

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



Authors: Sam Ellis, Joe Machamer, Mike MyLymuk, Dennis Peterson, Ana Sirianni
Thomson Instrument Company, Oceanside, CA



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, ExpiCHO™ and HEK293 Cell Lines. Thomson 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 complement 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.

Optimum Growth™ Flasks are available in the following sizes
125mL, 250mL, 500mL, 1.6L, 2.8L & 5L



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



HEK 293 Transient & CHO Stable cell lines were grown in the Thomson 1.6L Optimum Growth™ Flask compared to a 1L Standard Shake Flask

Materials and Methods

LakePharma compared the Thomson 1.6L Optimum Growth™ Flask to a 1L standard shake flask for overall cell growth and impact on protein yield.

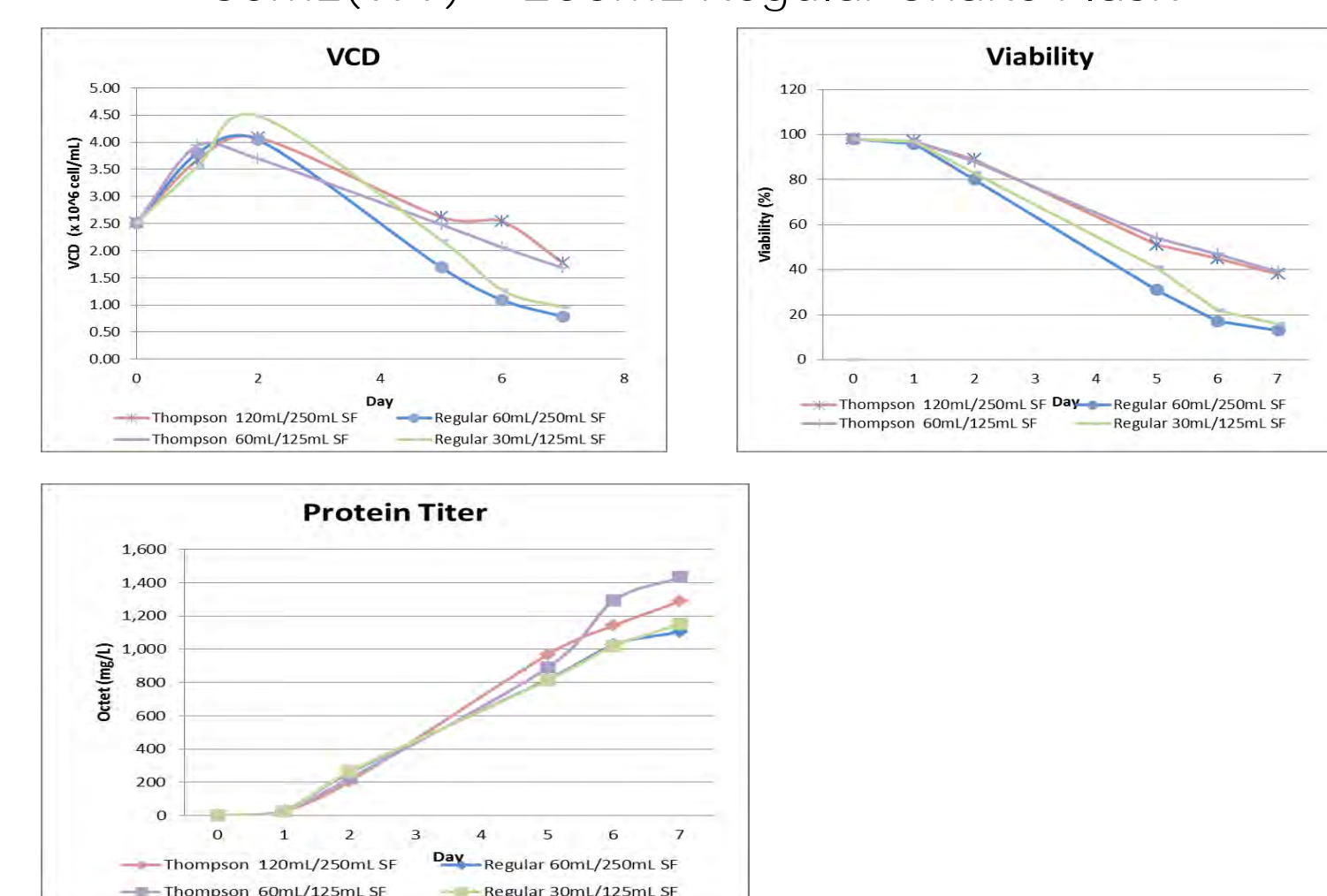
HEK293 Transient production were seeded at 0.8×10^6 cells/mL and at 37°C, with 5% CO₂. The cells were supplemented with feed on day 2 and harvested on day 7.

