Protocol for Transient Gene Expression in Adherent HEK293 Cells using PEI STAR™ Transfection Reagent

In Brief

PEI STAR™ (Cat. No. 7854) is a chemically defined, high-performance polyethylenimine (PEI) transfection reagent for cost-effective, affordable and scalable transient gene expression. PEI is a synthetic polymer with an exceptionally high positive charge density in pH-neutral solutions. Positively charged PEI binds strongly to negatively charged DNA and imparts a net cationic charge, allowing the DNA to enter cells. PEI is a non-viral vector commonly used to transfect HEK293 and CHO cells. Applications include production of recombinant proteins, antibodies and viruses.

Pre-dissolved, ready-to-use formulation of PEI STAR™ also available: PEI STAR-Go™ transfection reagent (Cat. No. 8174).

The following protocol provides guidelines for using this product.

Materials

- PEI STAR™, 1 mg/mL, pH neutralized, sterile-filtered
- HEK293 expression medium or DMEM maintained at 37°C transfection is inhibited by serum. Only use media that is reduced-serum, serum-free or chemically defined.
- HEK293 cells, ~50,000 cells/cm^2, seeded the day prior to transfection
- Transfection-grade plasmid DNA (pDNA) with gene of interest, 1 µg per 10^6 cells to be transfected

General Guidance

We list recommended reagent quantities per 10^6 cells on the table below. If performing many very similar transfections (e.g., making many similar viruses), we would recommend co-varying on these parameters over optimization ranges first.

Parameter	Recommended	Optimization Range
PEI STAR™ Concentration (per 10^6 cells)	3.0 µg	2.0 to 4.0 µg
DNA Concentration (per 10^6 cells)	1.5 µg	1.0 to 2.0 µg
PEI STAR™/DNA Complex Time	10.0 min	5.0 to 15.0 min

Even if performing **co-transfection**, keep the total DNA concentration range the same (1.0 to 2.0 µg/10^6 cells).

If high toxicity is observed, then reduce the quantity of PEI and/or DNA first. We **do not** recommend replacing the media post-transfection to reduce toxicity except as a last resort.

Before Transfection

Seed the cells the day before transfection to reach 50-80% confluence on the day of transfection. Seed approximately 50,000 cells per cm^2.

Transfection

1. Measure the cell density to determine the transfection parameters based on the number of viable cells.
2. Dilute 1.0 µg pDNA per 10^6 cells in 5% final culture volume of DMEM or media. Mix well.
3. Dilute 3.0 µg PEI STAR™ per 10^6 cells in 5% final culture volume of DMEM or media. Mix well.
4. Mix the solution of pDNA and PEI STAR™ together by quickly and briefly vortexing or inverting the tube several times.
5. Allow the mixture of pDNA and PEI STAR™ to rest for 10 minutes at room temperature.
6. Gradually add the pDNA and PEI STAR™ mixture to the cells dropwise while swirling the plate.
7. Incubate the cells at typical incubation conditions.

References

Boussif *et al* (1995) A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine. Proc.Natl.Acad.Sci.U.S.A. ***92*** 7297. PMID: 7638184.

Longo *et al* (2013) Transient mammalian cell transfection with polyethylenimine (PEI). Methods Enzymol. ***529*** 227. PMID: 24011049.