

Speed Improvement in Mid-Scale Biological Processes in: CHO, Hybridoma, HEK 293 and Cell Lines Using Single Use Optimum Growth Flasks 125mL-5L Flasks with Additional Features

ABSTRACT

Optimum Growth™ Flasks (patented) give excellent growth with space saving capability. By using Optimum Growth™ Flasks users are able to grow 25mL-3L of Cell Culture. The Optimum Growth™ Flasks have replaced expensive, disposable, Fernbach flasks and also small Wave® Bags (5L & 10L). The Optimum Growth™ Flasks also give high viability cultures with a great use of space as shown by our data in Insect, CHO, Hybridoma, and HEK293 Cell Lines. Transfer Caps (patented) allow for the flasks to be used as seed culture for Cell Bags and Bioreactors. We will show data from GPCR Proteins, Soluble Proteins, Vaccines and Antibodies.

INTRODUCTION

Thomson has recently introduced 125mL, 250mL, 500mL, 1.6L, and 2.8L Optimum Growth™ Flasks to compliment the industry standard 5L Optimum Growth™ Flask. These smaller flasks allow enhanced growth with the same footprint as conventional flasks, but with up to 300% higher working volume for maximum shaker spacer utilization. With the addition of the smaller flask sizes, Thomson Optimum Growth™ Flasks are scalable from 125mL to 5L. Transfer Caps (patented) allow easy, contamination free inoculation of larger vessels directly from Optimum Growth™ Flasks.

Available For
**125mL, 250mL, 500mL
1.6L, 2.8L & 5L
Optimum Growth™ Flasks**



**Optimum Growth Flasks™ Give Excellent
Growth with Space Saving Capability**

Lab Space is \$\$\$Expensive



Most Expensive Parking Spot, \$225,000!

Shaker SPACE 10x More Expensive



CHO Transient Cells

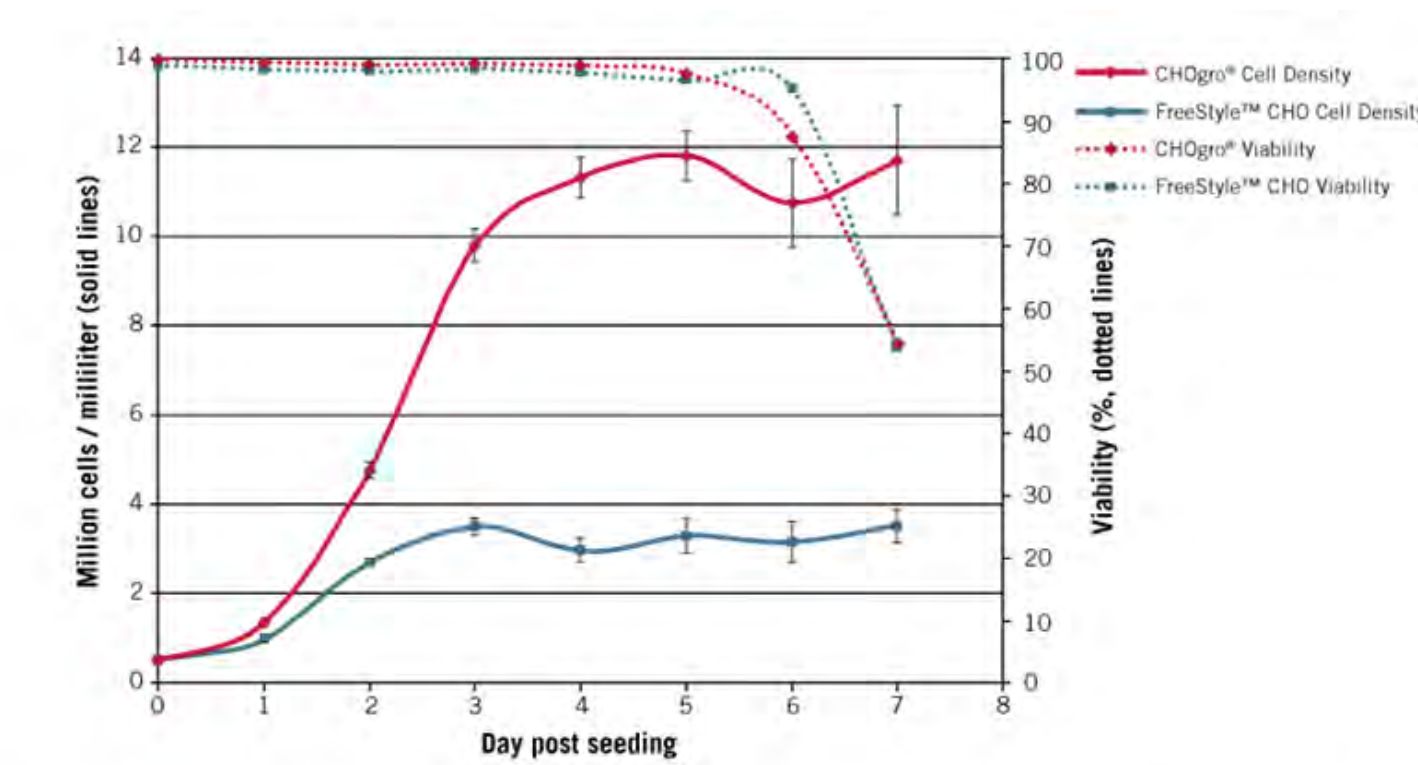
**CHOgro® Expression System:
High titer transient transfection system for
suspension CHO cells**



