Improving transient CHO and HEK-293 Expression Systems with a powerful transfection solution for high protein production yields: FectoPRO®

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Abstract

Development process for biotherapeutic protein production usually begins with generating a high-performing stable cell line which can be used for manufacturing. As this step takes a lot of time, transient transfection offers a great alternative to quickly produce milligram to gram quantities of recombinant proteins and antibodies. A various number of culture media are available for performing transient protein production in both CHO and HEK cells but the limiting factor often remains the transfection reagent. Therefore, Polyplus-transfection has developed a novel technologically advanced transfection solution named FectoPRO®. Here we show that FectoPRO® outperforms currently available PEI-based and lipid-based transfection reagents in all the transient expression systems tested, offering great transfection efficiency and amazing protein yields.

Protocol

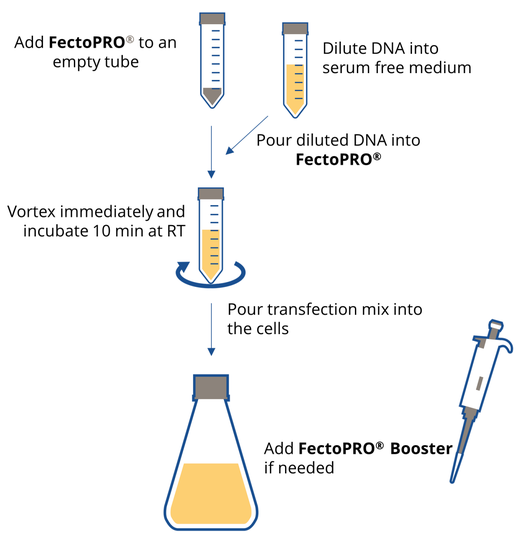
1- The day before transfection, prepare cell suspension at 1 x 10^6 cells/mL.

2- On the day of transfection, prepare the transfection mix in the serum free medium.

3- Add the FectoPRO®-DNA transfection mix to the cells, homogenize the culture.

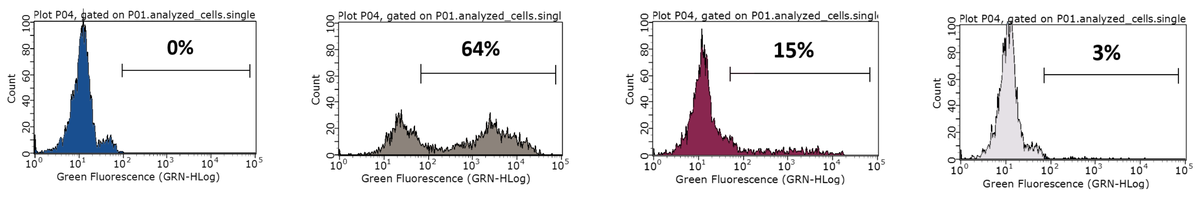
4- If FectoPRO® Booster is to be added, add it directly to the cell culture 0 to 4 hours post-transfection, homogenize.

5- Harvest protein or antibody when required.



Specifically developed for CHO cells

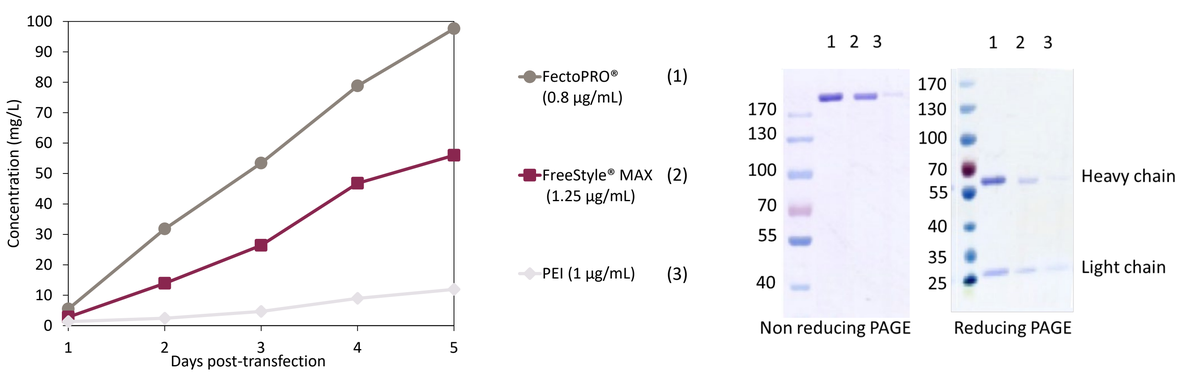
Superior transfection efficiency in CHO-K1



