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The Transfection Experts

CHOgro® Expression System:

High titer transient transfection system for suspension CHO cells

Mirus Bio LLC

What is the CHOgro® Expression System?

- **Efficient**: high transient protein titers with simple workflow
- **Convenient**: quick adaptation to CHO cell lineages
- **Optimized**: high density growth with minimal cell clumping
- **Worry-free**: no-commercial license required; animal origin-free

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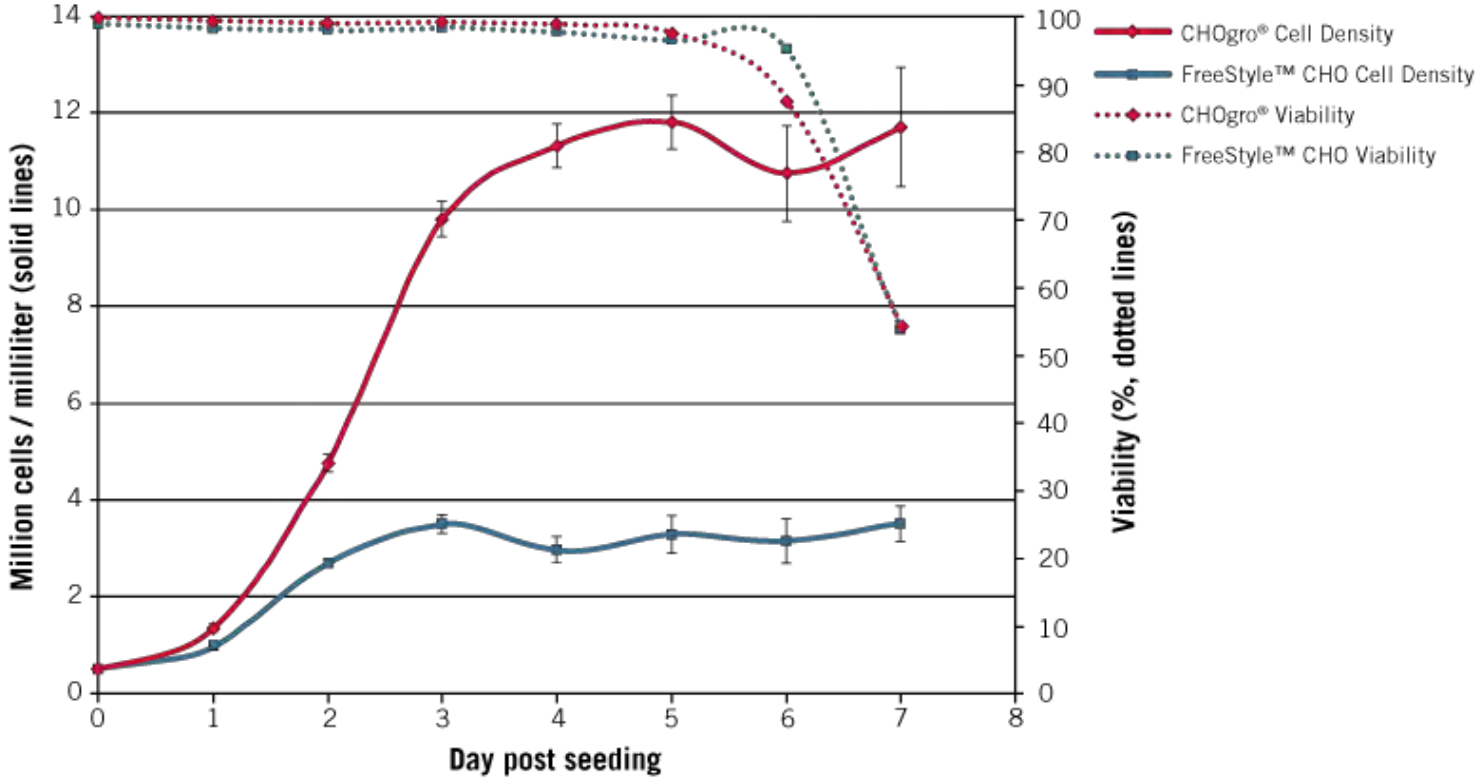
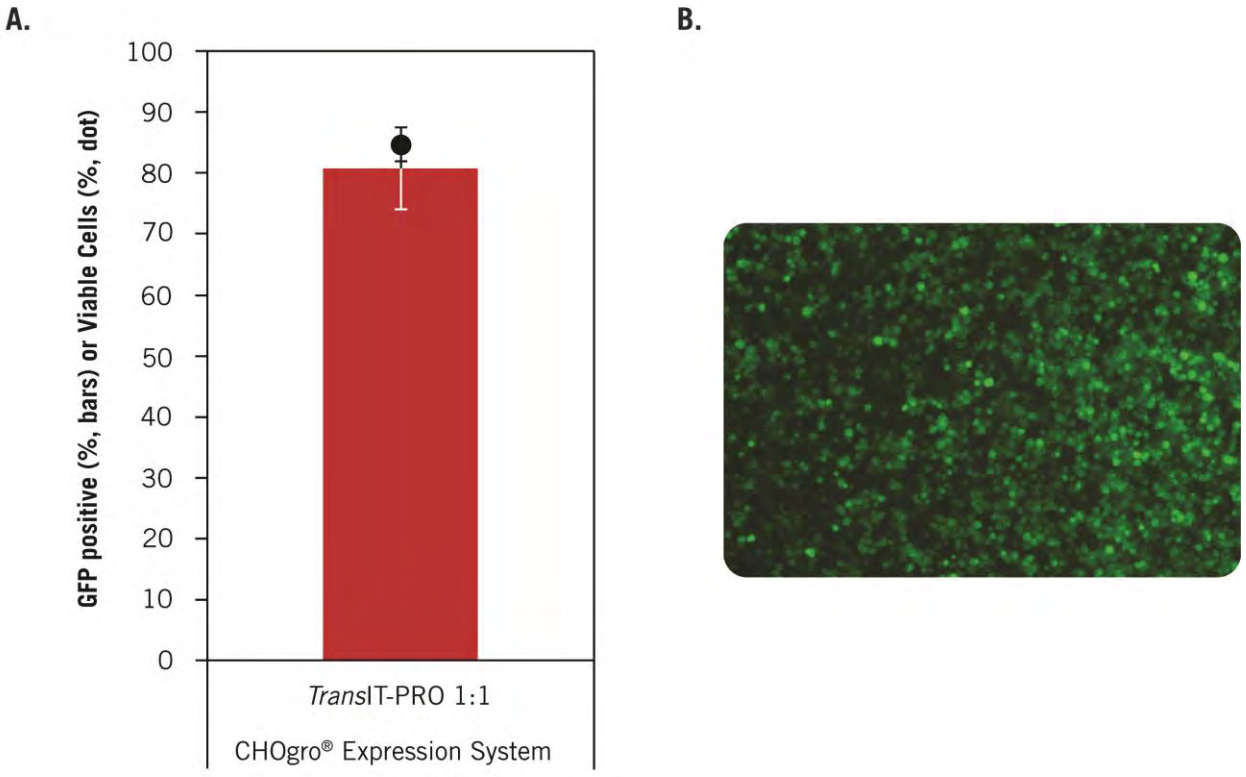
High Density Cell Growth Using CHOgro®



