Transient Gene Expression in HEK293 Suspensions using PEI STAR™ Transfection Reagent

Materials

- PEI STAR™, 1 mg/mL, pH neutralized, sterile-filtered
- HEK293 expression medium maintained at 37°C

Transfection is inhibited by serum. Use media that is reduced-serum, serum-free ("SFM") or chemically defined ("CDM"). Here are some popular media:

Vendor	Suitable Media
Thermo Fisher Scientific	Gibco™ Opti-MEM™ I Reduced Serum Media Gibco™ FreeStyle™ 293 Expression Medium Gibco™ Expi293™ Expression Medium (with Expi293F™ Cells)
Cytiva	HyClone HyCell TransFX-H HyClone SFM4Transfx-293 media
FUJIFILM Irvine Scientific	BalanCD HEK293 **(Recommended)**

- HEK293 cell culture at viable cell density of 1.5 to 2.0 x 10^6 cells/mL
- Transfection-grade plasmid DNA (pDNA) with gene of interest, 1 µg per mL of culture to be transfected
- Optional Positive Control: GFP-encoding pDNA

General Guidance

Cell densities used are for typical HEK293 cultures, with maximum viable cell densities of ~4.0 x 10^6 cells/mL. If using a high-density system, increase the values for cell density linearly. For example, Expi293™ media and Expi293F™ cells can support viable cell densities over 12 x 10^6 cells/mL instead of ~4.0 x 10^6 cells/mL, and so should be transfected at 3.15 x 10^6 cells/mL instead of 1.05 x 10^6 cells/mL.

The typical pDNA and PEI concentrations (1.0 and 3.0 mg/L, respectively) can achieve high transfection efficiency at viable cell densities from 1.0 x 10^6 cells/mL up to 5.0 x 10^6 cells/mL.

If performing the same expression many times, or many similar expressions, we would recommend co-varying on these parameters over corresponding ranges to find the optimal conditions:

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Parameter	Range
PEI Concentration	1.50 to 4.50 mg/(L final culture)
DNA Concentration	0.75 to 1.50 mg/(L final culture)
PEI/DNA Complex Time	5.0 to 15.0 minutes

Improvements in transfection efficiency are possible with changes outside the scope of this protocol. For further guidance on obtaining better yields please contact us at techsupport@bio-techne.com.

Before Transfection

Subculture and expand cells to obtain culture with viability greater than 95% and viable cell density between 1.5 to 2.0 x 10^6 cells/mL at time of transfection.

Transfection

1. Immediately prior to transfection, ensure that viability is greater than 95% and viable cell density is 1.5 to 2.0 x 10^6 cells/mL.
2. Dilute the viable cell density to 1.05 x 10^6 cells per mL.
3. Invert pDNA and PEI STAR™ 1 mg/mL reagent containers several times to mix well.
4. The final transfection concentration is 1 µg pDNA for each mL of culture to be transfected. First prepare 20 µg/mL of pDNA using 5% final culture volume in a clean vial. For example, 100 mL cell culture requires 100 µg pDNA, prepare 100 µg pDNA in 5 mL of fresh media.
5. To a clean vial, transfer 3 µL PEI STAR™ 1 mg/mL for each mL of culture to be transfected. Use media to dilute to 60 µg/mL (5% final culture volume).
6. Mix together diluted pDNA and PEI STAR™. Invert several times and allow to rest capped at room temperature for 10 minutes. Gently invert the closed container once immediately before use.
7. Use 10 mL of pDNA/PEI mixture for each 90 mL of culture to be transfected. Gradually add mixture to culture while mixing.
8. Incubate cells per typical conditions.

Post Transfection

If using a feed, booster, supplement, or enhancer, these can be added any time six hours post-transfection. Subcultures can also be prepared after six hours.

If using a GFP control, transfection efficiencies over 70% should be observed after 48 hours. For optimized procedures, efficiencies over 80% are reasonable.

Monitor expression levels and harvest upon observing plateaued titers. For typical processes, secreted proteins will be highest at 5 to 7 days post transfection.

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.

Support

Support is available by emailing us at techsupport@bio-techne.com.