ExpiCHO system: surpassing the performance of Expi293 in a transient CHO expression system

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Abstract and introduction

CHO cells are the predominant host for biotherapeutic protein expression, with roughly 70% of licensed biologics manufactured in CHO. Multiple attributes make CHO cells desirable for bioproduction, including the ability to adapt to high-density suspension culture in serum-free and chemically-defined media and the incorporation of post-translational modifications that are biologically active in humans. For these reasons, the ability to produce transient CHO-derived proteins early on during drug development is highly advantageous to minimize, as much as possible, changes in protein quality/function observed when moving from R&D to bioproduction. Unfortunately, CHO cells express lower levels of protein than HEK293 cells in existing transient systems, in some instances only 1% to 2% of the best 293-based systems, and only modest titer improvements are obtained through the optimization of individual components of existing CHO workflows. To address the significant unmet need for higher transient CHO protein titer, system-based approaches were employed whereby the latest advances in cell culture media, feeds, transfection reagents, and expression enhancements were optimized in conjunction with a new high-expressing CHO cell clone to generate a simple and robust workflow capable of generating g/L protein titers in 10-14 days. These advances will allow for unprecedented access to CHO-derived proteins early on during candidate selection and may serve to revolutionize the use of CHO cells for transient protein expression during the drug development process.

I. Generation of high-expressing ExpiCHO-S cells

ExpiCHO cells were transiently transduced and evaluated for protein expression using the Molecular Devices Clarys™ System. Selected clones were further evaluated via transfection with plasmids for multiple proteins.

Figure 1. Systems-based approach to increased transient protein expression

II. ExpiCHO Expression Medium and ExpiCHO™ Feed

ExpiCHO Expression Medium Attributes

- No supplementation required
- One medium for growth and transfection
- Minimal maintenance
- Optimized for transient transfection
- Chemically defined (CD)
- Animal-origin-free (AOF)
- Protein-free
- Mannose-resistant GM/cGMP
- Supports high-density cell growth
- Matched to a specific feed
- Free from regulatory/import/export limitations

Figure 2. Workflow for identifying high-expressing CHO clones. CHO cells were transiently transduced and evaluated for protein expression using the Molecular Devices Clarys™ System. Selected clones were further evaluated via transfection with plasmids for multiple proteins.

Figure 3. Characterization of ExpiCHO-S cells.

(A) ExpiCHO-S cells. (B) Stability of protein expression over 10 passages. (C) Growth and viability curves for ExpiCHO-S cells grown in serum-free shake flask culture.

Figure 4. Optimization of ExpiFectamine CHO Enhancer and ExpiCHO Feed addition

(A) Protein titer is reduced by 50% or more without the addition of the ExpiFectamine CHO enhancer reagent. (B) Two equal-volume feeds on Days 1 and 5 post-transfection double protein titer.

Figure 5. Characteristics of ExpiFectamine CHO Transfection Reagent

(A) When used in conjunction with ExpiCHO enhancer and feed, ExpiFectamine CHO Transfection Reagent generates greater than 27-fold higher titers than P38 protocol. (B) Despite the high-density of cells at the time of transfection, plasmid DNA levels as low as 0.5 µg/g of culture volume generate maximal protein titers, corresponding to half of the industry standard of 1.0 µg/g, plated DNA.

Figure 6. ExpiCHO protocol(s).

Figure 7. Kinetics of IgG expression, viability, and viable cell density.

- ExpiCHO system: Highest protein titer in half the time. (A) Standard protocol: Blue line High-titer protocol consisting of one feed and temperature shift to 25°C. Red line: Max titer protocol consisting of two feeds and temperature shift to 25°C. (B) Viable cell density post-transfection. (C) Viability post-transfection for troubleshooting red line.

Figure 8. Ultrahigh-density transfection for rapid protein expression

- Ultrahigh-density transfection can generate protein titers in half the time. (A) Standard protocol: Blue line High-titration transfection of ExpiCHO cells at density of 5x10⁶ cells/mL using the standard protocol. Blue line High density transfection of ExpiCHO cell at 6x10⁶ cells/mL. (B) High-titer protocol: Blue line High-density transfection at 10x10⁶ cells/mL.

Figure 9. Optimization of ExpiFectamine CHO Enhancer and ExpiCHO Feed addition

(A) Protein titer is reduced by 50% or more without the addition of the ExpiFectamine CHO enhancer reagent. (B) Two equal-volume feeds on Days 1 and 5 post-transfection double protein titer.

Figure 10. Protein quality and glycosylation patterns in ExpiCHO and Expi293™ systems

(A) SDS-PAGE of human IgG under non-reducing (left) and reducing (right) conditions. (B) Size exclusion chromatography of human IgG. (C) Human IgG glycans.

Conclusions

We describe a systems-based approach for enhancing levels of transient protein production in CHO cells that allows for the production of recombinant proteins at levels exceeding those of the Expi293 system while maintaining activity, purity, and glycosylation patterns comparable to those observed in stably transfected CHO-S cells. This performance enhancement was made possible through the incorporation of multiple novel reagents, including: (1) a high-expressing CHO cell clone, (2) a CADADF culture medium that allows for high-density CHO growth and transfection, (3) an optimized CHO cell transfection reagent, (4) a novel CHO feed optimized for transient transfection culture conditions, (5) a post-transfection enhancer solution, and (6) a simple-to-perform workflow.

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