Higher Cell Densities Leads to Higher Titers Using the CHOgro® Expression System.
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 106 cells/ml, 40 ml per 125 ml shake flask (Thomson). Cell counts (solid line) and viability (propidium iodide staining, dotted line) were measured daily. For more details www.mirusbio.com/CHOgro

*Trans*IT-PRO® in the CHOgro® Expression System Yields High Transfection Efficiency



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 106 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. For more details www.mirusbio.com/CHOgro

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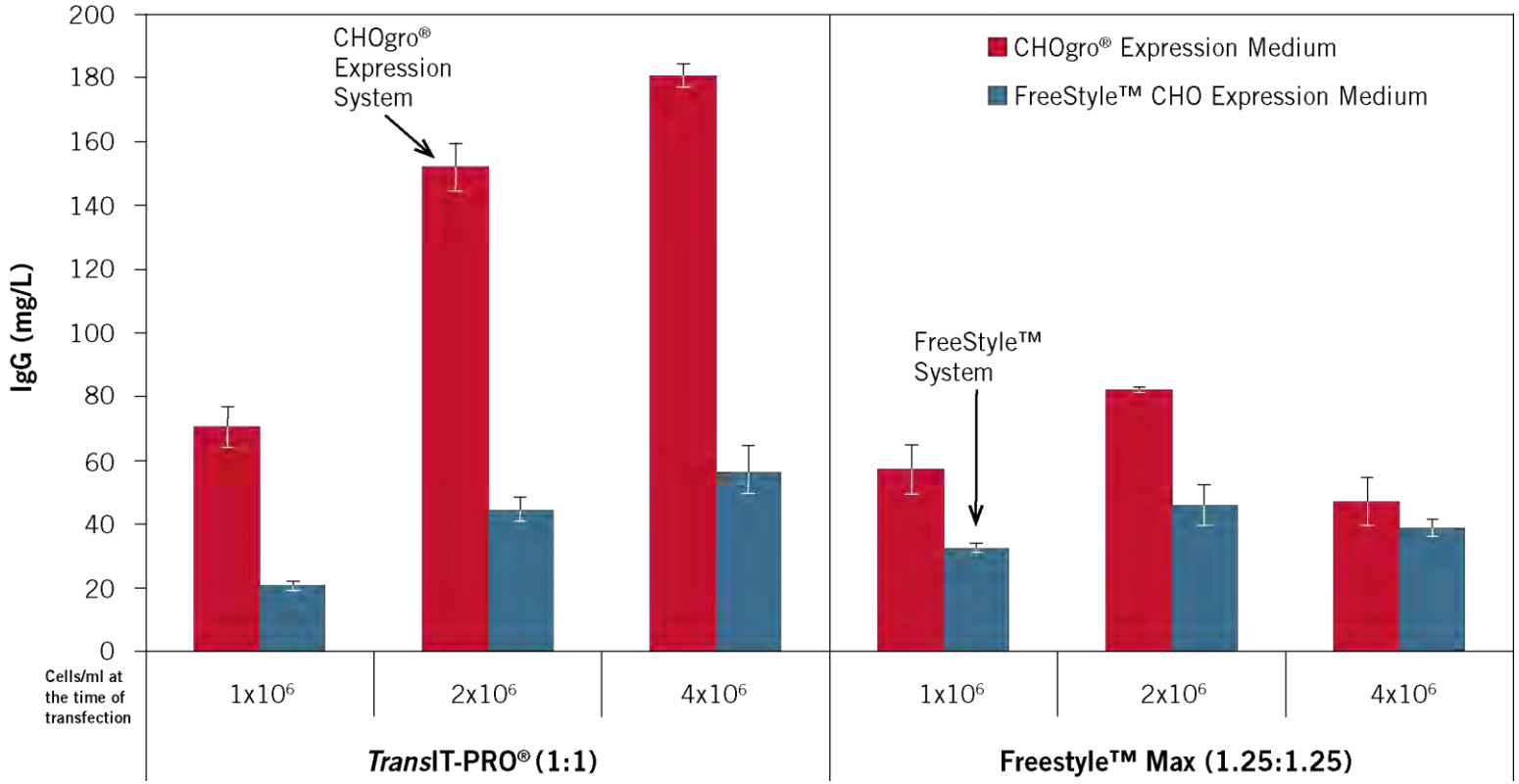
Transfection Optimization: Higher Titers with the CHOgro® Expression System



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. 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. For more details www.mirusbio.com/CHOgro

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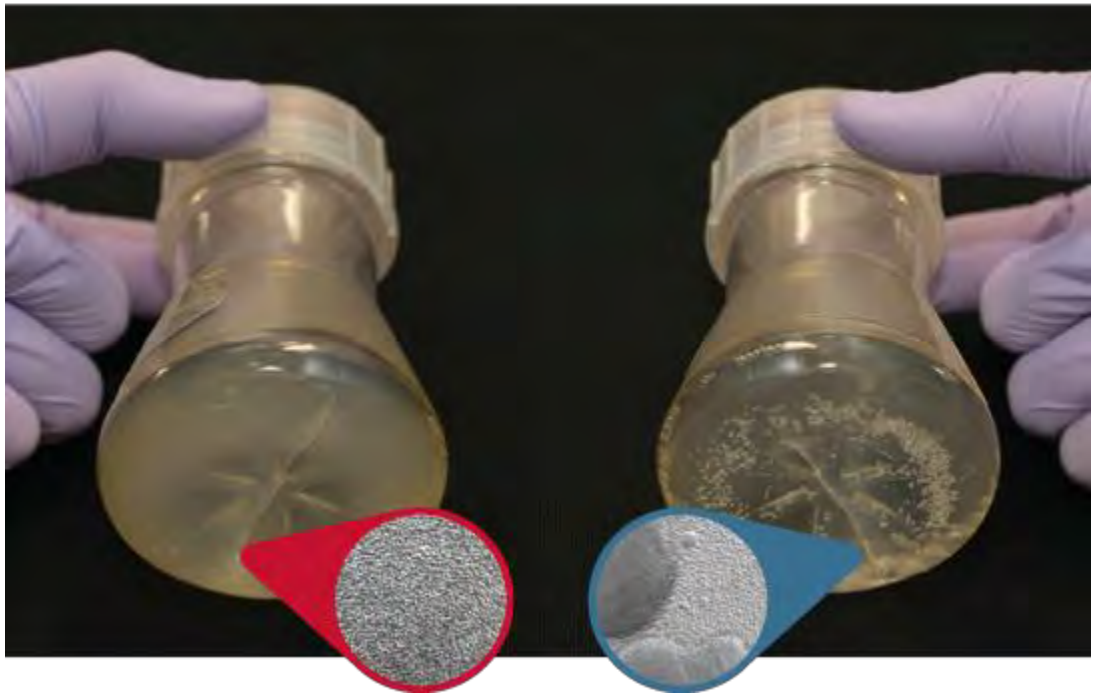
Minimal Cell Clumping Post-transfection