Non transfected cells | **FectoPRO®** | **FreeStyle™ Max** | **PEI**

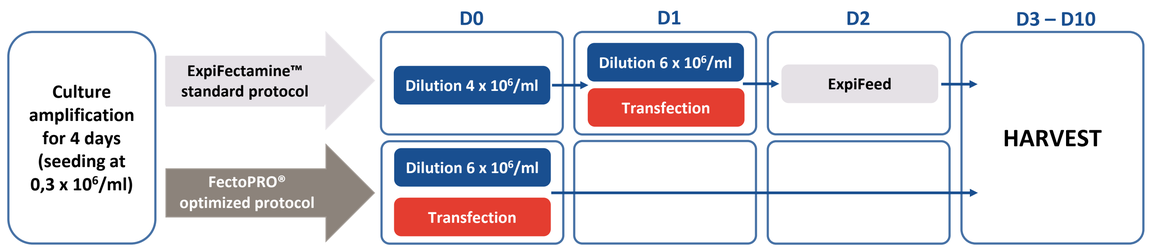
FectoPRO® shows a remarkable transfection efficiency in CHO-K1 cells in comparison to PEI and FreeStyle™ MAX. Suspension-adapted CHO-K1 cells were seeded following the recommended protocol, and transfected with FectoPRO® (0.8 µg DNA/mL), PEI (1 µg DNA/mL), and FreeStyle™ MAX (1.25 µg/mL) following the standard protocols. Transfection efficiency was determined by measuring the percentage of GFP-expressing cells by capillar cytometry 24 hours post-transfection.

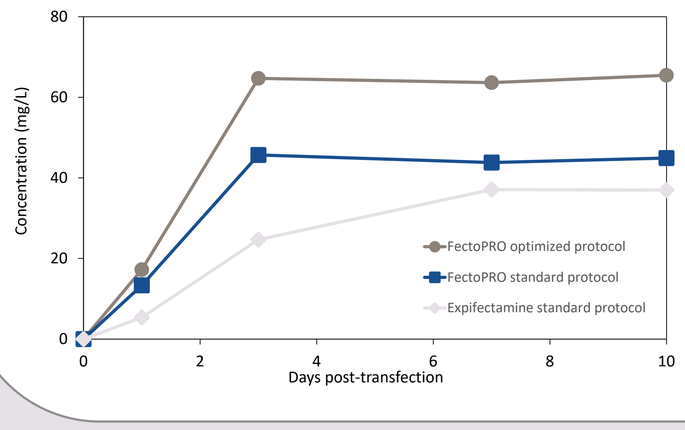
High-yield production of full mouse IgG in CHO-S cells



Significantly better yield of full mouse IgG is obtained with lower DNA amount when using FectoPRO® in comparison with FreeStyle™ Max and PEI. Mouse IgG production in CHO-S cells was achieved by co-transfection of plasmids coding for the Heavy chain & Light chain. Quantification was performed using protein G Biosensors (fortéBIO®). Qualitative analysis was done on 8% non reducing PAGE and 12% reducing PAGE 5 days post-transfection. *Data kindly provided by ProteoGenix SA.*

Higher yields obtained faster in the ExpiCHO™ system

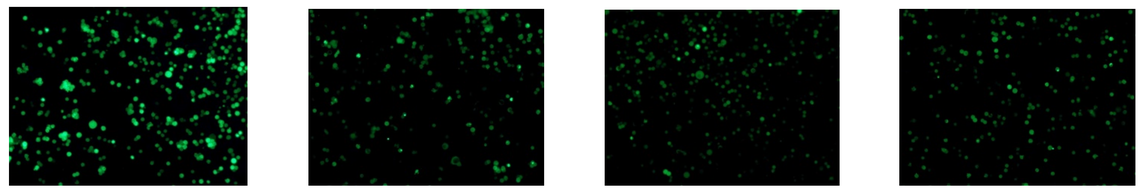




FectoPRO® protocol, to triple protein production yields in the ExpiCHO™ system compared to ExpiFectamine™-mediated transfection, as early as 3 days after transfection.
ExpiCHO-S™ cells were seeded following the recommended protocol in ExpiCHO™ Expression Medium, and transfected with FectoPRO® (0.8 µg DNA/mL) or ExpiFectamine™ CHO + Feed + Enhancer (0.8 µg DNA/mL) following the standard protocols. IgG_3-Fc production was assayed at different days using protein G Biosensors (fortéBIO® BLItz).
Detailed protocol available upon request.