CHO Stable pool production cells were seeded at 2.5×10^6 cells/mL and incubated at 37°C, with 5% CO₂. The cells received supplemental feeding of glucose as needed. Shift the temperature to 32°C on day 5 and harvest the cells on day 14.

Results

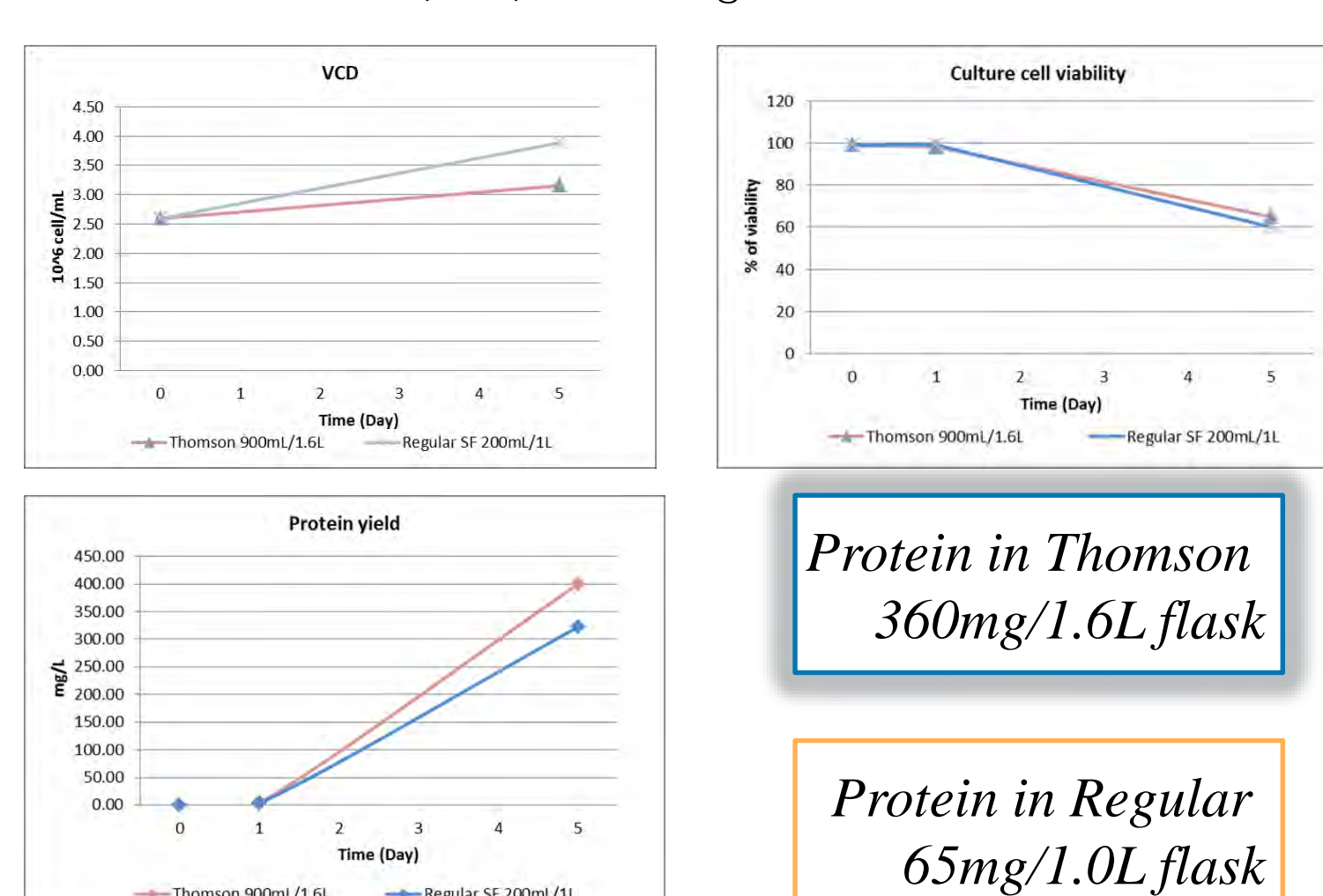
Case 1: HEK 293 Transient Production Results