Materials and Methods

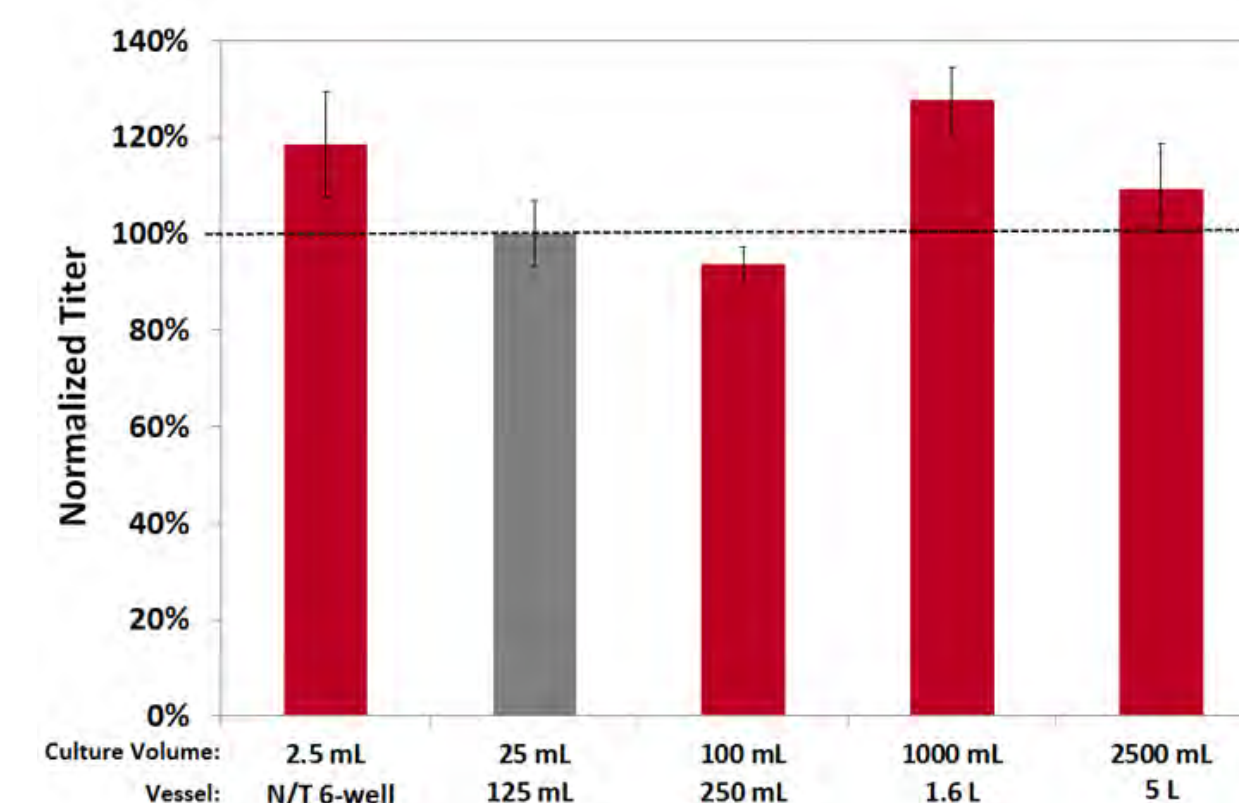


High Density Cell Growth Using CHOgro®



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 see www.mirus.com/CS026.

Scale-Up No Problem



Scale-up with CHOgro® Expression System from 2.5mL to 2.5L

Human IgG1 was produced by transient transfection with the *TransIT-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 10⁶ cells/ml in CHOgro® Expression Medium on an orbital shaker at the following volumes/culture vessels: 2.5 ml/non-tissue culture treated 6-well dish, 25 ml/125 ml Thomson flask, 100 ml/250 ml Thomson flask, 1000 ml/1.6 L Thomson flask, 2.5 L/5 L Thomson flask. At twenty-four hours post-transfection all cultures were moved to 32° C for the remainder of the experiment. Antibody levels were also analyzed from day 7 clarified supernatants using a human IgG ELISA (Zeptomatrix). All values are normalized to the 25ml volume sample and error bars represent the standard error of the mean of triplicate technical replicates.

Minimal Cell Clumping Post-transfection



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 manufacturer's protocol (reagent:DNA ratio). Pictures were taken of representative flasks and cells (inset) 6 days post-transfection. www.mirus.com/CS026

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 10⁶ cells/ml at the time of transfection
- hlgG1 expressing vector delivered using the *TransIT-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



