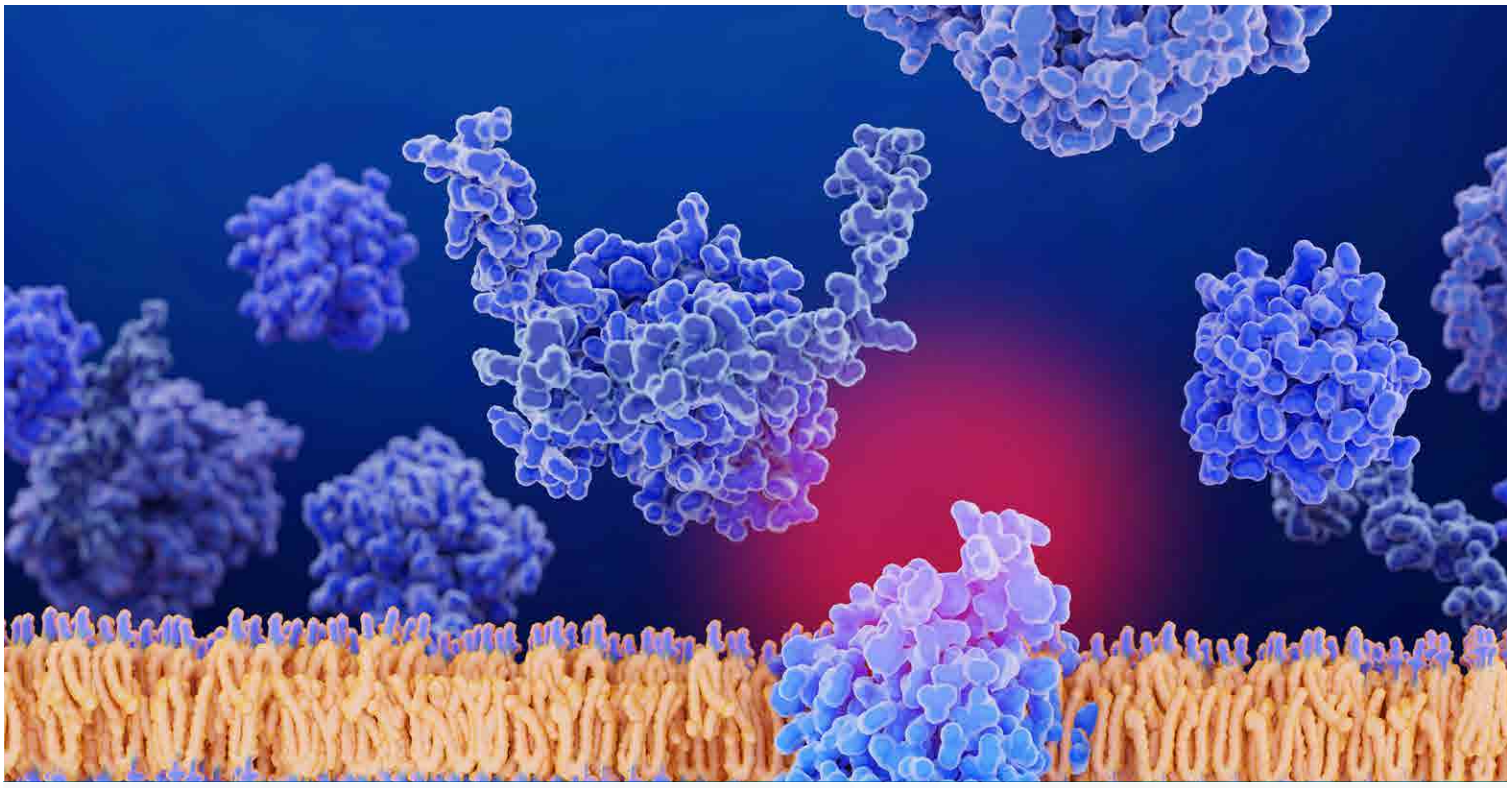
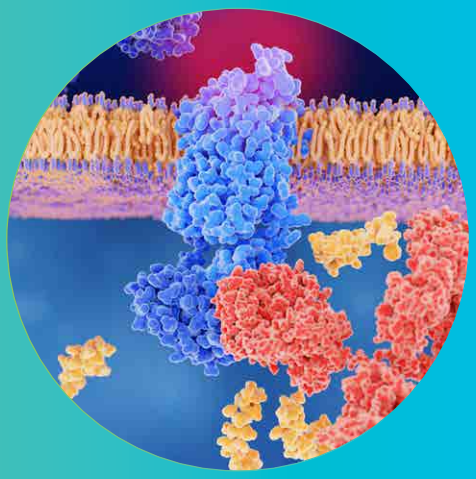


Focus: Protein Expression

Protein Expression is the process of generating a specific protein by manipulating the gene expression in a living organism. The organisms utilized for protein expression must be easy to culture, maintain, grow rapidly and produce large amounts of protein.