120mL (WV) - 250mL Thomson Optimum Growth™ Flask Vs
60mL (WV) - 250mL Regular Shake Flask



Case 2: HEK 293 Transient Production Results

900mL (WV) - 1.6L Thomson Optimum Growth™ Flask Vs
200mL (WV) - 1L Regular Shake Flask

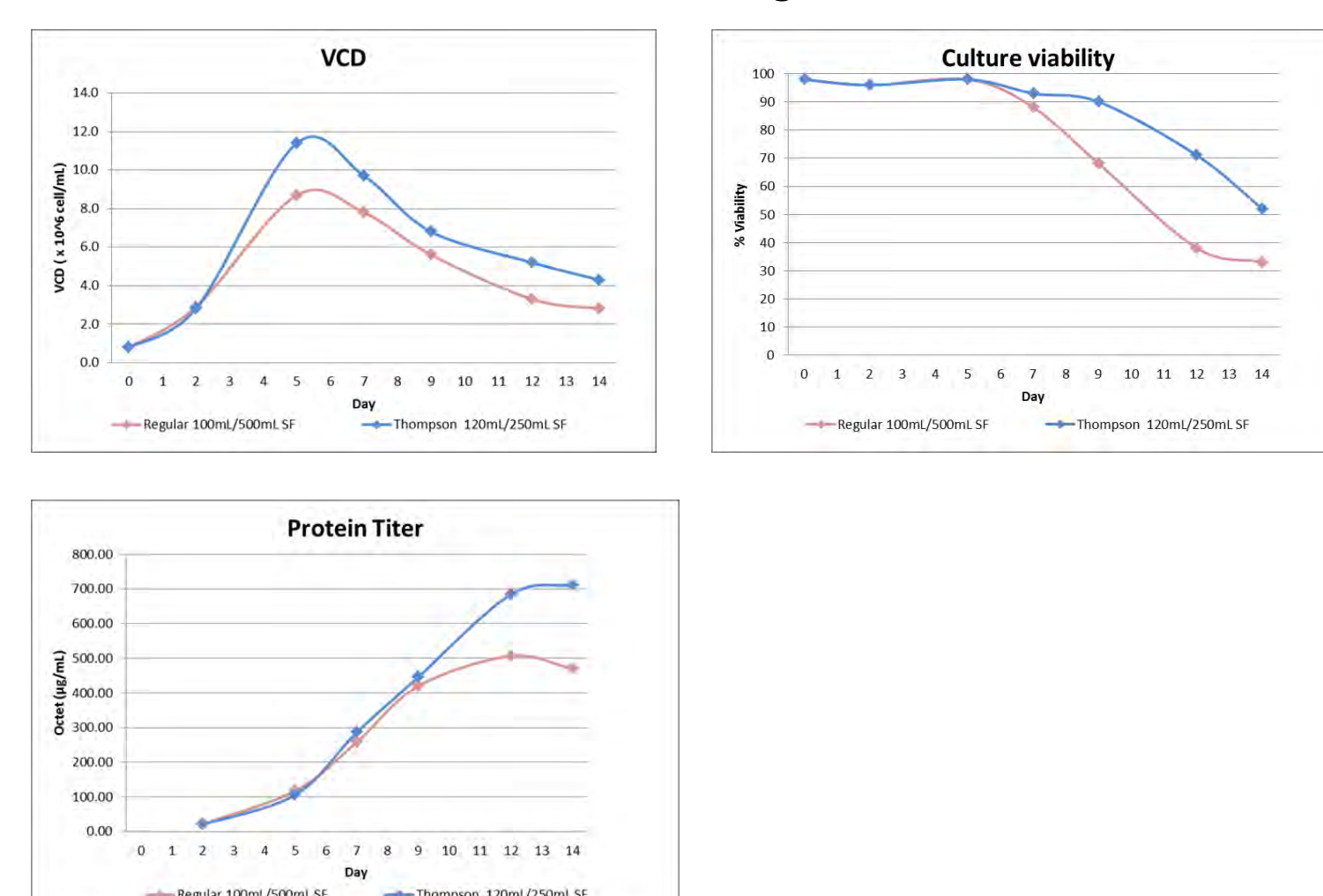


Protein in Thomson 360mg/1.6L flask

Protein in Regular 65mg/1.0L flask

CHO stable pool production results

120mL (WV) - 500mL Thomson Optimum Growth™ Flask Vs
100mL (WV) - 500mL Regular Shake Flask



Conclusions

Transient Protein Expression, HEK 293

- Both type of flasks support comparable peak VCD