Remarkably efficient in HEK-293 cells

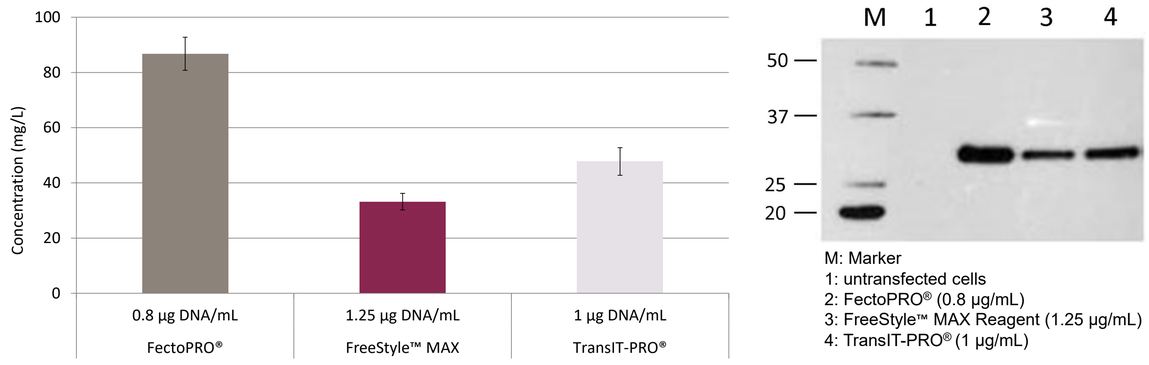
High transfection efficiency in HEK-293F cells



FectoPRO® | **FreeStyle™ MAX** | **TransIT-PRO®** | **PEI**

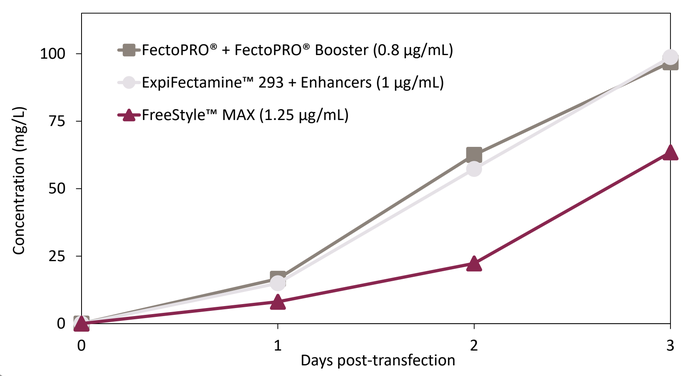
FectoPRO™ gives high transfection efficiency in suspension HEK-293F cells. HEK-293F cells were seeded at 1 x 10^6 cells/mL in 30 mL of FreeStyle™ 293 Expression Medium and transfected using a GFP expressing plasmid with FectoPRO™ (0.8 µg DNA/mL), PEI (1 µg/mL), FreeStyle™ MAX Reagent (1.25 µg DNA/mL) or TransIT-PRO® (1 µg DNA/mL). GFP expression was observed 24 hours after transfection using fluorescence microscopy.

Great protein production in HEK-293F cells



Significantly higher protein production yields are reached when using FectoPRO® in HEK-293F cells in comparison with competitors. FreeStyle™ 293F cells were seeded at 1 x 10^6 cells/mL in 30 mL of FreeStyle™ 293 Expression Medium and transfected with FectoPRO® and its competitors following the recommended protocols. Quantitation of IgG3-Fc fragment was performed by using protein G affinity column (HPLC) and qualitative analysis was done by Western Blot 72 hours post-transfection .

