Efficient and Consistent Transient Protein Production Using a High Quality Ready-to-Use PEI

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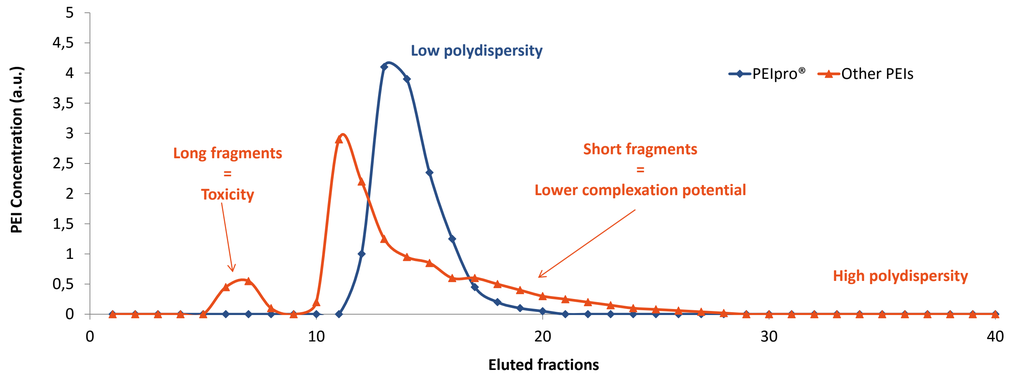
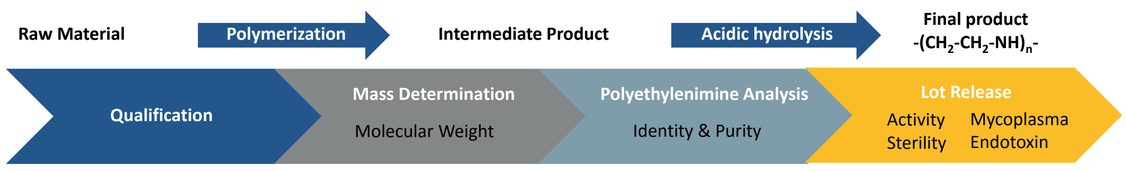
Abstract

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®, a high quality ready-to-use PEI optimized for transient protein expression and perfectly suitable for the development of bioproduction processes with great scale-up predictability. This reagent is also available with extra quality controls, ideal for use in GMP process. In this poster, we present experimental data showing the benefits of using PEIpro® for protein production, and its efficiency in comparison to other PEIs.

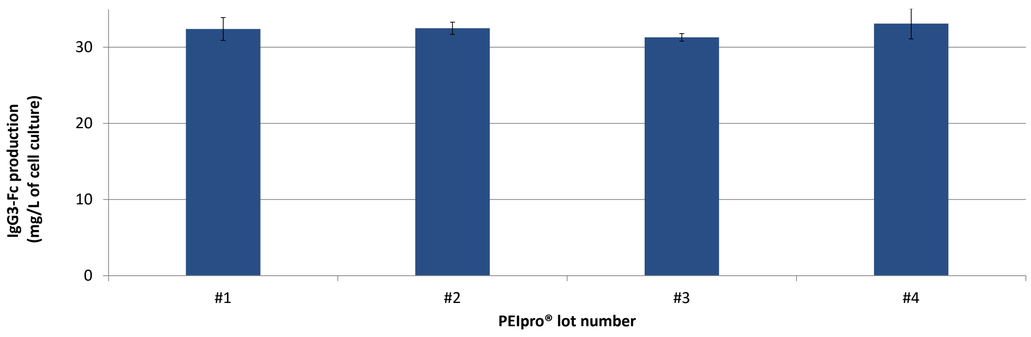
A High Quality Product Optimized for Transfection



Optimization process of PEIpro® chemistry. Whereas long polymer fragments lead to cell 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) and reproducibility in transfection.

