High Yield Protein Production System for Suspension CHO Cells

Simpler Workflow – Earlier Harvest – More Antibody

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INTRODUCTION

The history and utility of suspension Chinese hamster ovary (CHO) cells for biotherapeutic protein production is unparalleled. Advances in transient transfection technologies and pressure to shorten development timelines have created the opportunity for systems offering rapid generation of milligrams to grams of protein early in the drug discovery process. The high yield and low cost associated with improved transient gene expression methods enables researchers to determine, at an early stage, if drug candidates have desirable attributes and warrant the resources required to generate stable clonal cell lines.

To further increase the protein yields obtained by transient gene expression in suspension CHO cells, we developed the CHOgro® High Yield Expression System to improve upon our previous platform (Figure 1) by: (1) identifying cell culture additives (expression enhancers) that significantly increase cell productivity and (2) developing a streamlined protocol in which the steps of transfection, enhancer addition, and temperature shift are carried out on the same day. Through multiple rounds of screening and optimization, we identified the CHOgro® Titer Enhancer, which acts in synergy with the *Trans*IT®-PRO® Transfection Reagent and CHOgro® Expression Medium to increase antibody production.

Optimization of protein production parameters and process robustness were examined by a transfection complex formation time course and testing expression of various protein constructs in Freestyle™ CHO-S and ExpiCHO™ cells adapted to the CHOgro® Expression Medium. Scalability of transient transfection was also assessed in culture sizes ranging from 2 ml up to 2 L in shake flasks. Head-to-head comparisons of the CHOgro® High Yield Expression System to the ExpiCHO™ Expression System using six different antibody constructs show that higher or comparable protein titers are obtained at Day 7 and 14 post-transfection with the CHOgro® High Yield Expression System.

Higher Titers Faster:

MORE PRODUCT IN LESS TIME

Reach higher protein and antibody titers faster than ExpiCHO™ and FectoCHO™ Systems

Simple, Streamlined Workflow:

NO LICENSING, LESS MANIPULATION, REDUCE RISK OF CONTAMINATION

Same-day transfection, enhancer addition and temperature shift

Scalable:

SCALABLE SCREENING RESULTS

Reproducible titers from 2 ml to 2 L

FIGURE 1: Benefits of the CHOgro® High Yield Expression System.

Our results indicate that the attributes of the new and improved CHOgro® High Yield Expression System will help researchers obtain gram quantities of protein, simplify their workflow, and shorten their biotherapeutic development pipeline.

RESULTS

High Cell Growth and Viability Post-Transfection

To understand the effect of the CHOgro® Titer Enhancer on the health of high-density suspension CHO cells, we monitored cell viability and counts over seven days post-transfection using the CHOgro® High Yield Expression System. CHO-S cells were transfected on Day Zero with TransIT-PRO® alone or TransIT-PRO® and CHOgro® Titer Enhancer at a density ofCell growth and viability were not significantly affected by the presence of the enhancer (Figure 2). We concluded that the increase in antibody titers observed using the CHOgro® Titer Enhancer is not due to changes in cell viability or gene delivery efficiency (data not shown), but instead appear to be the result of alterations to cellular pathways that control recombinant protein expression.

Comparable Titers with CHO-S and ExpiCHO™ Cells

The CHOgro® High Yield Expression System was developed for use in both CHO-S and ExpiCHO™ cells. Figure 3 illustrates that with multiple protein constructs comparable titers are obtained using either suspension CHO cell line.

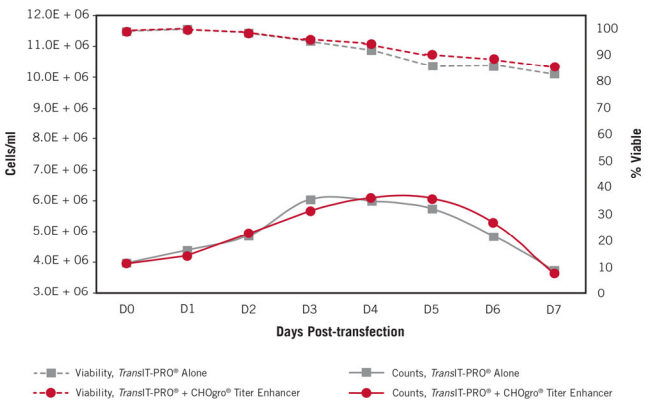

FIGURE 2: CHOgro® Titer Enhancer does not adversely affect cell growth and viability post-transfection. CHO-S cells were transiently transfected with *Trans*IT-PRO® Transfection Reagent. All cultures were shifted to 32 °C immediately post-addition of the transfection complexes and, where indicated, the CHOgro® Titer Enhancer was added to the culture.

