PEIpro®: a Well Characterized PEI Optimized for an Efficient and Reliable Transient Protein Production

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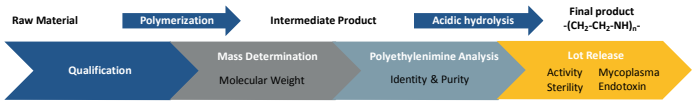
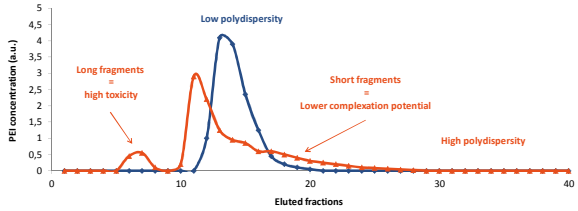
Abstract

Transient protein expression in mammalian cell lines has gained increasing relevance as it enables fast and flexible production of high-quality eukaryotic protein. Milligram amounts of protein can be produced within several days, meaning a significant shortening of process time in comparison to protein production from stable clones. However, to ensure the robustness of the process, it is absolutely necessary to have a reliable transfection solution. That's why we developed PEIpro®, an enhancement of the gold standard PEI, optimized for transient protein expression and perfectly suitable for the development of bioproduction processes with great scale-up predictability. In this poster, we present experimental data showing the benefits of using PEIpro® for protein production, and its efficiency in comparison to other PEIs.

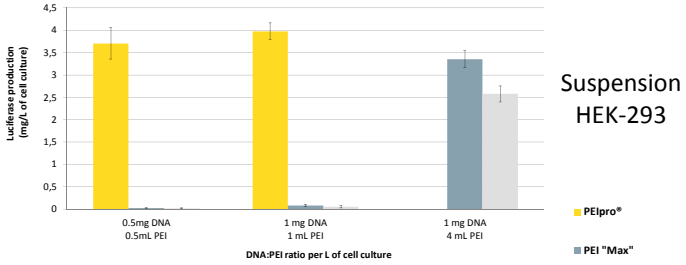
A PEI Optimized for Transfection

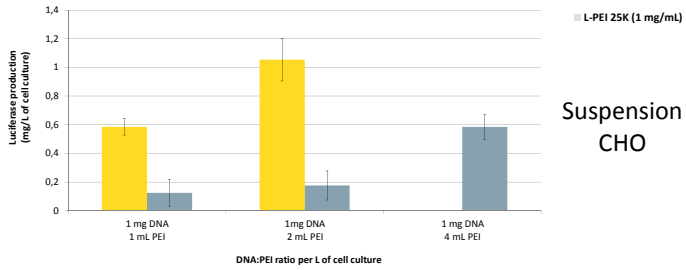


Manufacturing process of PEIpro® reagent. The linear form of PEIpro® and the manufacturing process developed by Polyplus-transfection® ensure a high, stable and reproducible amount of protonable amines available for transfection while providing a fully deacylated molecule and an extremely low polymer chain length variation.



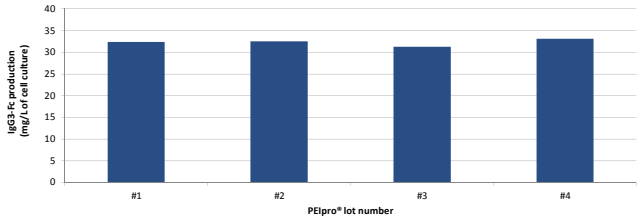
Optimization process of PEI polymer chemistry. Whereas long polymer fragments lead to toxicity and short fragments lead to lower complexation potential (in red), optimized PEI size with a low polydispersity index decreases toxicity, while increasing complexation potential (in blue).





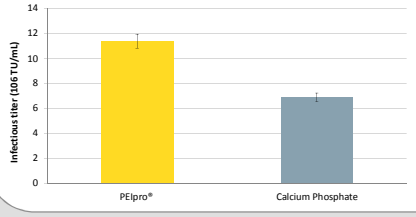
PEIpro® requires less reagent and similar to lower DNA amount compared to other PEIs. Suspension HEK-293 and CHO cells were seeded at 1×10^6 cells/mL in serum free medium and transfected with PEIpro®, PEI "Max" and L-PEI 25 kDa (Polysciences, Warrington, PA) resuspended at 1 mg/mL. Luciferase expression was assayed 48 h after transfection using a conventional luciferase assay.

Great Lot to Lot Consistency



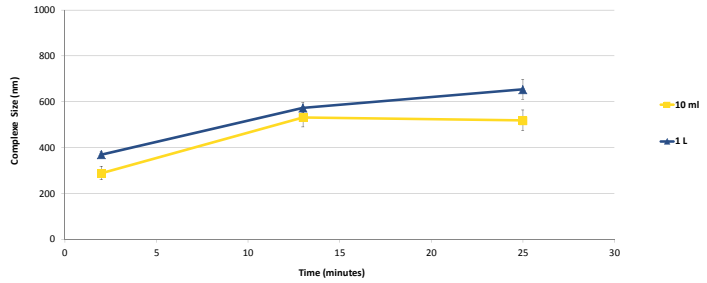
Excellent lot-to-lot protein yield reproducibility using PEIpro®. Suspension HEK-293 cells were seeded at 1×10^6 cells/mL in serum-free medium and transfected with PEIpro® following the standard protocol. IgG3-Fc production was assayed 48 h after transfection using protein G affinity quantification (HPLC).