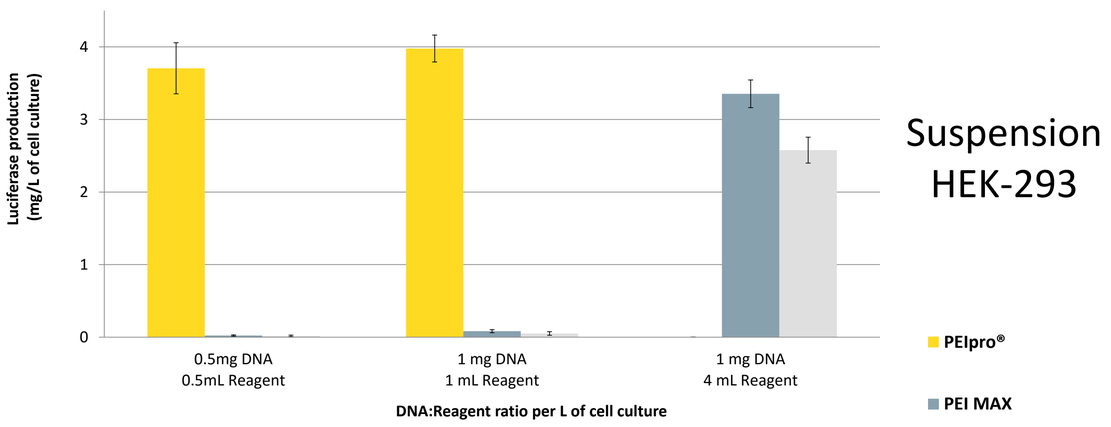


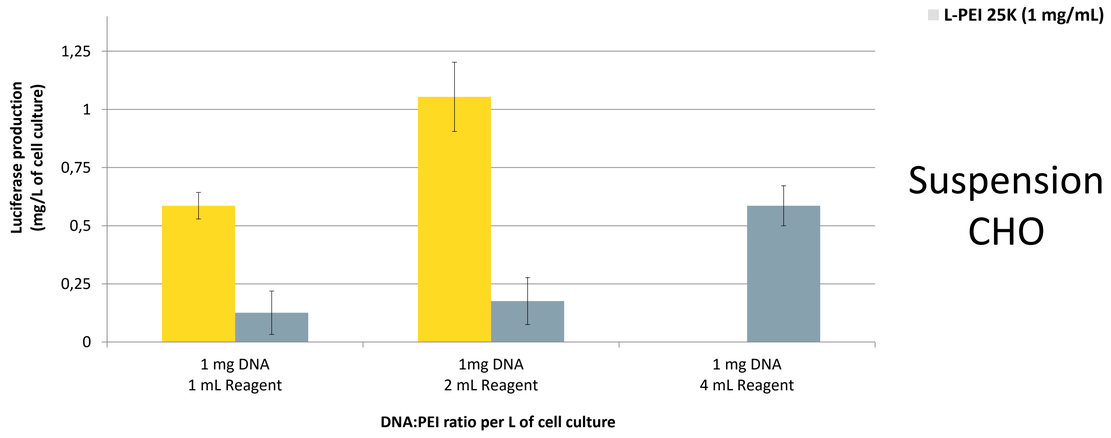
Manufacturing process of PEIpro® reagent. The linear form of PEIpro® and the manufacturing process developed by Polyplus-transfection® ensure a high, stable and reproducible amount of protonable amines available for transfection while providing a fully deacylated molecule and an extremely low polymer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



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).

