Development and Optimization of CHOgro® Transient Expression Technologies for High Titer Antibody Production in Suspension CHO Cells

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Abstract

During early stage drug development, quickly obtaining relevant candidate proteins through transient transfection can accelerate drug discovery. High titers are often obtained from Human Embryonic Kidney (HEK) 293 derived cell types; however, the use of different host cells between early stage transient and later stable protein production is a concern and can lead to the advancement of false-positive candidates. Chinese hamster ovary (CHO) cells are a desirable target cell type due to growth characteristics and a history of regulatory approval; however, their use has been hampered by low transient gene expression levels. To address this short-coming, we have created a robust and simple CHO transient protein expression system enabled by critical media attributes such as high density cell growth, quick adaptation and minimization of cell clumping post-transfection. The CHOgro® Expression System was developed through systematic optimization of transfection protocol parameters including: cell density, transfection reagent, media formulation and culture temperature leading to a commercially accessible high titer CHO transient transfection platform. Through this optimization antibody titers increased 2-10 fold over existing technologies with higher amounts of antibody secreted per cell. Six different representative antibody constructs were tested using the CHOgro® Expression System. Notably, even CHO cells maintained in other commercially available media formulations (e.g. FreeStyle™ CHO Expression Medium) can be seamlessly adapted with a full media exchange to the CHOgro® Expression Medium 24 hours prior to transfection and yield multi-fold increases in transient expression levels. With the CHOgro® Expression System high protein titers can now be achieved in suspension CHO cells through high density transient transfection.

High Density Growth

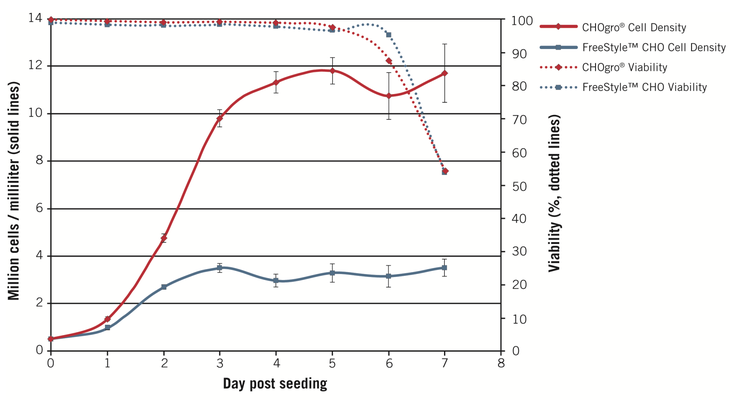


Figure 1. Suspension CHO Cells Grow to High Density in the CHOgro® Expression Medium.
Triplicate flasks of FreeStyle™ CHO-S cells were seeded in CHOgro® Expression Medium (red line) or FreeStyle™ CHO Expression Medium (blue line) at a cell density of 0.5 x 10^6 cells/ml, 40 ml per 125 ml shake flask (Thomson). Cell counts (solid line) and viability (propidium iodide staining, dotted line) were measured daily using a Guava easyCyte™ 5HT flow cytometer (EMD Millipore). Error bars represent the standard deviation of three readings of biological triplicates.