- Thomson Optimum Growth™ Flask maintained higher VCD and viability
- Thomson Optimum Growth™ Flasks showed a boost in protein yield of more than 20%

Stable Pool Protein Expression, CHO

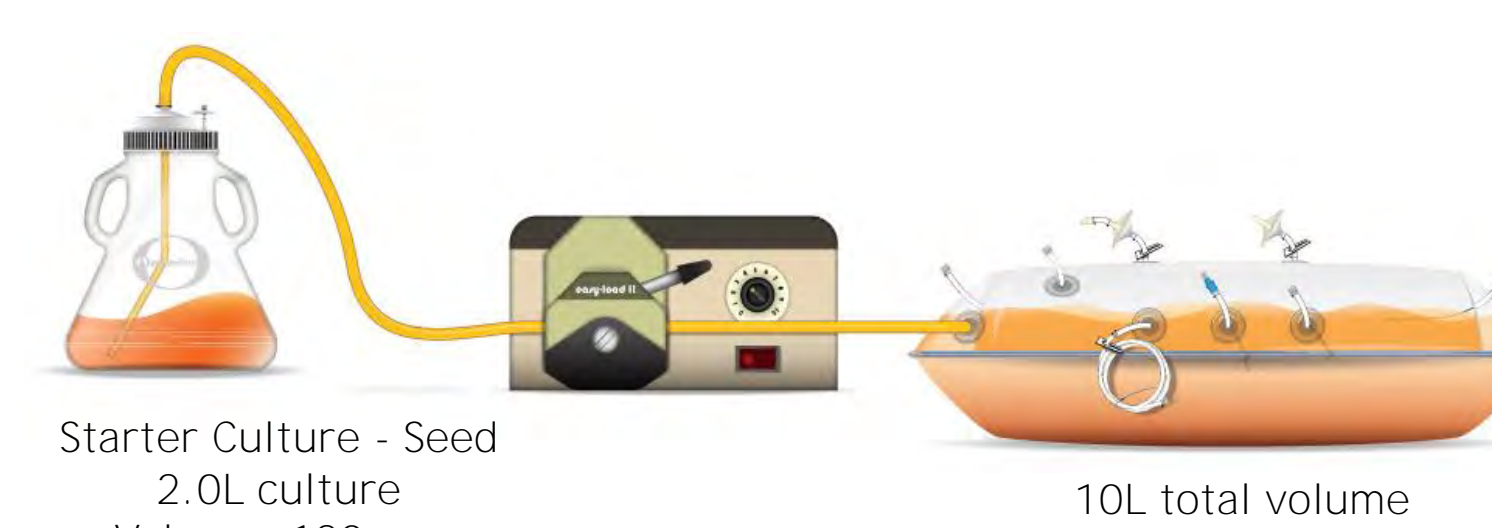
- Thomson Optimum Growth™ Flasks supported higher PCD: 11.4×10^6 cell /mL vs 8.7×10^6 cell /mL
- Thomson Optimum Growth™ Flasks maintained higher culture VCD and viability
 - Extended culture life for 2 days based on the harvesting criteria
- Thomson Optimum Growth™ Flasks resulted in a 50% titer increase