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



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). Pictures were taken of representative flasks and cells (inset) 6 days post-transfection.
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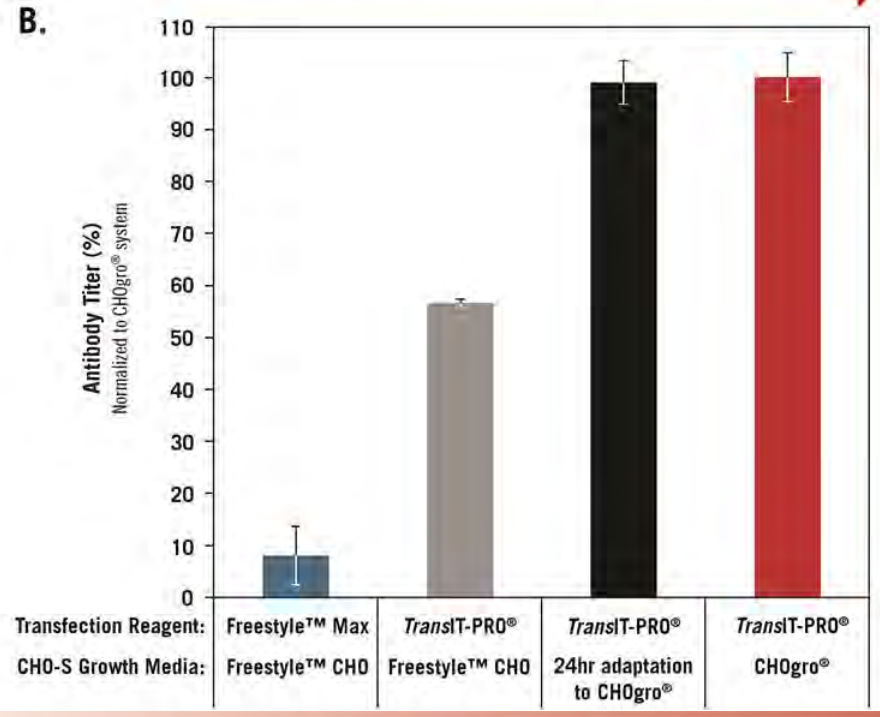
Medium Exchange to CHOgro® = Quick Adaptation



CHOgro® Media Exchange Leads to Higher Protein Production. FreeStyle™ CHO-S cells were cultured in FreeStyle™ CHO Expression Medium or CHOgro® Expression Medium. Twenty four 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. (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 (ZeptoMatrix). Data is normalized to the complete CHOgro® Expression System (red bar). For more details www.mirusbio.com/CHOgro

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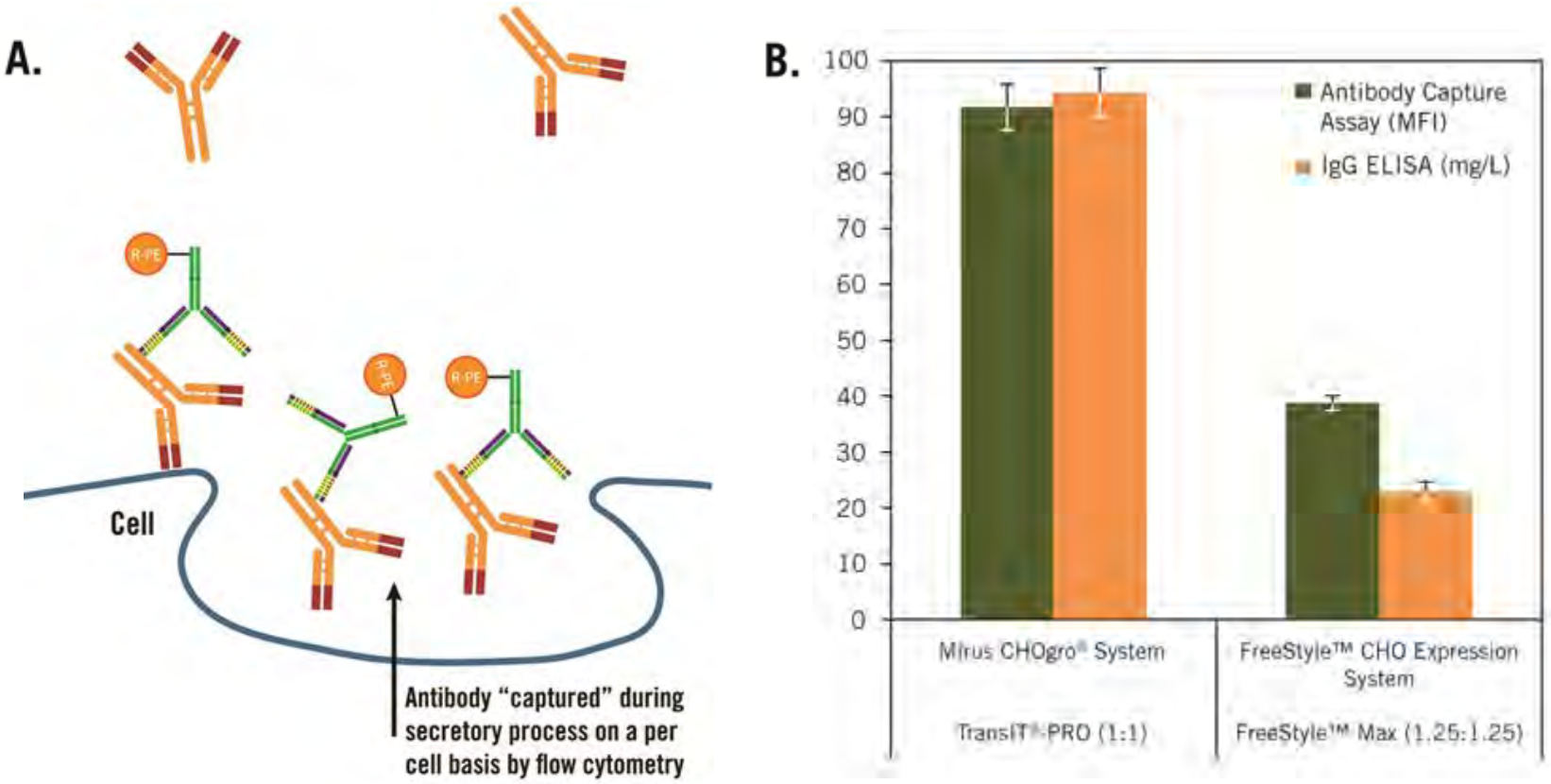
More Antibody Secreted Per Cell



