

Attaining High Transient Titters in CHO Cells

Case Study Involving the Use of the ExpiCHO™ Mammalian Transient Expression System

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The ability to produce transient CHO-derived proteins early during biotherapeutic development is highly desirable to mimic, as closely as possible, the final critical quality attributes of the protein when manufactured at bioproduction scales. Unfortunately, CHO cells typically express lower levels of protein than HEK293 in transient systems, in some instances 50–100 times less than the best 293-based systems, and only modest titer improvements are obtained through the optimization of individual components of existing transient CHO workflows.

To address the significant unmet need for higher transient CHO protein titers, we employed systems-based approaches whereby the latest advances in cell culture media, feeds, transfection reagents, and expression enhancers were optimized in conjunction with a new high-expressing CHO cell clone.

The goal was to generate a simple and robust workflow for transient protein expression in CHO cells capable of generating gram-per-liter protein titers in 10–14 days, titers previously attainable only by stably expressing cells in a bioreactor setting. These advances allow for unprecedented access to CHO-derived proteins during therapeutic candidate selection and may serve to

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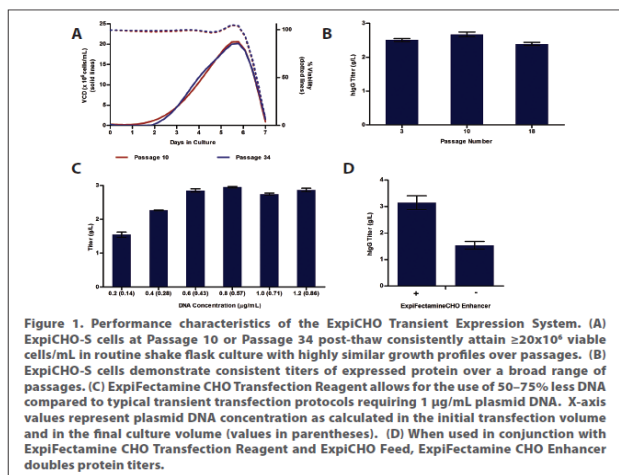
revolutionize the use of CHO cells for transient protein expression during the early stages of drug development.

Maximizing Transient Titers

Typical yields for monoclonal antibodies produced in transient CHO systems are in the 1–20 mg/L range, forcing researchers to perform large-scale transfections, multiple transfections, or both, to generate enough material for their studies. To attain gram per liter levels of transiently expressed proteins in CHO, the ExpiCHO™ mammalian transient expression system was developed through the optimization of each critical system component followed by design-of-experiment (DoE) of the entire system to obtain maximum protein expression levels.

As a first step in this process, a new, high-expressing CHO clone (ExpiCHO-S) was isolated from existing CHO-S cell banks and matched to a new chemically defined and animal origin-free cell culture media (ExpiCHO Expression Medium). When maintained in ExpiCHO Expression Medium, ExpiCHO-S grow rapidly (doubling time ~17 hours) to high density ($>20 \times 10^6$ cells/mL) in standard shake flask culture.

ExpiCHO-S cells show excellent stability of growth (*Figure 1A*) and protein expression (*Figure 1B*) over passages, ensuring consistent performance over time. ExpiCHO Expression Medium enables transfection at 6.0×10^6 cells/mL and when used in conjunction with ExpiCHO Feed maintains high cell viability (70–80% at the time of harvest) throughout protein production runs up to 14 days, allowing for exceptionally clean supernatants requiring minimal/no preprocessing prior to protein purification.



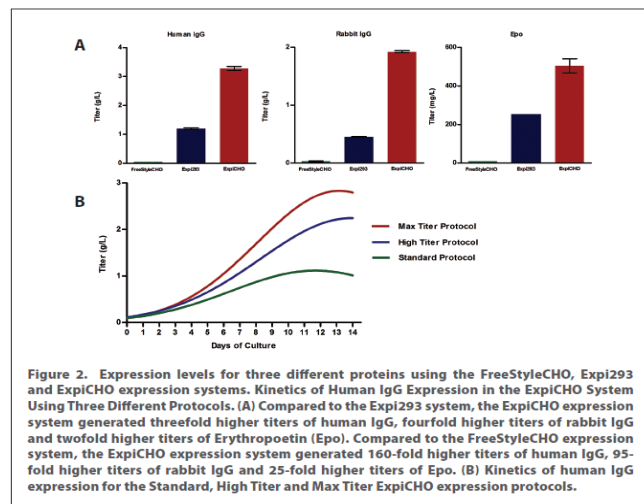
To take advantage of the high density growth of the ExpiCHO-S cells, a new transfection reagent, ExpiFectamine CHO, was developed that allows for high-efficiency transfection of ExpiCHO-S cell cultures without the need for media exchange. Additionally, ExpiFectamine CHO requires significantly less

plasmid DNA than typical transient transfection protocols that use 1µg/mL DNA to reach maximum titers (*Figure 1C*).

