CHOgro® Expression System

At Mirus Bio, we know it's all about expression. The CHOgro® Expression System is a transient transfection platform that finally gets high protein titers with robust cell growth in the most relevant CHO cells.

- **Efficient** – Enables high protein titers with simple workflow
- **Convenient** – Quick adaptation to CHO cell line lineages
- **Optimized** – High density growth with minimal clumping post-transfection
- **Worry-free** – No commercial license required; animal origin free

Available as a complete system, or components sold separately.

Mirus

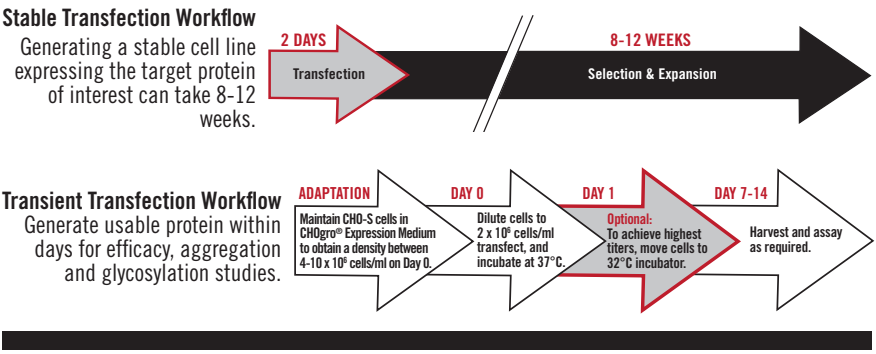
www.mirusbio.com/CHOgro

MORE THAN TRANSFECTION

The shift of therapeutics towards biologics has created a need for mammalian systems that can quickly produce high titer proteins in a consistent, reproducible manner. Transient transfection is an attractive option for early stage biologics development because generation of target proteins in usable quantities is much faster and easier compared to stable cell line generation. Suspension 293 and CHO cells are the cell factories of choice because of their growth characteristics and ability to produce post-translationally modified and active proteins.

HEK-293 systems traditionally produce higher yields than CHO cell systems and thus are favored for early stage development. However, the majority of biologics are produced in CHO cells because of safety and regulatory concerns, necessitating an inconvenient switch of host cell lines during the drug development process.

Transient Transfection Allows for Decreased Timelines to Useable Protein



With 20 years of experience in developing and commercializing high performance transfection products and technologies, Mirus Bio introduces a system to address the issues from early stage work to final drug development. The new CHOgro® Expression System is a platform developed to produce substantially higher titers through an integration of media and transfection enhancements and to allow preclinical development to be performed in suspension CHO cells.

The CHOgro® Expression System enables researchers to reach critical stages faster by decreasing time to produce usable protein and maximize target protein yields through transient transfection. The system is animal origin free and designed for high and reproducible protein yield in suspension CHO cells. Using this system will give you the following advantages:

√ High Titers – Increase titers from 2-10 fold over existing technologies
√ Simplicity – No optimization required
√ Worry-free – ALL components are animal origin free; no commercial license required
√ No Cell Clumping Post-transfection – Obtain accurate cell counts & high viability
√ Quick Adaptation – CHO-S cells are transfection-ready within 24-hours of media exchange

*Trans*IT-PRO® Transfection Reagent

High Efficiency Transfection

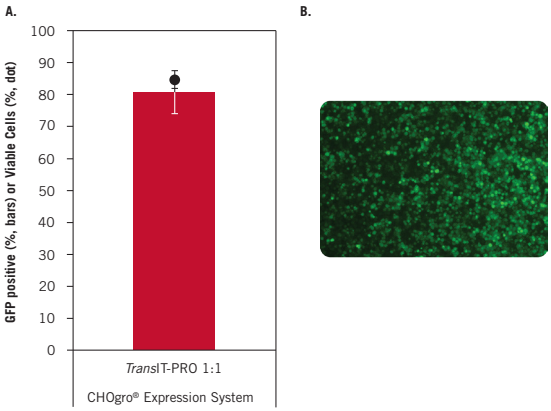


FIGURE 1. GFP was expressed in CHO-S cells by transient transfection using the *Trans*IT-PRO® Transfection Reagent (1:1). (A) GFP efficiency and cell viability (propidium iodide) were measured 48 hours post-transfection using a Guava easyCyte™ 5HT flow cytometer (EMD Millipore). (B) Images were captured using a Zeiss Axiovert inverted fluorescence microscope.