Thomson Flasks for Scalable Expi-CHO™ Protein Expression

Data Provided by Thermo Fisher

Use of Thomson Optimum Growth™ 5L Flasks for ExpiCHO™ WAVE scale up.

Optimum Growth™ 5L flasks support robust ExpiCHO-S™ cell growth and provide a suitable source of cell expansion for easy inoculation of WAVE bags for high volume transient expression runs.

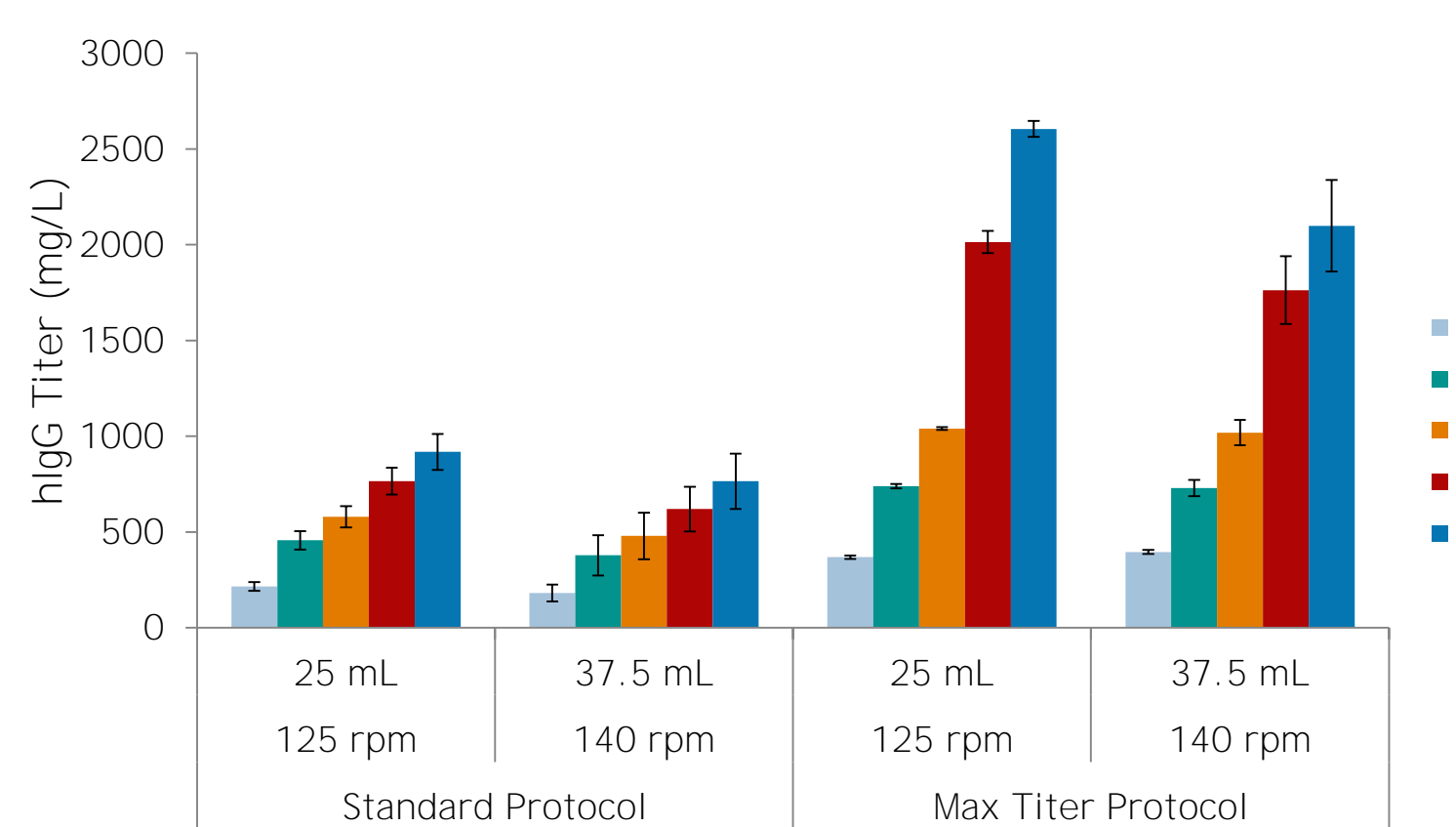


Starter Culture - Seed 2.0L culture Volume: 120rpm
10L total volume

Scale-Up No Problem

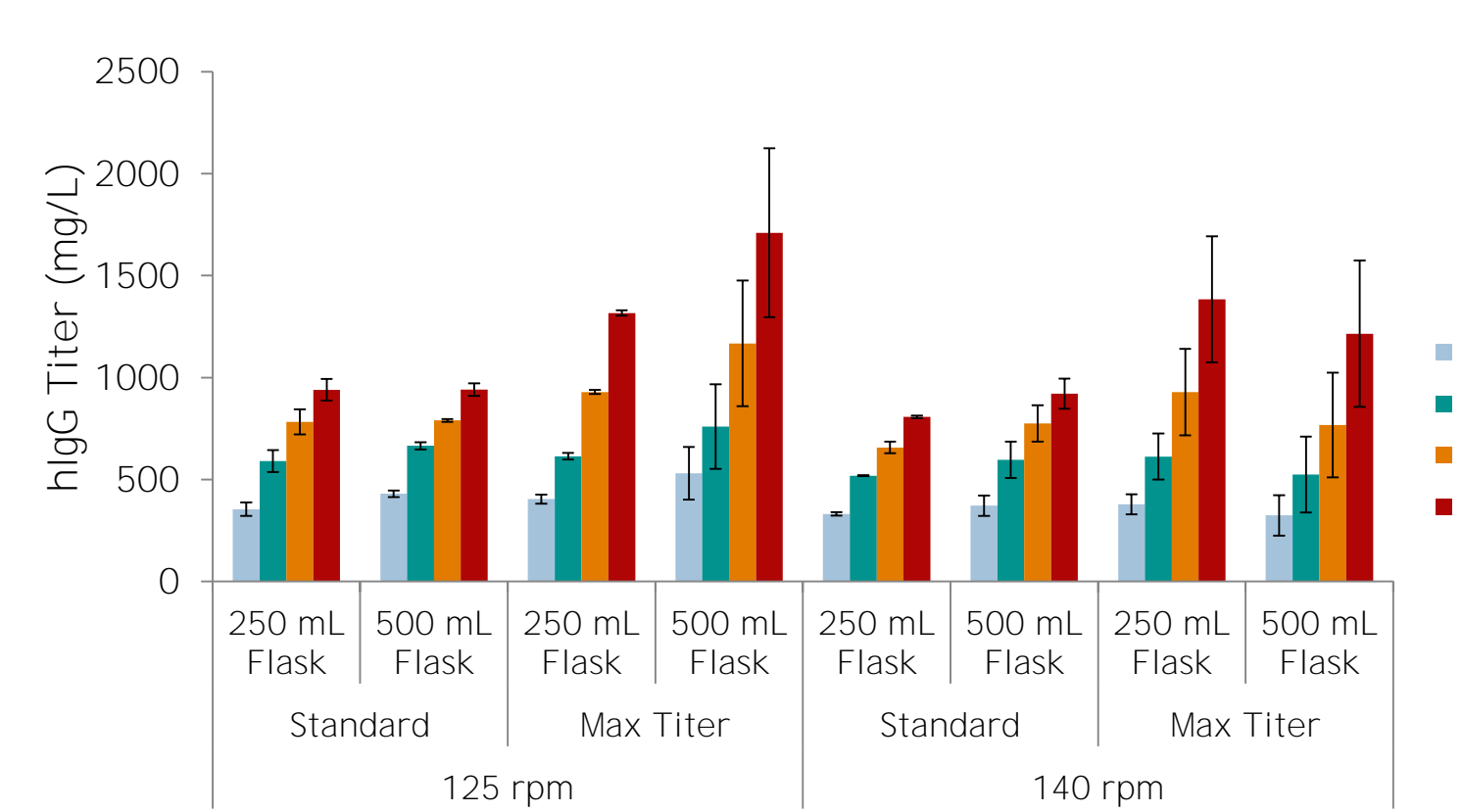
ExpiCHO™ Transient Expression System in Thomson 125mL shake flasks