Lastly, a cocktail of transfection enhancers (ExpiCHO Enhancer) was developed to work in conjunction with ExpiFectamine CHO transfection reagent and ExpiCHO Feed, allowing for a further twofold increase in protein titers while still allowing for high cell viability throughout the expression run (*Figure 1D*).

System Comparisons

When combined into a single expression system and optimized via multifactorial DoE, the protein yield improvements enabled by each of the ExpiCHO system were synergistic, generating protein titers over 3 g/L for human IgG, 2 g/L for rabbit IgG, and 500 mg/L for erythropoietin (*Figure 2A*); these values represent 25–160 fold increases in protein expression levels compared to the existing FreeStyleCHO expression system and 2–4 fold increases compared to the Expi293™ expression system, the current standard in transient transfection systems.



Compared to the Expi293 expression system where protein is typically harvested 5–7 days post-transfection, the ExpiCHO expression system generates similar or greater titers on days 5–7 post-transfection as the Expi293 system, however, the ExpiCHO system continues to produce protein for up to 14 days post-transfection, leading to significantly higher overall titers for many proteins (*Figure 2B*).

The ExpiCHO expression system provides unprecedented protocol flexibility, allowing researchers to tailor the ExpiCHO workflow to their needs/time lines/equipment availability using one of three different protocols (*Figure 2B*): 1) the Standard protocol requires a single feed on Day 1 post-transfection and 37°C incubation throughout the expression run, a protocol identical in the number of steps to the Expi293 expression system; 2) The High Titer protocol requires a single feed and a shift to 32°C one day post-transfection; and 3) the Max Titer

protocol requires feeds on Day 1 and Day 5 post-transfection with a shift to 32°C one day post-transfection.

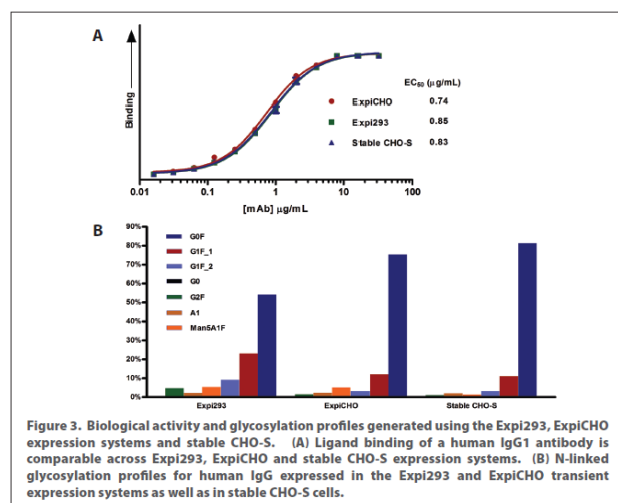
Protein expression results are readily scalable for each of the three protocols, with consistent titers generated in volumes ranging from 30 mL to 1 L culture volumes tested to date.

This exceptional flexibility not only allows for ease of scheduling expression runs within the workweek, but also offers two temperature conditions for protein expression, providing multiple options for hard to express or aggregation-prone proteins. Indeed, certain proteins have been shown to express better at 37°C than 32°C (data not shown), even though expression levels in the Max Titer protocol at 32°C are typically 2–3 fold higher than at 37°C for many proteins.

Protein Functionality and Glycosylation Patterns

Although high protein yields are desirable, resultant proteins are less valuable if they are aggregated, misfolded, degraded or improperly glycosylated. The quality of the proteins expressed in the ExpiCHO System was compared to the quality of the same proteins expressed in the Expi293 expression system or in a stably transfected CHO-S cell line, a cell line commonly used for GMP protein production. N-linked carbohydrates for a recombinant human IgG were compared.

The data demonstrate highly comparable glycosylation patterns for antibody expressed in the ExpiCHO system or by stable CHO-S, but, not surprisingly, quite distinct from HEK293 transiently-derived protein generated in the Expi293 expression system (*Figure 3A*). Antibody fucosylation levels have the potential to impact antibody Fc-related effector functions; no differences in the levels of GO (non-fucosylated) variants were observed between any of the systems.



The antibody produced in each system was tested for activity in a ligand binding assay. Resultant EC₅₀ values were within 15% for antibody generated in the different expression systems indicating comparable biological activity between ExpiCHO, Expi293 and stable CHO-derived protein (*Figure 3B*).

The results demonstrate that the ExpiCHO transient expression system can deliver 25–100 fold or greater improvements in functional protein yields compared to existing CHO-based transient transfection systems and 2–4 fold increases compared to Expi293, the current standard in transient transfection.

To achieve these yields, the ExpiCHO transient expression system incorporates multiple advances in protein expression technology into a flexible, easy-to-follow protocol capable of generating gram-per-liter titers in a transient CHO system, allowing researchers to start in CHO and stay in CHO during biotherapeutic development.

Acknowledgements

We would like to thank Michael Gillmeister for his assistance with glycosylation profiling and Jeffrey Fein for protein activity assessments.

The ExpiCHO™ and Expi293™ Expression Systems are for Research Use Only. Not for use in diagnostic procedures.

