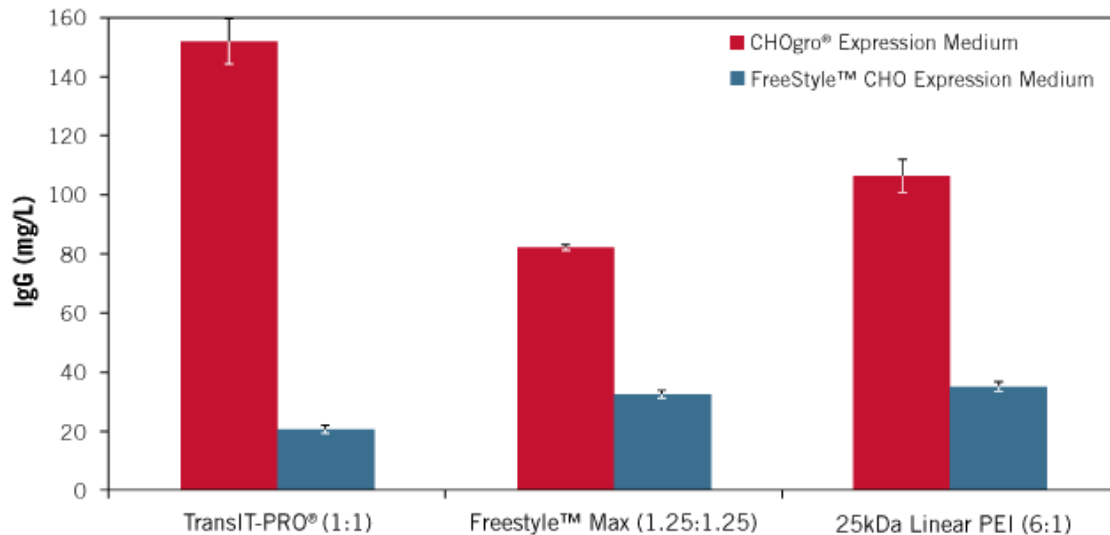
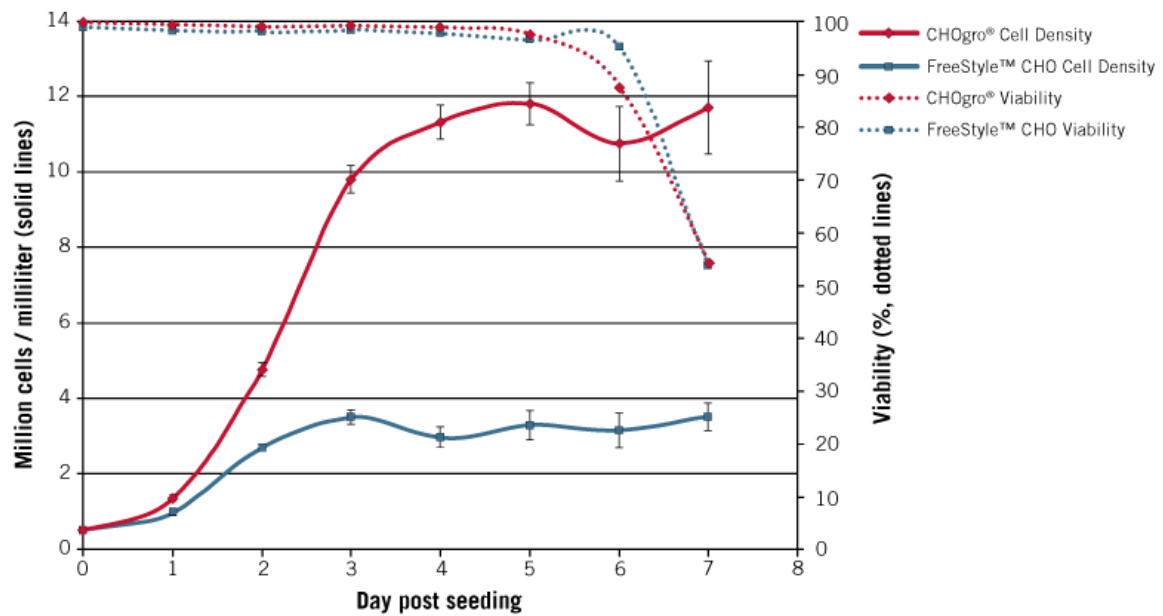