Two different transfection volumes (nominal and nominal + 50%) were tested in a pilot study using the ExpiCHO Standard and Max Titer protocols at different shake speeds. Up to 1g/L titers were obtained using the Standard protocol and greater than 2.5g/L using the Max Titer protocol. As typical, shake speeds and volumes should be matched appropriately for optimal results.



ExpiCHO™ Transient Expression System in Thomson 250mL and 500mL Optimum Growth™ Flasks

Human IgG was expressed using the ExpiCHO Standard and Max Titer protocols at two different shake speeds. Up to 1g/L titers were obtained using the Standard protocol and greater than 1.5g/L using the Max Titer protocol (note: titers for the Max Titer protocol are lower due to experimental takedown on day 11 post-transfection instead of day 12-14).



Optimum Growth™ 250mL Flasks with XPICHO & HEK293 Cells in shaker



Insect Cells

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

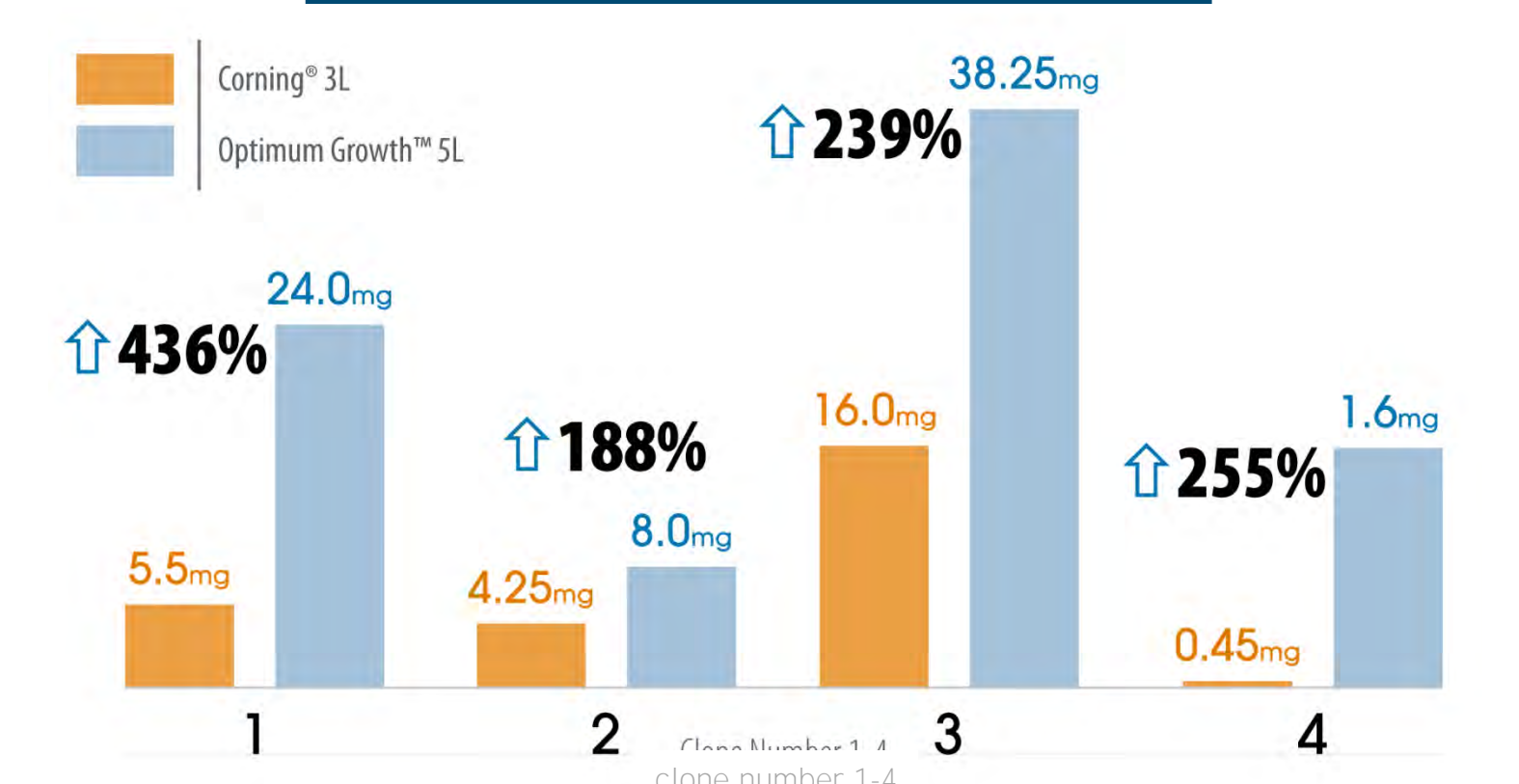


Materials and Methods

New York Structural Genomics compared protein expression in insect cells between the Corning® 3L Flasks with 2L of media in duplicate to 3L of media in one Thomson Optimum Growth™ 5L flask. High Five cells at 1×10^6 /mL were infected with 500µL of P3 virus per liter of culture. The culture conditions were 100rpm shaking speed, 50mm orbit using 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