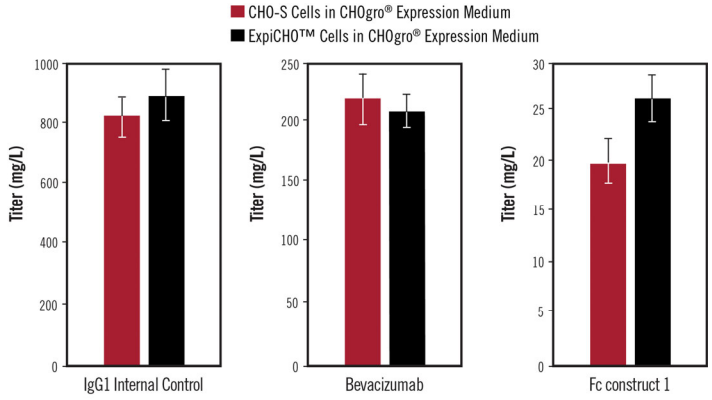

FIGURE 3: CHO-S or ExpiCHO™ cells yield similar titers using the CHOgro® High Yield Expression System. Both cell lines were transfected with plasmid encoding an IgG1 internal control antibody, Bevacizumab, or Fc-fusion construct. Day 14 supernatants were analyzed with an IgG ELISA.

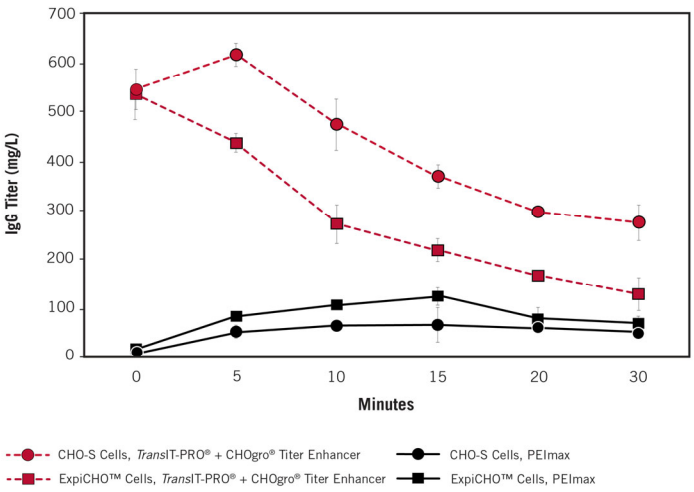

FIGURE 4: Complex formation time is a key parameter for achieving high titers with the CHOgro® High Yield Expression System. Transfection complexes, formed with either *Trans*IT-PRO® or PEImax, were incubated at the indicated times before addition to cultures of CHO-S or ExpiCHO™ cells. Day 7 supernatants were analyzed with an IgG ELISA.

Transfection Complex Formation Time Is a Key Factor

Among the parameters assessed, the time the transfection reagent is allowed to incubate with DNA before addition of transfection complexes to the cultures (i.e. transfection complex formation time) was identified as a critical factor to optimize for achieving high titers using the CHOgro® High Yield Expression System. As shown in Figure 4, optimal complex formation time is less than five minutes for both CHO-S and ExpiCHO™ cells transfected with TransIT-PRO® and CHOgro® Titer Enhancer. Using either cell line, the CHOgro® High Yield Expression System outperforms PEImax. Importantly, optimal complex formation times are system-specific and should not be used interchangeably between protocols with different transfection reagents.

1000-fold Scalability

Scalability of transient transfection was assessed in culture sizes ranging from two milliliters up to two liters in shake flasks. Comparable titer concentrations were obtained from the smallest to the largest volume formats (Figure 5), which suggests the CHOgro® High Yield Expression System can be integrated into diverse research and manufacturing workflows. We typically perform screens in 2 ml of culture medium per well in 6-well non-tissue culture treated plates. These small-scale experiments accurately depict larger volumes and increase experimental throughput.