More Antibody is Secreted on Per-cell basis 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). **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 (ZeptoMatrix). For more details www.mirusbio.com/CHOgro

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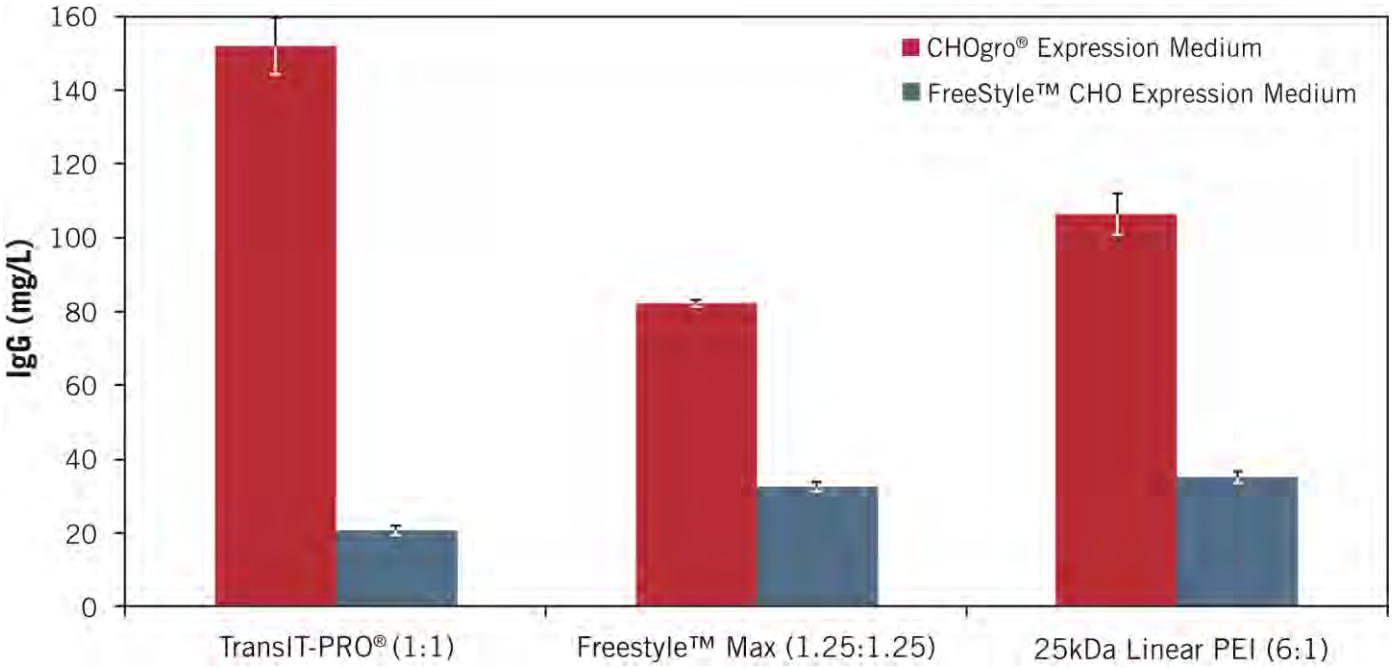
CHOgro® Expression Medium Supports Multiple Transfection Technologies



CHOgro® Expression Medium Yields Multi-fold Increases in Antibody Titer. Human IgG1 was produced by transient transfection using TransIT-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.