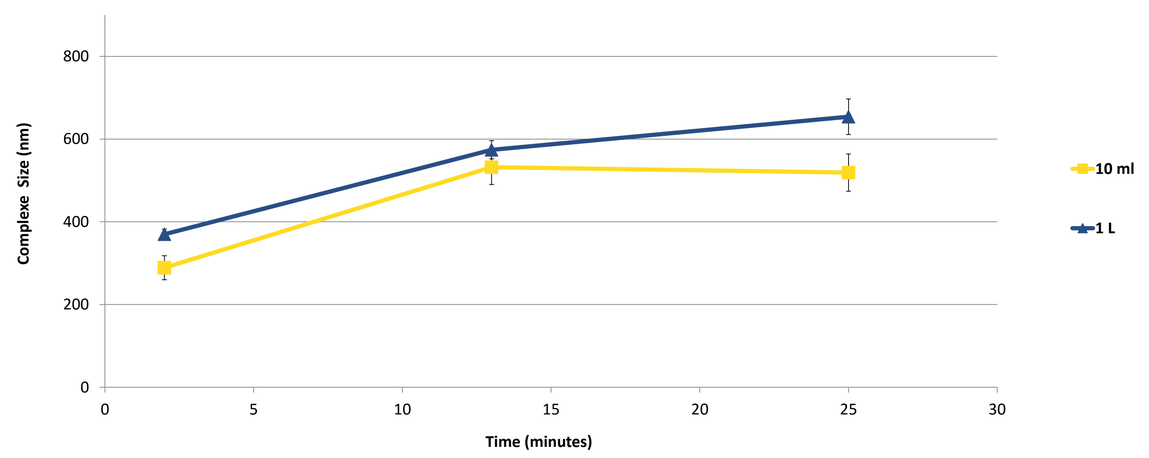
Superior Protein Production Efficiency



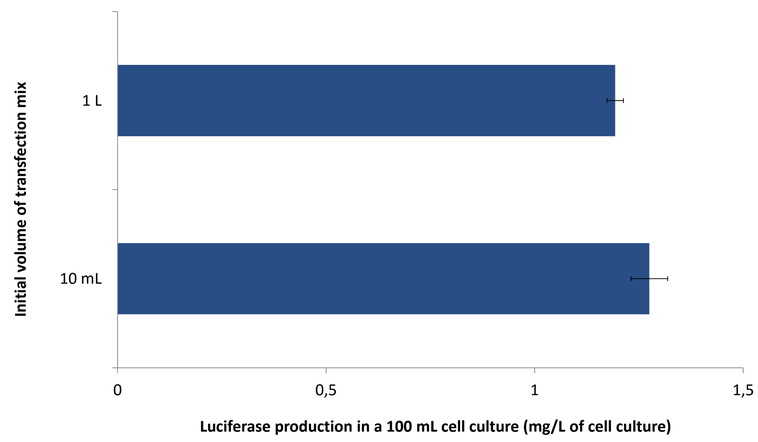


PEIpro® requires less reagent and similar to lower DNA amount compared to other PEIs. Suspension HEK-293 and CHO cells were seeded at 1×10^6 cells/mL in serum free medium and transfected with PEIpro®, PEI MAX and L-PEI 25 kDa (Polysciences, Warrington, PA) resuspended at 1 mg/mL. Luciferase expression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

Easily Scalable for Large Protein Production

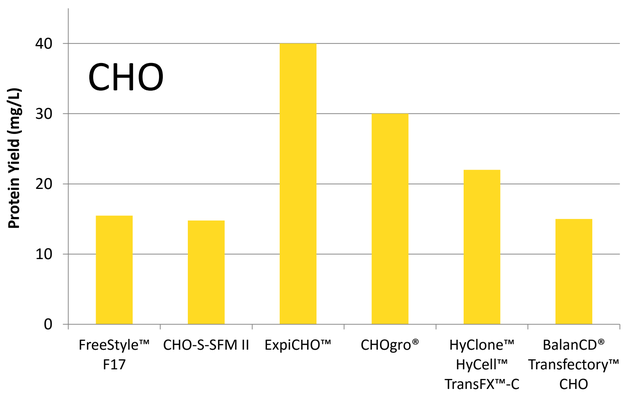


DNA-PEIpro® complex size is identical, independently from the volume of transfection mix preparation. Complexes were prepared with a DNA concentration of 0.01 mg per mL of complex volume at a DNA:Reagent ratio of 1:4, either in 10 mL or 1 L. The size of the complexes was then measured every ten minutes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



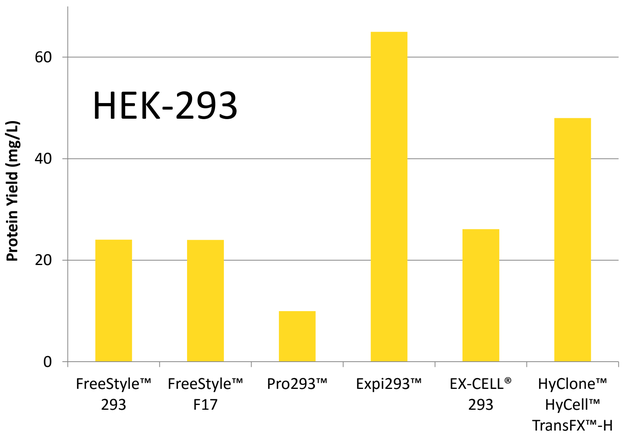
Scale-up protein production results with PEIpro® are highly predictable. Suspension CHO cells were seeded at 1×10^6 cells/mL in 100 mL serum-free medium.
DNA-PEIpro® complexes were prepared with a DNA concentration of 0.01 mg per mL of complex volume at a DNA:Reagent ratio of 1:4, either in 10 mL or 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



CHO 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 high quality product 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 components
- Robust and 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**

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