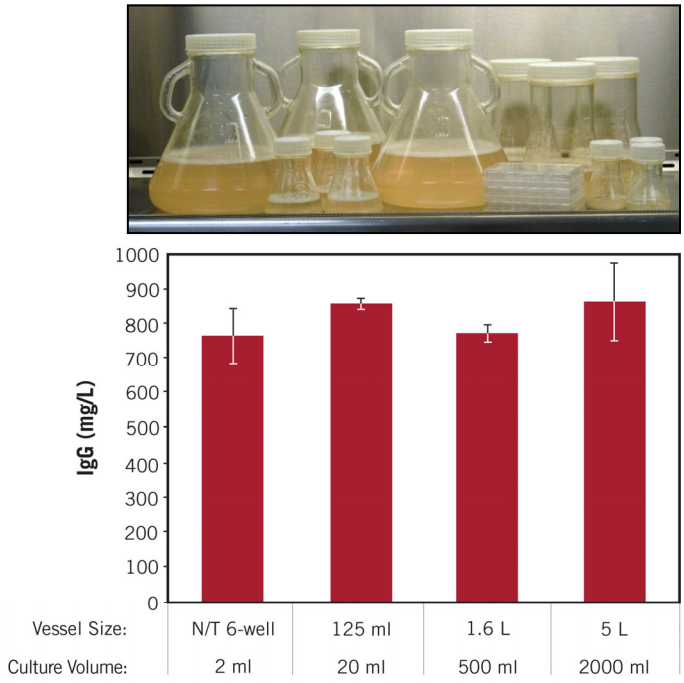

FIGURE 5: CHOgro® High Yield Expression System enables broad scalability, 1000-fold. Human IgG1 was produced by transient transfection in the following volumes/culture vessels: 2 ml/non-tissue culture treated 6-well dish, 20 ml/125 ml Thomson flask, 500 ml/1.6 L Thomson flask, 2000 ml/5 L Thomson flask. Day 14 supernatants were analyzed with an IgG ELISA.

CHOgro® High Yield Versus ExpiCHO™

Head-to-head comparisons of the CHOgro® High Yield Expression System to the ExpiCHO™ Expression System were performed using six different therapeutically relevant antibody constructs (Table 1). For these constructs, a higher or comparable protein titer was obtained using the CHOgro® High Yield Expression System at both Day 7 and 14 post-transfection (Figure 6).

METHODS

Cell Culture

FreeStyle™ CHO-S cells (ThermoFisher Scientific) or ExpiCHO-S cells (ThermoFisher Scientific) were cultured in CHOgro® Expression Medium supplemented with 4 mM L-Glutamine and 0.3% Poloxamer 188 (Mirus Bio). Cells were cultivated at 37 °C in a humidified incubator with 8% CO_2 and shaking. Cell counts and viability (via propidium iodine staining) were measured using a Guava easyCyte™ 5HT flow cytometer (EMD Millipore).

Molecule Name	Target	Company
hIgG1 Internal Control	Confidential	Mirus Bio
Alemtuzumab	CD52	Ilex Oncology; Millennium and Berlex
Bevacizumab	VEGF	Genentech and BioOncology
Cetuximab	EGFR	Bristol-Myers Squibb and ImClone
Rituximab	CD20	Genentech and IDEC
Trastuzumab	HER2	Genentech

TABLE 1: Representative Antibody Targets Used in Figure 6

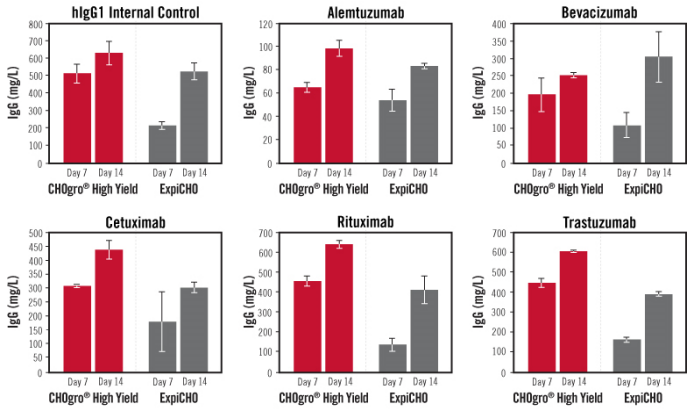

FIGURE 6: The CHOgro® High Yield Expression System outperforms the ExpiCHO™ Expression System in production of multiple antibody constructs.