Sustained protein production with low DNA amount in the Expi293™ system

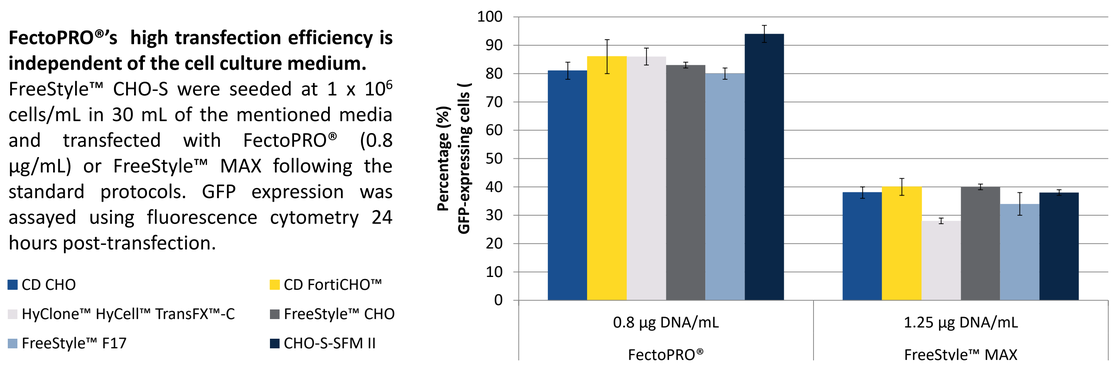


FectoPRO® allows a sustained protein production with yields similar to ExpiFectamine™ 293 while using 20% less DNA. Expi293F™ cells were seeded following the recommended protocol in Expi293™ Expression Medium, and transfected with FectoPRO® + FectoPRO® Booster (0.8 µg DNA/mL), ExpiFectamine™ 293 + Enhancers (1 µg DNA/mL), or FreeStyle™ MAX (1.25 µg/mL) following the standard protocols. IgG_3-Fc production was assayed at different days by protein G affinity quantification (HPLC).

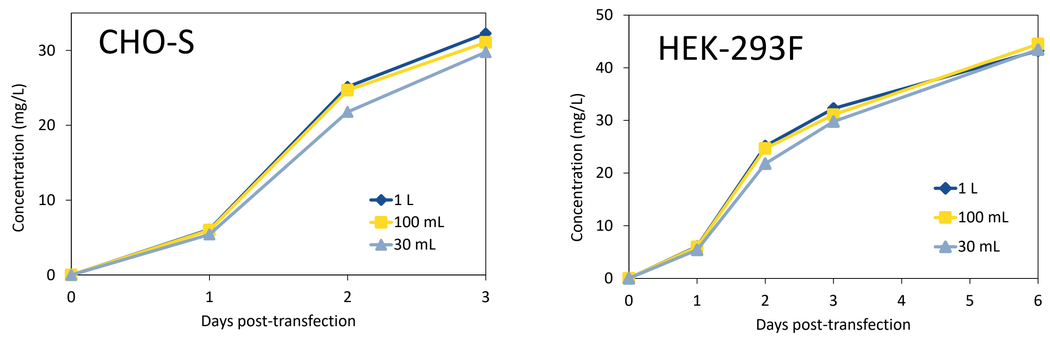
Easily implementable in a production process

Compatible with various synthetic media

FectoPRO®'s high transfection efficiency is independent of the cell culture medium. FreeStyle™ CHO-S were seeded at 1 x 10^6 cells/mL in 30 mL of the mentioned media and transfected with FectoPRO® (0.8 µg/mL) or FreeStyle™ MAX following the standard protocols. GFP expression was assayed using fluorescence cytometry 24 hours post-transfection.



Great scalability for antibody production



A perfect scalability for protein production is observed with FectoPRO® in both CHO and HEK-293 cells. FreeStyle™ CHO-S and HEK-293F cells were seeded at 1 x 10^6 cells/mL in either 30 mL, 100 mL or 1 L of their recommended FreeStyle™ media and transfected with an IgG_3-Fc expressing plasmid using FectoPRO®+ FectoPRO® Booster (0.5 µg DNA/mL). Quantification was performed every day using protein G affinity column (HPLC).

Conclusion

Advantages of FectoPRO®

- Amazing antibody yields in CHO & HEK-293 suspension cells, including high cell density systems
- Cost-effective Transient Gene Expression using low DNA amount (<1 µg/mL of cell culture)
- Sustained protein and antibody production over several days
- Easily scalable from a few mL to several liters of cell culture
- Compatible with various mammalian expression media and cell systems

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