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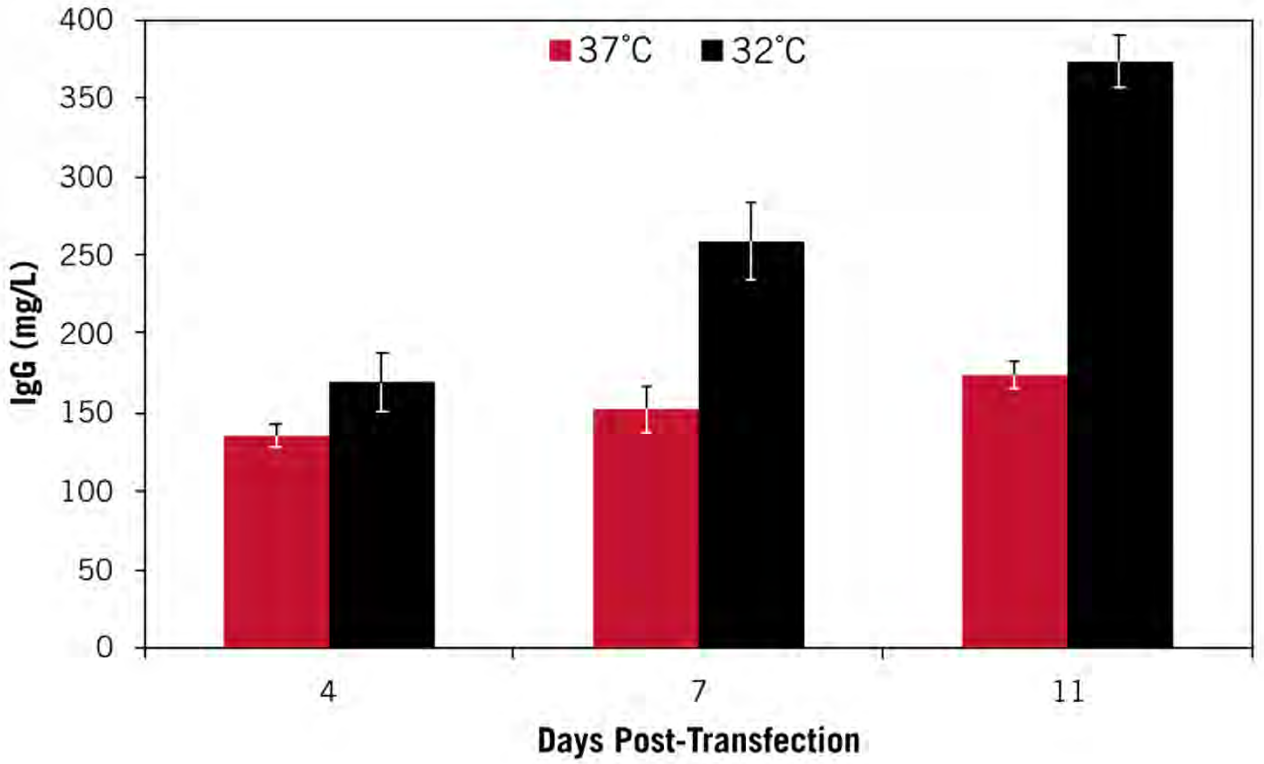
Temperature Shift to 32°C Increases Yield



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.

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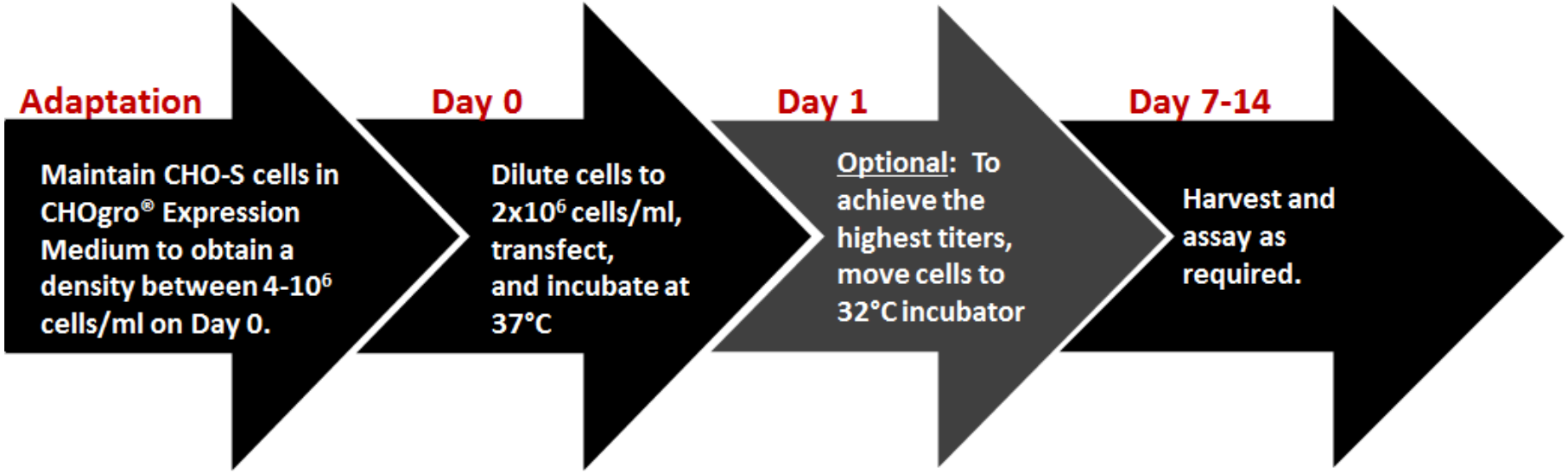
Optimized Process Workflow