Transfection Optimization: Cell Density

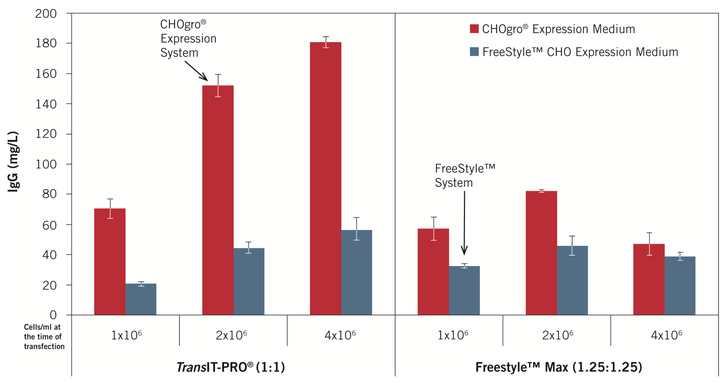


Figure 2. Higher Cell Densities Leads to Higher Titers Using the CHOgro® Expression System.
Human IgG1 was produced by transient transfection using *Trans*IT-PRO® (1:1) or FreeStyle™ MAX (1.25:1.25) transfection reagents according to the manufacturers' protocol (reagent:DNA ratio, volume:weight). Transfections were performed using 1 µg plasmid DNA per milliliter of culture and cell densities of 1, 2 or 4 x 10^6 cells/ml at the time of transfection. FreeStyle™ CHO-S cells were cultured in CHOgro® Expression Medium (red bars) or FreeStyle™ CHO Expression Medium (blue bars) and plated into non-treated 6-well plates (2ml/well) for transfection. (A) Day 6 supernatants were clarified and analyzed using a human IgG ELISA (Zeptometrix). Error bars represent the standard deviation of triplicate technical replicates. The arrows the standard protocol for the CHOgro® Expression System and FreeStyle™ CHO Expression System are designated by the arrows.

High Transfection Efficiency

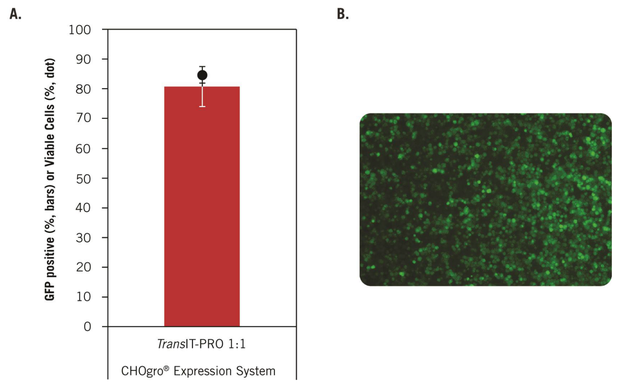


Figure 3. High Efficiency Transfection Using the *Trans*IT-PRO® Transfection Reagent.
Human IgG1 was produced by transient transfection using the *Trans*IT-PRO® Transfection Reagent (1:1) (reagent:DNA ratio, volume:weight) using 1 µg plasmid DNA per milliliter of culture and cell a density of 2 x 10^6 cells/ml in the CHOgro® Expression Medium at the time of transfection. FreeStyle™ CHO-S cells were cultured in CHOgro® Expression Medium and plated into non-treated 6-well plates (2ml/well) for transfection. A. GFP levels and cell viability (propidium iodide) were measured 48 hours post-transfection using a Guava easyCyte™ 5HT flow cytometer (EMD Millipore). B. Images were captured using a Zeiss Axiovert inverted fluorescence microscope.

More Antibody Secretion

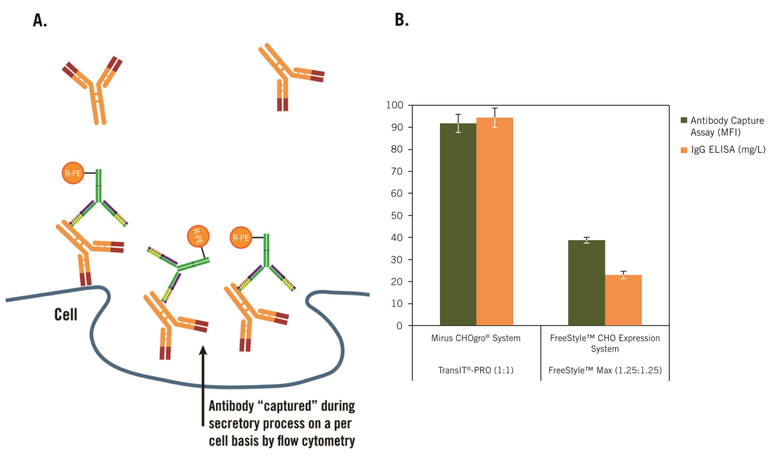


Figure 4. More Antibody is Secreted Per-Cell With the CHOgro® Expression System.
Human IgG1 was produced by transient transfection using *Trans*IT-PRO® (1:1) or FreeStyle™ MAX (1.25:1.25) transfection reagents according to the manufacturers' protocol (reagent:DNA ratio). Transfections were performed using 1 µg plasmid DNA per milliliter of culture and cell densities of 2 x 10^6 cells/ml or 1 x 10^6 cells/ml for the CHOgro® or FreeStyle™ System, respectively, at the time of transfection. FreeStyle™ CHO-S cells were cultured in CHOgro® Expression Medium or FreeStyle™ CHO Expression Medium and plated into non-treated 6-well plates (2ml/well) for transfection. (A) Cells were analyzed using antibody capture. Briefly, an aliquot of cells was washed, and incubated with an anti-IgG-PE antibody and blocking agent, washed and assayed for fluorescence. (B) Fluorescence was measured using a Guava easyCyte™ 5HT flow cytometer. Antibody levels were also analyzed from day 6 clarified supernatants using a human IgG ELISA (Zeptometrix). Error bars represent the standard deviation of triplicate technical replicates.