2.8L	1.4L	150 / 110
5L	2.0L - 3.0L	120 / 90

CHO Stable Cells, CHO Transient, HEK 293 Transient		
Flask Size	Best Fill Volume	*RPM in 1"/2"
125mL	24mL	120 / 90
250mL	50mL	120 / 90z
500mL	100mL	120 / 90
1.6L	400mL	100 / 80
2.8L	900mL	100 / 80
5L	1.2L	90 / 70

Initial ExpiCHO™ Fill Volume CHO Transient		
Flask Size	Best Fill Volume	*RPM in 1"/2"
125mL	50mL	150 / 110
250mL	100mL	150 / 110
500mL	150mL	150 / 110
1.6L	400mL-750mL	150 / 110
2.8L	900mL-1.2L	150 / 110
5L	1.2L-1.6L	150 / 110

Final Volumes after ExpiCHO™ feeds CHO Transient		
Flask Size	Best Fill Volume	*RPM in 1"/2"
125mL	63mL (6.5mL feed)	150 / 110
250mL	120mL (10mL feed)	150 / 110
500mL	180mL (15mL feed)	150 / 110
1.6L	700mL-900mL (100mL feed)	150 / 110
2.8L	1.1L-1.4L (150mL feed)	150 / 110
5L	1.4L-2.0L (200mL feed)	150 / 110

Insect Cells		
Flask Size	Best Fill Volume	*RPM in 1"/2"
125mL	63mLs-75mL	150 / 110
250mL	150mL	150 / 110
500mL	250mL	150 / 110
1.6L	900mL	150 / 110
2.8L	1.4L	150 / 110
5L	2.0L-3.0L	135 / 90

Microbes/E. coli		
Flask Size	Best Fill Volume	*RPM in 1"/2"
125mL	63mL	250 / 150
250mL	125mL	250 / 150
500mL	250mL	250 / 150
1.6L	900mL	250 / 150
2.8L	1.4L	250 / 150
5L	2.0L-3.0L	250 / 150

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
2.8L	1.4L	120 / 90
5L	2L	120 / 90

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

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