Transient Transfection

CHO cells were transfected at 4 x 10^6 cells/ml in CHOgro® Expression Medium with 1 µg/ml plasmid DNA using either the *Trans*IT-PRO® Transfection Reagent (Mirus Bio) at a 1:1 (vol:wt) reagent-to-DNA ratio, or with PEImax (Polysciences) at a 4:1 reagent-to-DNA ratio. CHOgro® Titer Enhancer was added to the TransIT-PRO® Transfection Reagent at 20 µl per 1 ml of culture. Cultures were shifted to 32 °C immediately post-addition of the transfection complexes to the culture. The transfections conducted with the ExpiCHO™ Expression System (ThermoFisher Scientific, Figure 6) followed the Max Titer Protocol: 6 x 10^6 cells/ml ExpiCHO™ cells (ThermoFisher Scientific) cultured in ExpiCHO™ Expression Medium (ThermoFisher Scientific) were transfected using the Expifectamine™ CHO Transfection Kit (ThermoFisher Scientific) at a 3.2:1 reagent-to-DNA and 1 µg plasmid DNA/ml of culture; Expifectamine™ CHO Enhancer and Feed (ThermoFisher Scientific) were added at 24 hours post-transfection and cultures were shifted to 32 °C, and at Day 5 a second volume of the ExpiCHO™ Feed (ThermoFisher Scientific) was added to the appropriate flasks.

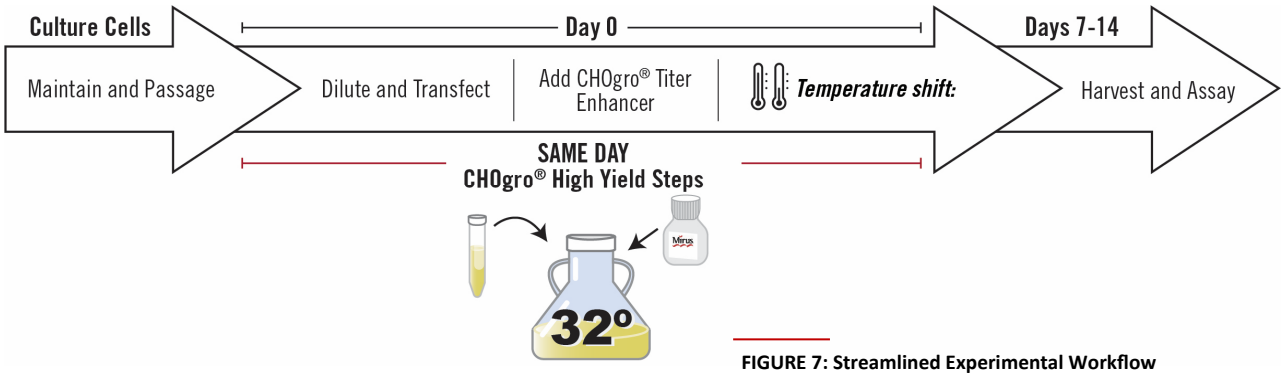

FIGURE 7: Streamlined Experimental Workflow

Determination of IgG Titer

Post-transfection, supernatants were analyzed using a standard sandwich human IgG ELISA. In the Figures, the error bars represent the standard deviation of triplicate technical replicates, or in Figure 4, the range of duplicate samples.

CONCLUSION

The CHOgro® High Yield Expression System was engineered to maximize transient protein production in suspension CHO cells, while still maintaining a simple and cost-effective workflow (Figure 7). The ability to add expression enhancers at the time of transfection and immediately shift cell cultures to hypothermic conditions provides researchers with more flexibility in the timing of their experiments, saves time and reduces the risk of contamination caused by repeated handling of the culture.

Generate High Antibody Titers Like a PRO

The **CHOgro® High Yield Expression System** is the most advanced and cost-effective transient transfection system for high-yield protein production in suspension CHO cells. Our second-generation system features the **CHOgro® Titer Enhancer** which provides rapid, industry-leading protein yields.

HIGH YIELD – Reach higher antibody titers in seven days – faster than the ExpiCHO Expression System

SIMPLE WORKFLOW – Same-day transfection, enhancer addition and temperature shift

WORRY FREE – No commercial license required; animal origin-free

Description	Size	Cat. No.
CHOgro® High Yield Expression System CHOgro® Expression Medium (2 x 1L), CHOgro® Titer Enhancer (20 ml), TransIT-PRO® Transfection Reagent (1 ml), CHOgro® Complex Formation Solution (100 ml), Poloxamer 188 Solution (100 ml), L-Glutamine Solution (100 ml)	1 System	MIR 6270
CHOgro® Transfection and Titer Enhancer Kit CHOgro® Titer Enhancer (20 ml), TransIT-PRO® Transfection Reagent (1 ml)	1 Kit	MIR 6225
CHOgro® Expression Medium	1 L	MIR 6200
CHOgro® Expression Medium, Polybag	10 L	MIR 6202
CHOgro® Expression Medium, Dry Powder	10 L	MIR 6201
CHOgro® Complex Formation Solution	100 ml	MIR 6210
Poloxamer 188 Solution	100 ml	MIR 6230
L-Glutamine Solution	100 ml	MIR 6240
Human IgG1 Expression Control	100 µg	MIR 6250