CHOgro® Expression System

CHOgro® Expression System

- CHOgro® Expression Medium (2 x 1 L)
- *Trans*IT-PRO® Transfection Reagent (1 ml)
- CHOgro® Complex Formation Solution (100 ml)
- Poloxamer 188 (100 ml)
- L-glutamine (100 ml)

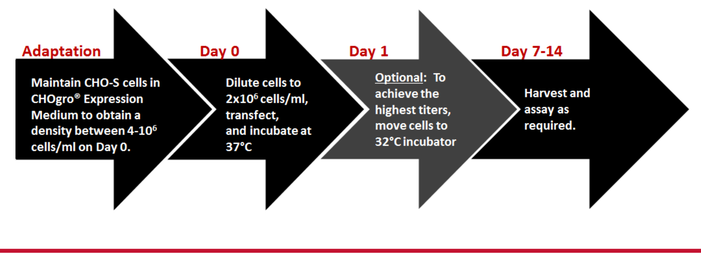
NOTE: Available as a full kit or all components can also be purchased separately.

Human IgG1 Expression Control (MIR 6250), a premix of heavy and light chain expressing plasmid constructs, is also available as a positive control for your transfection experiments.

CHOgro® Expression Medium

CHOgro® Expression Medium is a chemically defined, hydrolysate-free and animal-origin-free medium. CHOgro® is formulated to provide high density cell growth, and many suspension CHO cells (e.g. Freestyle™ CHO-S) can easily and quickly grow in CHOgro® Expression Medium with minimal adaptation.

Optimized Process Flowchart



Competitor Comparison

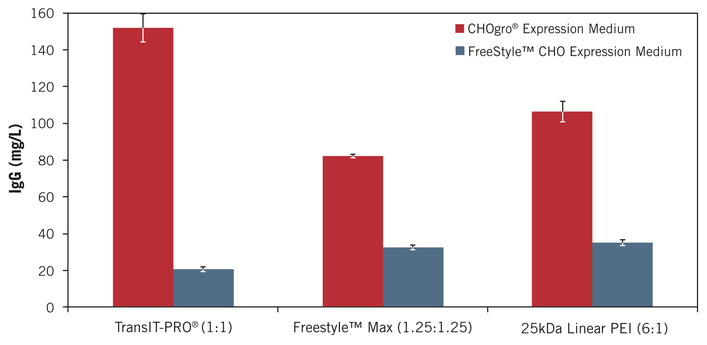


Figure 5. CHOgro® Expression Medium Yields Multi-fold Increases in Antibody Titer.
Human IgG1 was produced by transient transfection using *Trans*IT-PRO® (1:1), FreeStyle™ MAX (1.25:1.25) or 25kDa linear PEI (6:1) transfection reagents according to the manufacturers' or published protocol (reagent:DNA ratio). Transfections were performed using 1 µg plasmid DNA per milliliter of culture and cell densities of 2 x 10^6 cells/ml or 1 x 10^6 cells/ml for the CHOgro® Expression Medium (red bars) or FreeStyle™ Expression Medium (blue bars), respectively, at the time of transfection. FreeStyle™ CHO-S cells were cultured in CHOgro® Expression Medium or FreeStyle™ CHO Expression Medium and plated into non-treated 6-well plates (2ml/well) for transfection. Antibody levels were also analyzed from day 6 clarified supernatants using a human IgG ELISA (Zeptometrix). Error bars represent the standard deviation of triplicate technical replicates.