CHOgro® Expression Medium

Suspension CHO Cells Grow to High Density

cGMP

ISO Compliant

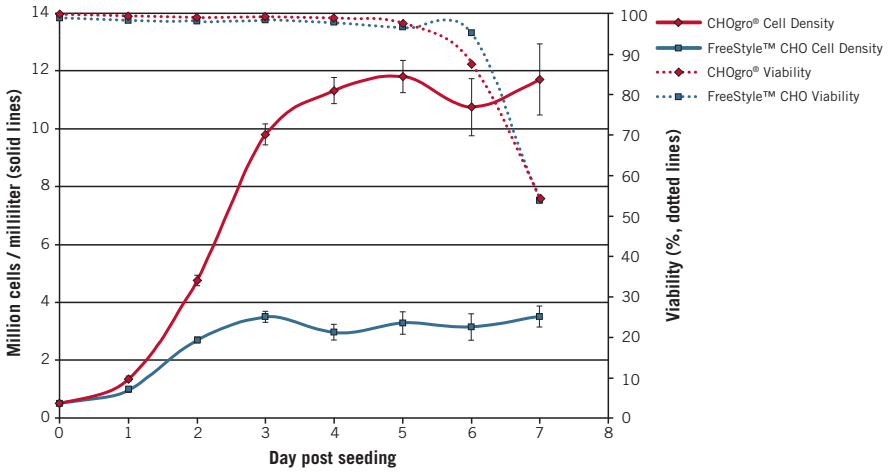


FIGURE 2. Triplicate flasks of FreeStyle™ CHO-S cells were seeded in CHOgro® Expression Medium (red line) or FreeStyle™ CHO Expression Medium (blue line) at cell density of 0.5 x 10^6 cells/ml, 40 ml per 125 ml shake flask (Thomson). Cell counts (solid line) and viability (propidium iodide staining, 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.

Less Cell Clumping is Observed in CHOgro® Expression Medium Post-transfection

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

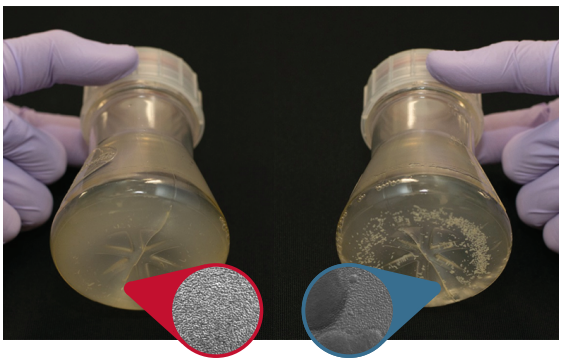


FIGURE 3. FreeStyle™ CHO-S cells were cultured in CHOgro® Expression Medium or FreeStyle™ CHO Expression Medium and seeded into a 125 ml shake flask (20 ml culture volume, Thomson) for transfection. Cells were transfected according to manufacturer's protocol. Pictures were taken of representative flasks and cells (inset) 6 days post-transfection.

CHOgro® Media Exchange Leads to Higher Protein Production

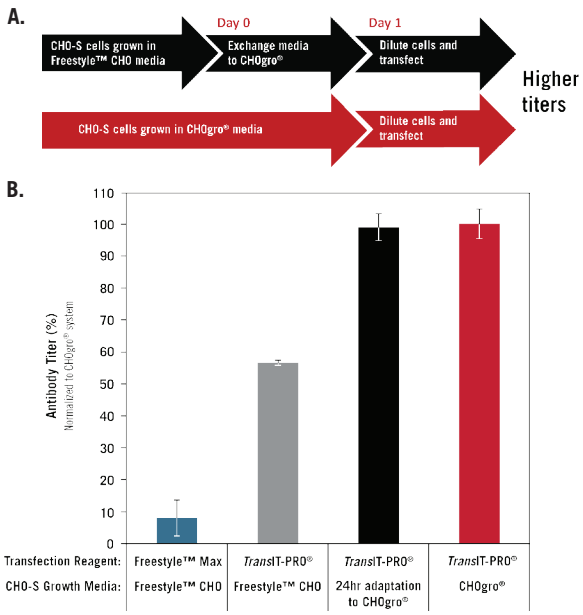


FIGURE 4. FreeStyle™ CHO-S cells were cultured in FreeStyle™ CHO Expression Medium or CHOgro® Expression Medium. (A) Workflow schematic of media exchange of CHO-S cells from FreeStyle™ CHO Expression Medium to CHOgro® Expression Medium (black arrow) or the normal CHOgro® Expression System (red arrow) (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.

