

Optimized PEI Mediated Reverse Transfection Methodology for Transient Transfection of HEK293 Cells

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Non-viral vectors are increasingly used for gene delivery due to their safety and flexibility. Polyethyleneimine (PEI) is widely applied in transfection, but high PEI and DNA concentrations can induce cytotoxicity, necessitating careful optimization of the PEI ratio. This study aimed to optimize PEI-mediated reverse transfection for HEK293 cells and compare its efficiency with the conventional forward transfection method. For reverse transfection, plasmid DNA (pEGFP-N1) and PEI (25 kDa branched) were separately diluted in PBS and then gently mixed to promote complexation. The PEI-DNA mixture was incubated at room temperature. HEK293 cells were seeded at 1×10^5 cells/well in a 6-well plate during this period. The PEI-DNA transfection mixtures were added to the freshly seeded cells, mixed gently, and incubated overnight at 37°C in a 5% CO₂ humidified incubator. 18 hours post transfection, the transfection medium was replaced with fresh cell culture medium. Transfection efficiency was assessed 72 hours post-transfection using fluorescence microscopy. PEI ratios (1:1, 1:2, 1:3, 2:3, and 2:6) were tested for both forward and reverse transfections. Paired t-tests showed significant differences in efficiency between the methods at ratios 1:2, 1:3, 2:3, and 2:6, with *p*-values of 0.0055, 0.0012, 0.0068, and 0.0034 (*p* < 0.05). Significant differences between the two methods highlight the critical influence of transfection methodology on efficiency. Based on the observed results, a 1:3 DNA to PEI ratio in reverse transfection showed optimal efficiency and eliminated the need for prior cell seeding, streamlining the workflow compared to forward transfection. Higher DNA ratios (2:4 and 2:6) increased efficiency but caused notable cytotoxicity, as evidenced by cell death observed visually 48 hours post-transfection. This underscores the need to optimize the DNA:PEI ratio to achieve optimal transfection efficiency and mitigate cytotoxic effects.

Keywords: *PEI, Reverse Transfection, HEK293, PEI: DNA Ratio*

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