Insect Cells

Insect Cells in Thomson 5L Optimum Growth™ and 3L Corning® Flasks

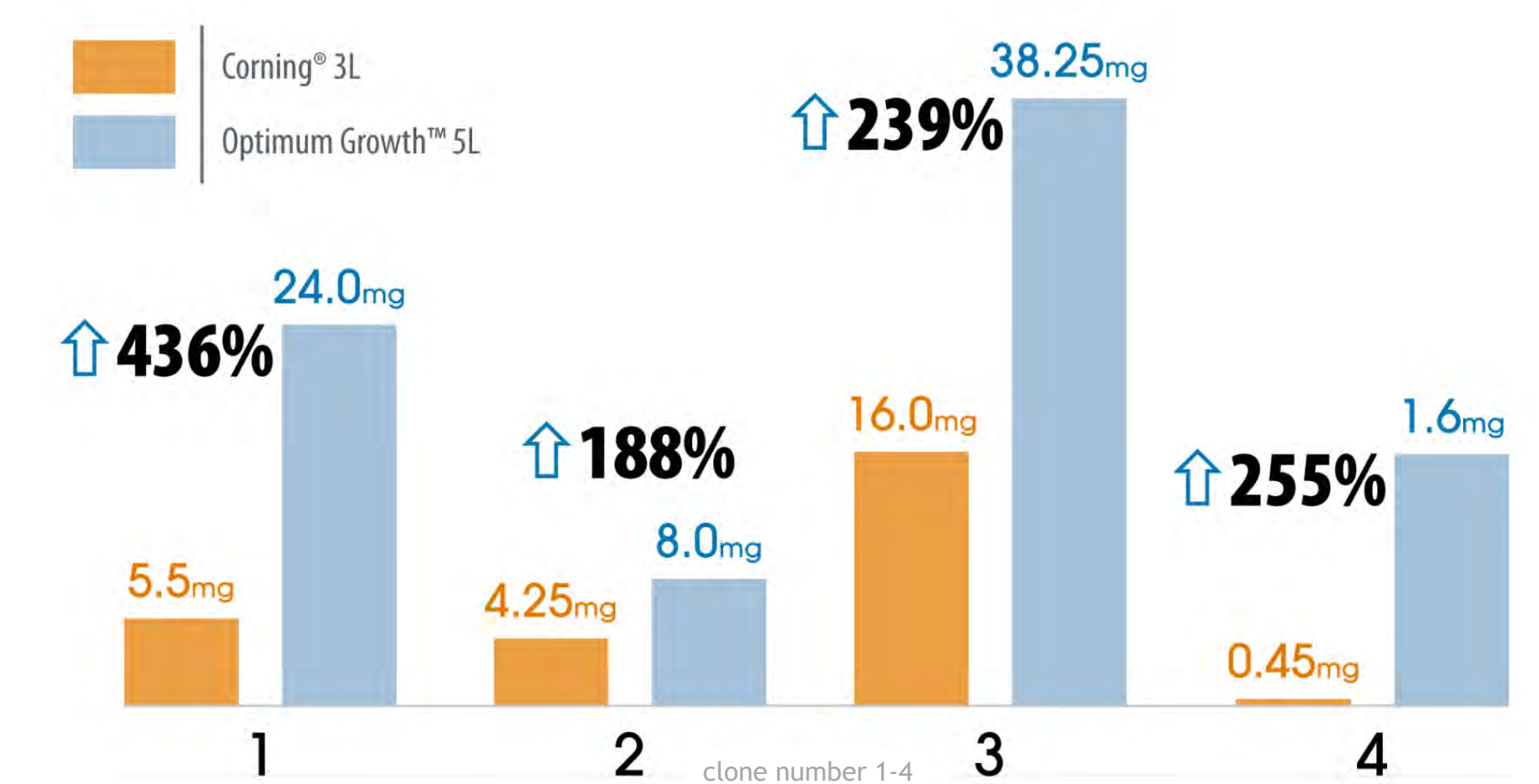


Materials and Methods

New York Structural Genomics compared protein expression in insect cells between two Corning® 3L Flasks with 2L of media in each flask totaling 4L and 3L of media in one Thomson Optimum Growth™ 5L flask. High Five cells at 1x10⁶/mL were infected with 500µL of P3 virus per liter of culture. The culture conditions were 100rpm shaking speed, 50mm orbit, and Express Hi5® media from Invitrogen™. Secreted targets were harvested at 96 hours post infection.

Results

**214% Yield Increase From Insect Cells
Protein Production/Flask**



The average increase in protein production was 214% across 8 clones. Data from Clones 5-8 not shown.

Conclusions