CHOgro® Expression System

Ideal for Biotherapeutic Protein Production in Suspension CHO Cells

Increases in Product Titer are Observed at Longer Time Points with Mild Hypothermic Conditions

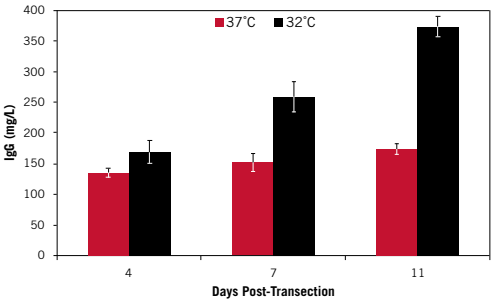


FIGURE 5. Cells were transfected at a density of 2 x 10^6 cells/ml in 20 ml of CHOgro® Expression Medium in 125 ml shake flasks (Thomson). Antibody levels were analyzed from day 4, 7 and 11 clarified supernatants using a human IgG ELISA (ZeptoMetrix). All flasks were incubated at 37°C for 24 hours; at the timepoint designated, parallel flasks were switched to 32°C for the remainder of the experiment. Error bars represent the standard deviation of triplicate technical replicates.

High Cell Density Leads to Increased Titers

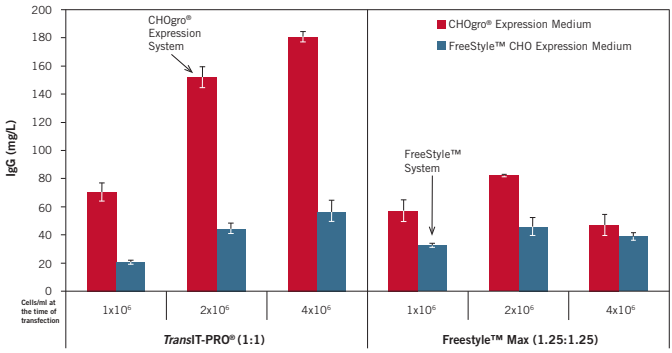


FIGURE 6. Human IgG1 was produced by transient transfection at 37°C using *Trans*IT-PRO® (1:1) or FreeStyle™ MAX. Day 6 supernatants were clarified and analyzed using a human IgG ELISA (ZeptoMetrix). Error bars represent the standard deviation of triplicate technical replicates.

Titers of Different Antibody Vector Constructs

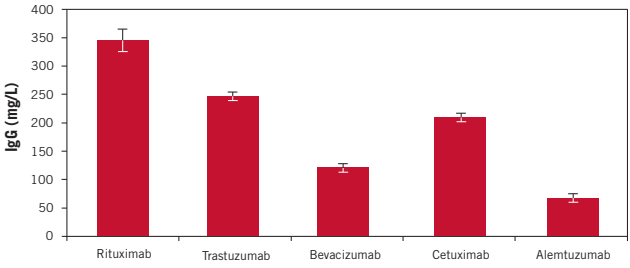


FIGURE 7. Five different antibody constructs were produced by transient transfection using a temperature shift to 32° C and *Trans*IT-PRO®. Day 11 supernatants were clarified and analyzed using a human IgG ELISA (ZeptoMetrix). Error bars represent the standard deviation of triplicate technical replicates.

CHOgro® Expression System

Product List

SYSTEM AND INDIVIDUAL COMPONENTS	DESCRIPTION	PRODUCT NO.	QUANTITY
CHOgro® Expression System 	Complete System Includes: - CHOgro® Expression Media (2 L) - *Trans*IT-PRO® Transfection Reagent (1 ml) - CHOgro® Complex Formation Solution (100 ml) - Poloxamer 188 Solution (100 ml) - L-Glutamine Solution (100 ml)	MIR 6260	1 Kit
CHOgro® Expression Medium  cGMP ISO Compliant	Chemically defined, hydrolysate-free, animal origin free growth medium supporting transient transfection and robust growth. *10 Liter polybag †Dry powder format, prepares 10 liters	MIR 6200 MIR 6202 MIR 6201	1 Liter *10 Liter †10 Liter
*Trans*IT-PRO® Transfection Reagent 	Low toxicity, animal origin free, transfection reagent for high, reproducible protein production in suspension CHO and HEK 293 cells	MIR 5740	1 ml
CHOgro® Complex Formation Solution 	An animal origin free solution for complex formation with *Trans*IT-PRO® Transfection Reagent	MIR 6210	100 ml
Poloxamer 188 Solution 	Required supplement for CHOgro® Expression Medium, 10% solution	MIR 6230	100 ml
L-Glutamine Solution 	Required supplement for CHOgro® Expression Medium, 200mM solution in 0.85% NaCl	MIR 6240	100 ml

ACCESSORY, SOLD SEPARATELY NOT INCLUDED WITH KIT		PRODUCT NO.	QUANTITY
Human IgG1 Expression Control 	Positive control plasmid DNA mixture of heavy and light chains to verify antibody expression	MIR 6250	100 µg

Mirus®