Perfectly Suited for Virus Production

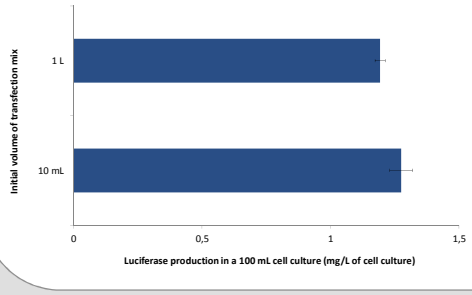


PEIpro® gives higher virus titers than Calcium Phosphate. Lentiviruses were produced in adherent HEK-293 cells grown in serum-free culture medium, using 15 µg DNA and 30 µL PEIpro® per 75 cm² flask. Viral titers were determined by flow cytometry of supernatants 48 h after transfection.

Easily Scalable for Large Protein Production

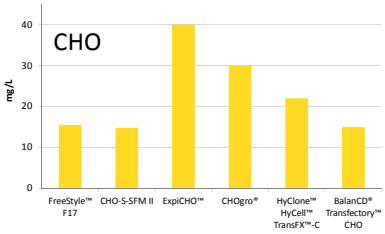


DNA-PEIpro® complex size is identical, independently from the volume of transfection mix preparation. Complexes were prepared with a DNA concentration of 0.01 mg per mL of complex volume at a DNA:Reagent ratio of 1:4, either in 10 mL or 1 L. The size of the complexes was then measured every ten minutes using the Zetasizer Nanometer ZS (Malvern Instrument, Malvern, UK).



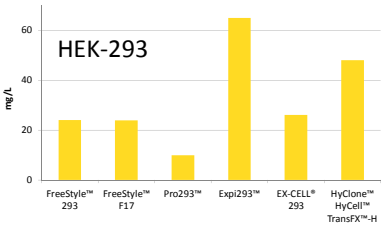
Scale-up protein production results with PEIpro® are highly predictable. Suspension CHO cells were seeded at 1×10^6 cells/mL in 100 mL serum-free medium.
DNA-PEIpro® complexes were prepared with a DNA concentration of 0.01 mg per mL of complex volume at a DNA:Reagent ratio of 1:4, either in 10 mL or 1 L. For the transfection, only 10 mL of transfection mix were added to the 100 mL culture. Luciferase expression was assayed 48 h after transfection using a conventional luciferase assay.

Compatible with Various Synthetic Media



Culture medium	Protein yields using PEIpro®
ExpiCHO™ (Life Technologies™)	++++
CHOgro® (Mirus®)	+++
HyClone™ HyCell™ TransFx™-C (GE Healthcare™)	+++
FreeStyle™ F17 (Life Technologies™)	++
CD FortiCHO™ (Life Technologies™)	++
BalanCD® Transfectory™ CHO (Irvine Scientific™)	++
CHO-S-SFM-II (Life Technologies™)	++
FreeStyleCHO™ (Life Technologies™)	+
Pro-CHO™ 4 (Lonza®)	+
CD CHO (Life Technologies™)	-
PowerCHO™ 2 (Lonza®)	-
HyClone™ CDM4 CHO (GE Healthcare™)	-

PEIpro® is compatible with several CHO synthetic culture media. Suspension CHO cells were seeded following the recommended protocol in serum-free media and transfected with PEIpro® using the standard conditions. IgG3-Fc production was assayed 48 h after transfection using protein G affinity quantification (HPLC).



Culture medium	Protein yields using PEIpro®
Expi293™ (Life Technologies™)	++++
HyClone™ HyCell™ TransFx™-H (GE Healthcare™)	+++
FreeStyle™ 293 (Life Technologies™)	++
FreeStyle™ F17 (Life Technologies™)	++
EX-CELL®293 (Sigma-Alfrich®)	++
Pro293™ (Lonza®)	+
CD293 (Life Technologies™)	-

PEIpro® is compatible with several HEK-293 synthetic culture media. Suspension HEK-293 cells were seeded following the recommended protocol in serum-free media and transfected with PEIpro® using the standard conditions. IgG3-Fc production was assayed 48 h after transfection using protein G affinity quantification (HPLC).

Conclusion

Advantages of PEIpro®

- A PEI optimized for transfection, suitable for protein and virus production
- Ideal for the development of bioproduction processes
- Manufactured according to a well-established process
- Synthetic reagent free of any animal-origin component
- Robust product with a great lot-to-lot reproducibility and a long shelf life.
- Highest quality PEI available with extra Quality Controls (identity, potency, safety and purity) and supplied with extensive documentation, **PEIpro®-HQ: Ideal for use in GMP processes**

www.polyplus-transfection.com