One Thomson 5L Optimum Growth™ Flask with 3L of working volume produced 214% more protein across eight clones than 4L of working volume in two 3L Corning® flasks. Using Thomson 5L Optimum Growth™ Flasks reduced the shaker space required by 50% while increasing titers.

Recommended Fill Volumes & Shake Speeds

CHO Stable Cells, CHO Transient, HEK 293 Transient

Flask Size	Best Fill Volume	*RPM in 1"/2"
125mL	63mL	150/110
250mL	150mL	150/110
500mL	250mL	150/110
1.6L	900mL	150/110
5L	2.0L - 3.0L	120/90

Hybridoma Cells

Flask Size	Best Fill Volume	*RPM in 1"/2"
125mL	36mL	70/50
250mL	75mL	70/50
500mL	150mL	70/50
1.6L	480mL	70/50
5L	1.5L	80/60

Insect Cells

Flask Size	Best Fill Volume	*RPM in 1"/2"
125mL	63mL-75mL	150/110
250mL	150mL	150/110
500mL	250mL	150/110
1.6L	900mL	150/110
5L	2.0L - 3.0L	135/90

Microbes/E.coli

Flask Size	Best Fill Volume	*RPM in 1"/2"
125mL	63mL	250/110
250mL	125mL	250/110
500mL	250mL	250/110
1.6L	900mL	250/110
5L	2.0L - 3.0L	250/150

* 1" orbit = 25mm and 2" orbit = 50mm

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