Minimal Cell Clumping

CHOgro® Expression System:
CHO-S cells
CHOgro® Expression Media
*Trans*IT-PRO® Transfection Reagent

Freestyle™ CHO System:
CHO-S cells
Freestyle™ CHO Expression Media
Freestyle™ MAX Transfection Reagent

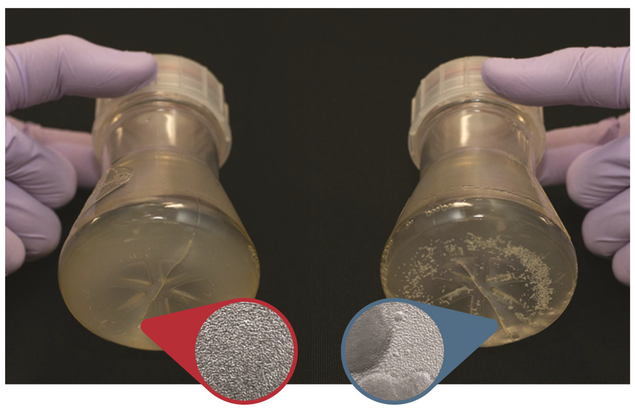


Figure 6. Less cell clumping is observed with the CHOgro® Expression System.
FreeStyle™ CHO-S cells were cultured in CHOgro® Expression Medium or FreeStyle™ CHO Expression Medium and seeded into a 125 ml shake flask (20ml culture volume, Thomson) for transfection. Human IgG1 was produced by transient transfection using *Trans*IT-PRO® (1:1) or FreeStyle™ MAX (1.25:1.25) transfection reagents according to the manufacturers protocol (reagent:DNA ratio). Transfections were performed using 1 µg or 1.25 µg plasmid DNA per milliliter of culture and cell densities of 2 x 10^6 cells/ml or 1 x 10^6 cells/ml for the CHOgro® or FreeStyle™ System, respectively, at the time of transfection. Pictures were taken of representative flasks and cells (inset) 6 days post-transfection.

Representative IgG1 Antibodies

Molecule Name	Target	Companies
Rituximab	CD20	Genentech and IDEC
Bevacizumab	VEGF	Genentech and BioOncology
Cetuximab	EGFR	Bristol-Myers Squibb; ImClone
Trastuzumab	HER2	Genentech
Alemtuzumab	CD52	Ilex Oncology; Millenium and Berlex

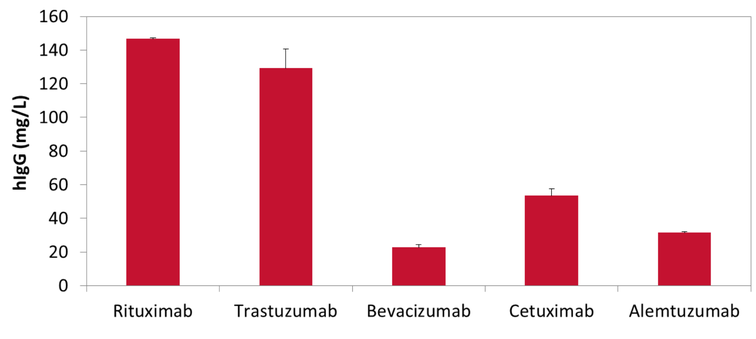


Figure 7. Titers of different antibody vector constructs using the CHOgro © Expression System.
Five different antibody constructs were produced by transient transfection using *Trans*IT-PRO® at a 1:1 reagent:DNA ratio. Transfections were performed using 1 µg plasmid DNA per milliliter of culture and a cell density of 2 x 10^6 cells/ml at the time of transfection. FreeStyle™ CHO-S cells were cultured in CHOgro® Expression Medium and plated into non-treated 6-well plates (2ml/well) for transfection. Day 6 supernatants were clarified and analyzed using a human IgG ELISA (ZeptoMetrix). Error bars represent the standard deviation of triplicate technical replicates.