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Assessing Linearity Across Culture Vessels

- FreeStyle™ CHO-S cells adapted to CHOgro® Expression Medium
- Culture vessels ranging from 6-well plate to 5L Thomson Flask
- Cells seeded at 2 x 10^6 cells/ml at the time of transfection
- hIgG1 expressing vector delivered using the *Trans*IT-PRO® Transfection Reagent
- All flasks were incubated at 37°C for 24 hours and subsequently shifted to 32°C for the remaining incubation, all flasks shaking at 120 rpm

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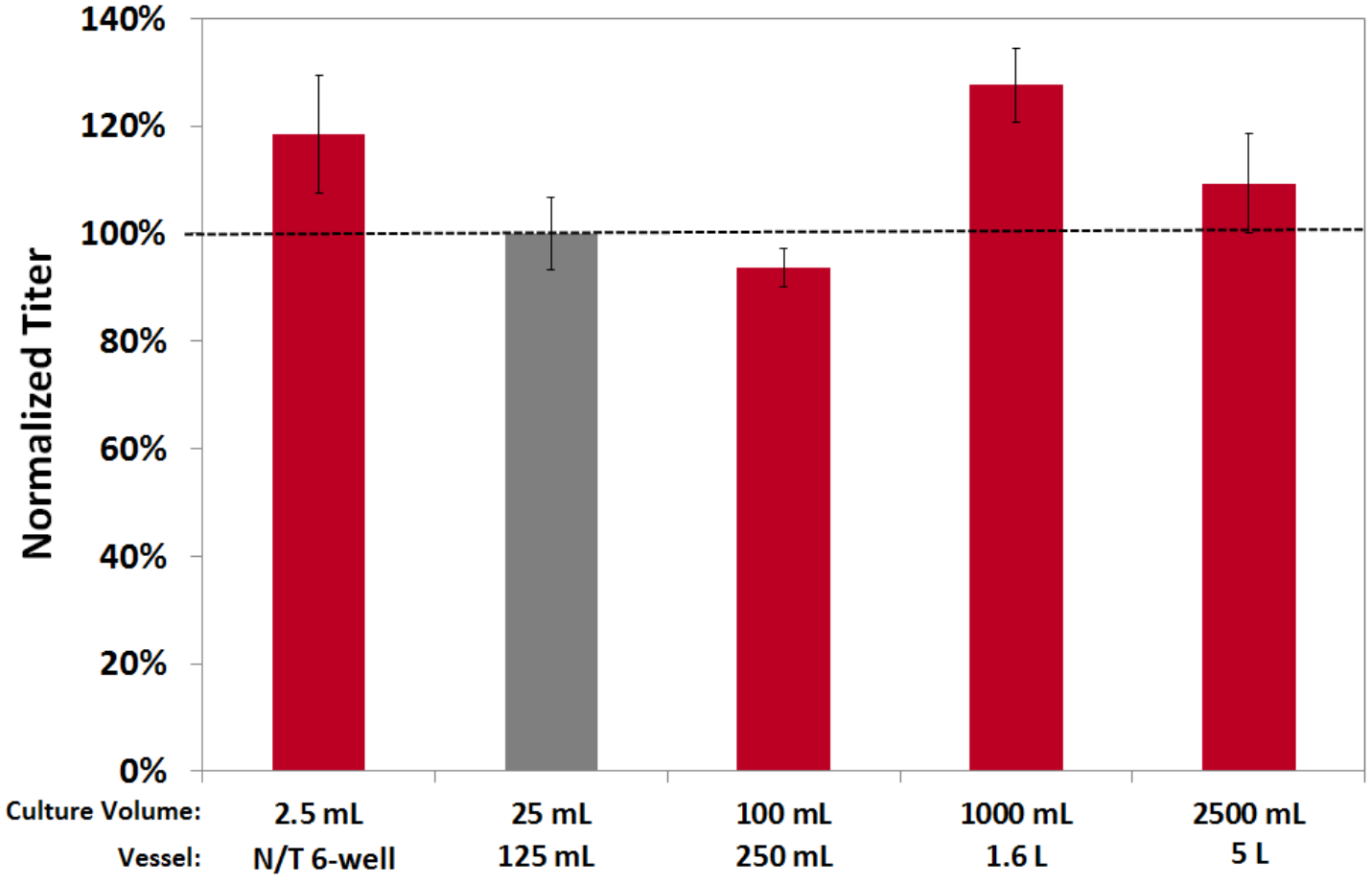
Scale-up: No problem



