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

Mathieu Porte, Jonathan Havard, Valérie Moreau, Jelena Vjetrovic, Fabrice Stock, Patrick Erbacher
Polyplus-transfection, Bioparc, 850 Boulevard S. Brant, 67400 Illkirch, France

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 10⁶ 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

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 (1.5 µg DNA/mL) following the standard protocols. Transfection efficiency was determined by measuring the percentage of GFP-expressing cells by capillary 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(Fecto®). Qualitative analysis was done on 8% non-reducing PAGE and 12% reducing PAGE 5 days post-transfection. Data directly provided by ProteGenics SA.

Remarkably efficient in HEK-293 cells

High transfection efficiency in HEK-293 cells

FectoPRO® gave high transfection efficiency in suspension HEK-293 cells. HEK-293 cells were seeded at 1 x 10⁶ cells/mL in 30 ml of Freestyle™ Expression Medium and transfected using a GFP expressing plasmid with FectoPRO® (0.8 µg DNA/mL), PEI (1 µg/mL), Freestyle® MAX Reagent (1.5 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-293 cells

Significantly higher protein production yields are reached when using FectoPRO® in HEK-293 cells in comparison with competitors. Freestyle™ 293F cells were seeded at 1 x 10⁶ cells/mL in 30 ml of Freestyle™ 293 Expression Medium and transfected with FectoPRO® and its competition following the recommended protocols. Quantification of IgG-Fc fragment was performed by using protein G affinity column (HPH) 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. ExpiFectamine™ 293 cells were seeded following the recommended protocol in Expi293™ Expression Medium, and transfected with FectoPRO® and its competition following the recommended protocols. Quantification of IgG-Fc fragment was performed at different days by protein G affinity quantification (HPH).

Easily implementable in a production process

Compatible with various synthetic media

FectoPRO® high transfection efficiency is independent of the cell culture medium. Freestyle™ CHO-S cells were seeded at 1 x 10⁶ 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-293 cells were seeded at 1 x 10⁶ cells/mL in either 100 mL or 3 L of their recommended Freestyle™ media and transfected with an IgG-Fc expressing plasmid using FectoPRO®+ FectoPRO® Booster (0.5 µg DNA/mL). Quantification was performed every day using protein G affinity column (HPH).

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

www.polyplus-transfection.com