## Figures and Data

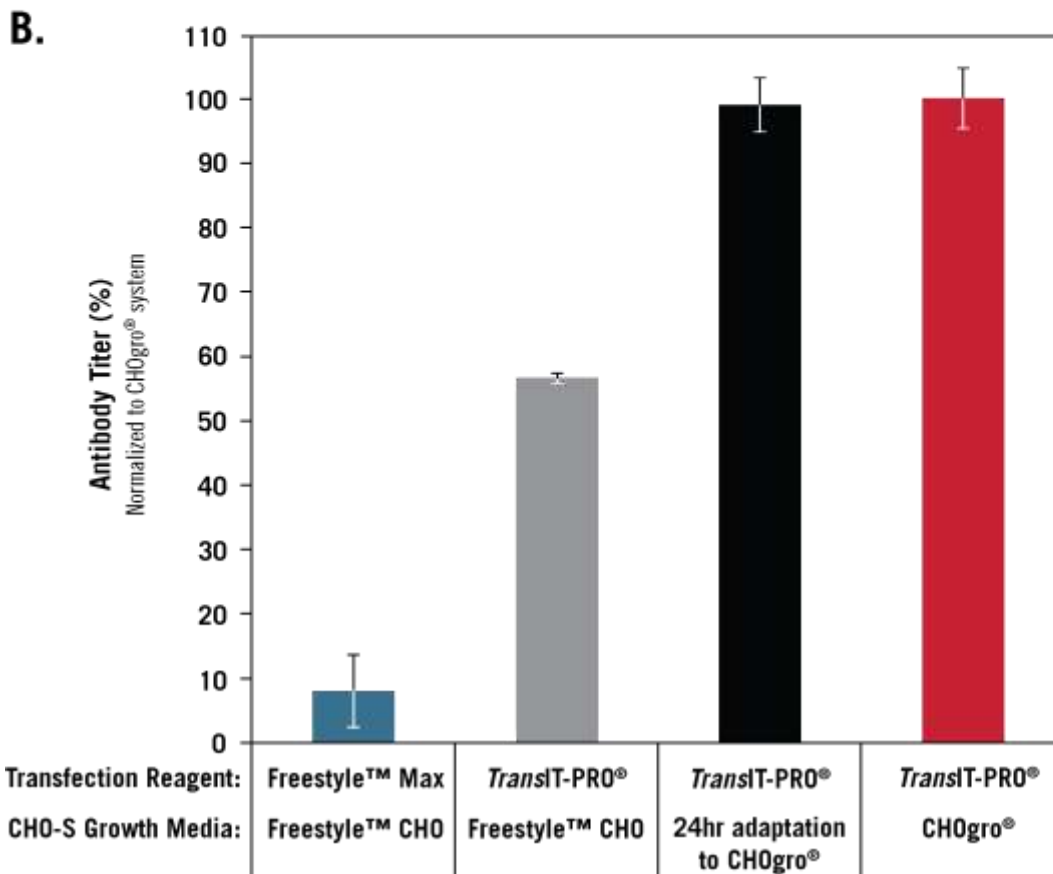
[CHOgro® Expression Medium Yields Multi-fold Increases in Antibody Titer](#)  
[Suspension CHO Cells Grow to High Density in the CHOgro® Expression Medium](#)  
[CHOgro® Media Exchange Leads to Higher Protein Production](#)  
[Less Cell Clumping is Observed with the CHOgro® Expression System](#)



**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  $2 \times 10^6$  cells/ml or  $1 \times 10^6$  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.



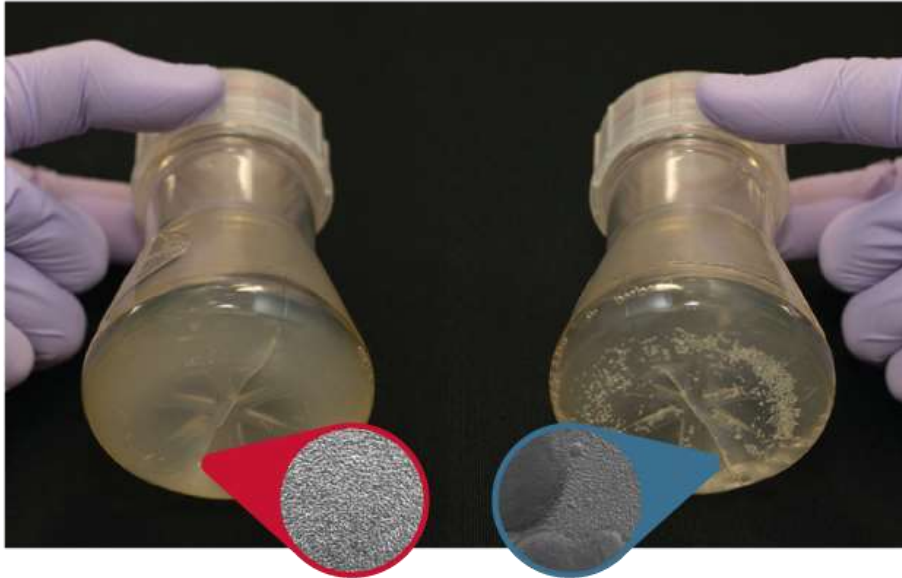
**Suspension CHO Cells Grow to High Density in the CHOgro® Expression Medium.** Triplicate flasks of FreeStyle™ CHO-S cells were seeded in CHOgro® Expression Medium (red line) or FreeStyle™ CHO Expression Medium (blue line) at cell density of  $0.5 \times 10^6$  cells/ml, 40 ml per 125 ml shake flask (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.



**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. The cells were allowed to grow and adapt for 24 hours prior to transfection with FreeStyle™ MAX (1.25:1.25) or TransIT-PRO® (1:1) transfection reagents according to the manufacturers' protocol (reagent:DNA ratio) and a human IgG1 encoding construct. Transfections were performed using 1 µg plasmid DNA per milliliter of culture and cell densities of  $1 \times 10^6$  cells/ml for cells transfected with FreeStyle™ Max and  $2 \times 10^6$  cells/ml for cells transfected with TransIT-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.

**CHOgro® Expression System:**  
CHO-S cells  
CHOgro® Expression Media  
*TransIT-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 *TransIT-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  $2 \times 10^6$  cells/ml or  $1 \times 10^6$  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.