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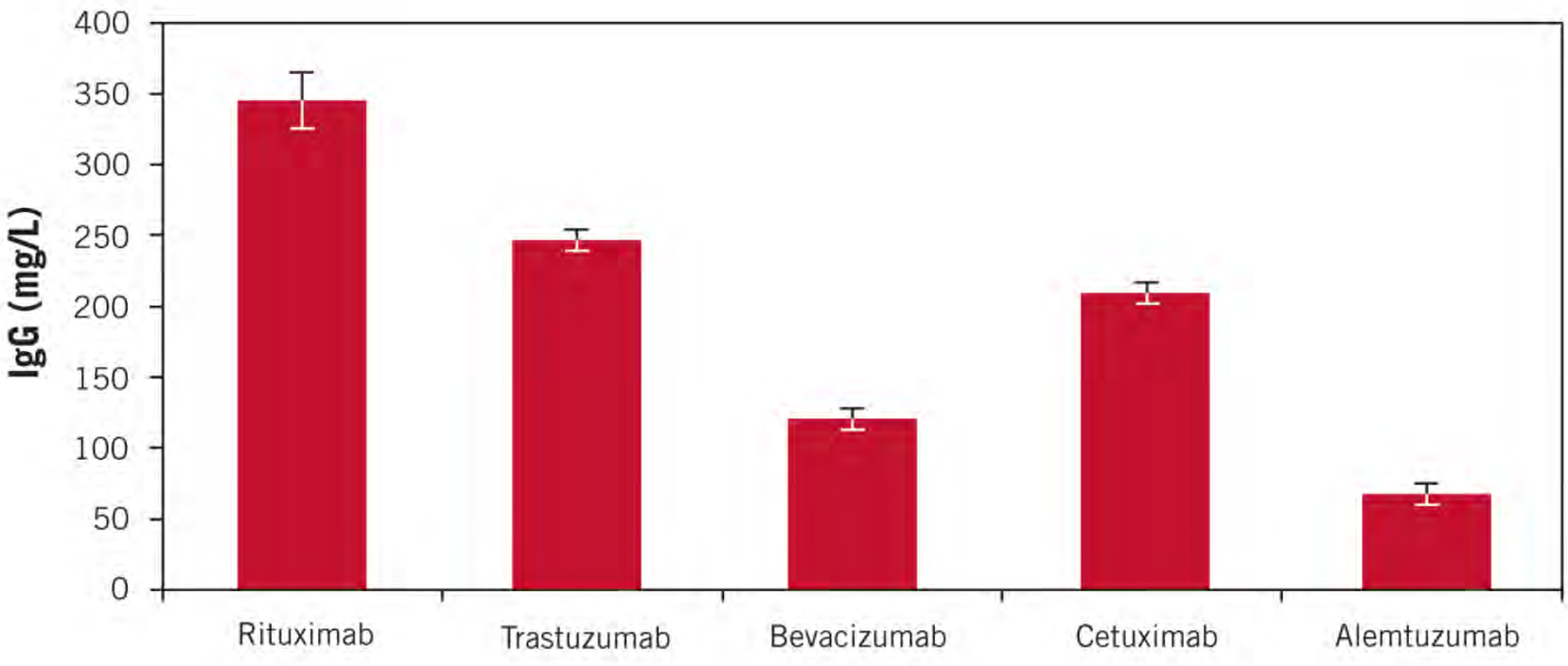
Representative IgG1 Antibody Production



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.

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Summary

Robust growth: Increased cell density growth and viability

Higher titers: 2-10 fold over existing technologies (e.g. FreeStyle™ Max)

Quick adaptation: CHO-S cells are ready within 4-24 hrs of media change

Scale-up: reproducible titers from 2.5 mL to 2.5 L

Peace of mind: no-commercial license required; animal origin-free

CHOgro® Expression System

CHOgro® Expression System components:

- CHOgro® Expression Media (2L)
- CHOgro® Complex Formation Solution (100mL)
- *Trans*IT-PRO® Transfection Reagent (1mL)
- Poloxamer 188 (100 mL)
- L-Glutamine (100mL)

** All components are also sold separately*

Related products:

- Human IgG1 Expression Control (100µg)

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