV. Vortex quickly twice (50% vortex)
 - V. Incubate transfection solution 10min at 22 °C
4. Wash the cells two times with 1X PBS.
5. Add the ~550 ul transfection solution onto the cells (dropwise), while gently swirling it.
6. Incubate 3h at 37C/5% CO₂ incubator.
7. Replace media with complete media (+Antibiotics; +10% FBS)
8. Cells can be harvested 24h, 48h, 72h or 96h post transfection according to the experiment.