Temperature Shift Increases Long-term Expression

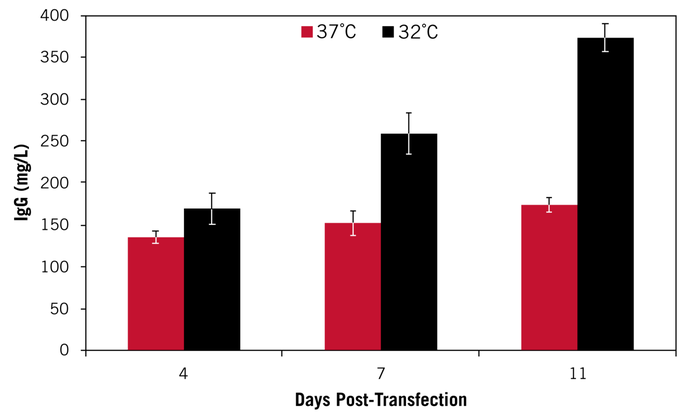


Figure 8. Increases in Product Titer are observed at longer time points with mild hypothermic conditions
Human IgG1 was produced by transient transfection with the *Trans*IT-PRO® Transfection Reagent and 1 µg plasmid DNA per milliliter of culture at a 1:1 reagent:DNA ratio. Cells were transfected at a density of 2 x 10^6 cells/ml in 20 ml of CHOgro® Expression Medium in 125 ml shake flasks (Thomson). Antibody levels were also analyzed from day 4, 7 and 11 clarified supernatants using a human IgG ELISA (Zeptometrix). All flasks were incubated at 37°C for 24 hours, at that point designated parallel flasks were switched to 32° C for the remainder of the experiment. Error bars represent the standard deviation of triplicate technical replicates.

Medium Exchange to CHOgro®

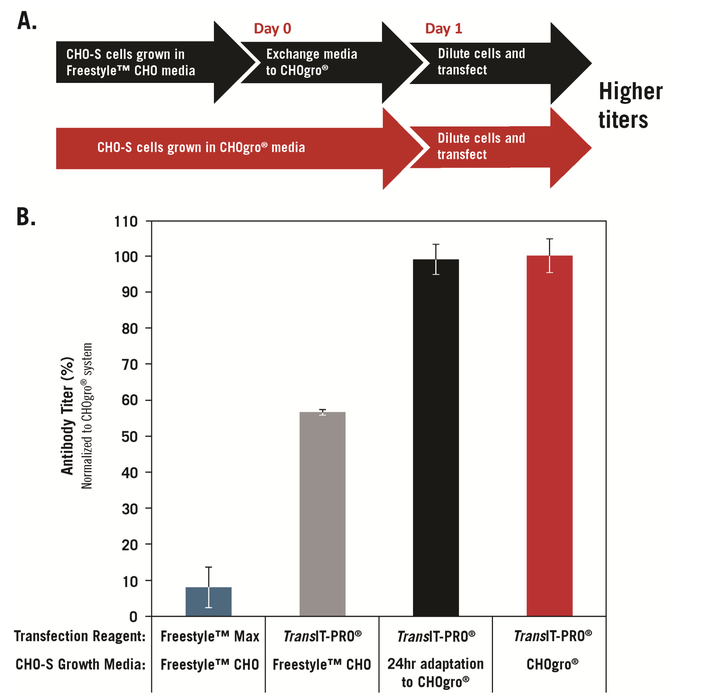


Figure 9. Media Exchange Leads to Higher Protein Production.
FreeStyle™ CHO-S cells were cultured in FreeStyle™ CHO Expression Medium or CHOgro® Expression Medium and 24 hours prior to transfection a subset of the cells grown in FreeStyle™ CHO Expression Medium were spun down and exchanged with 100% fresh CHOgro® Expression Medium. The cells were allowed to grow and adapt for 24 hours prior to transfection with FreeStyle™ MAX (1.25:1.25) or *Trans*IT-PRO® (1:1) transfection reagents according to the manufacturers protocol (reagent:DNA ratio) and a hIgG1 encoding construct. Transfections were performed using 1 µg plasmid DNA per milliliter of culture and cell densities of 1 x 10^6 cells/ml for cells transfected with FreeStyle™ Max and 2 x 10^6 cells/ml for cells transfected with *Trans*IT-PRO®. All cells were plated into non-treated 6-well plates (2ml/well) for transfection. (A) Workflow schematic of media exchange of CHO-S cells from FreeStyle™ CHO Expression Medium to CHOgro® Expression Medium (black arrow) or the normal CHOgro® Expression System (red arrow) (B) Day 6 supernatants were clarified and analyzed using a human IgG ELISA (Zeptometrix). Data is normalized to the complete CHOgro® Expression System (red bar). Error bars represent the standard deviation of triplicate technical replicates.

Conclusions

- **High Titers-** Increase titers from 2-10 fold over existing technologies
- **Simple-** No optimization required
- **Minimal cell clumping post-transfection-** Obtain accurate cell counts and high viability
- **Regulatory friendly-** ALL components are free of animal derived materials
- **Quick Adaptation-** CHO-S cells are transfection ready within 24 hours of media exchange