For more information on products that fit each step in this workflow, visit **vwr.com/protein-expression.**

2020



High-yield protein production system for suspension CHO cells

Simpler workflow – earlier harvest – more antibody

By Anthony Lauer, Austin Storck, and Laura Juckem, Mirus Bio LLC, Madison, Wisconsin USA

INTRODUCTION

The history and utility of suspension Chinese hamster ovary (CHO) cells for biotherapeutic protein production is unparalleled. Advances in transient transfection technologies and pressure to shorten development timelines have created the opportunity for systems offering rapid generation of milligrams to grams of protein early in the drug discovery process. The high yield and low cost associated with improved transient gene expression methods enables researchers to determine, at an early stage, if drug candidates have desirable attributes and warrant the resources required to generate stable clonal cell lines.

To further increase the protein yields obtained by transient gene expression in suspension CHO cells, we developed the CHOgro® High Yield Expression System to improve upon our previous platform (Figure 1) by: (1) identifying cell culture additives (expression enhancers) that significantly increase cell productivity; and (2) developing a streamlined protocol in which the steps of transfection, enhancer addition, and temperature shift are carried out on the same day. Through multiple rounds of screening and optimization, we identified the CHOgro® Titer Enhancer, which acts in synergy with the *Trans*IT®-PRO® Transfection Reagent and CHOgro® Expression Medium, to increase antibody production.

Optimization of protein production parameters and process robustness were examined by a transfection complex formation time course and testing expression of various protein constructs in Freestyle™ CHO-S and ExpiCHO™ cells adapted to the CHOgro® Expression Medium. Scalability of transient transfection was also assessed in culture sizes ranging from 2mL up to 2L in shake flasks. Head-to-head comparisons of the CHOgro® High Yield Expression System to Competitor A's System using six different antibody constructs show that higher or comparable protein titers are obtained at Day 7 and 14 post-transfection with the CHOgro® High Yield Expression System.

Higher Titers Faster:
MORE PRODUCT IN LESS TIME
Reach higher protein and antibody titers faster than Competitor Systems

Simple, Streamlined Workflow:
NO LICENSING, LESS MANIPULATION, REDUCE RISK OF CONTAMINATION
Same-day transfection, enhancer addition, and temperature shift

Scalable:
SCALABLE SCREENING RESULTS
Reproducible titers from 2mL to 2L

FIGURE 1: Benefits of the CHOgro® High Yield Expression System.

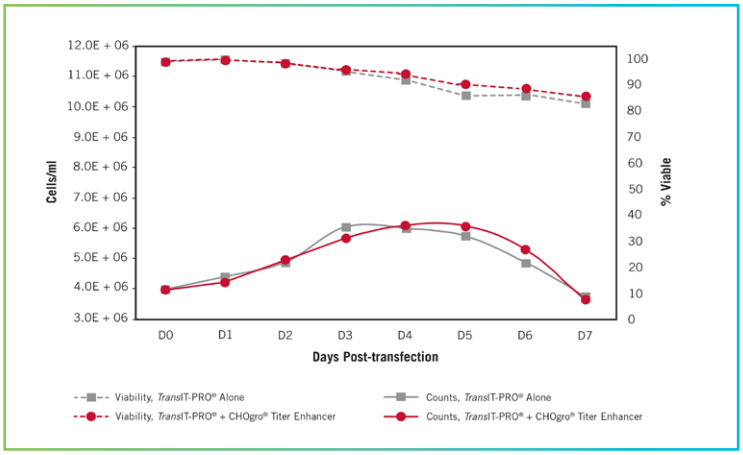


FIGURE 2: CHOgro® Titer Enhancer does not adversely affect cell growth and viability post-transfection. CHO-S cells were transiently transfected with *Trans*IT-PRO® Transfection Reagent. All cultures were shifted to 32 °C immediately post-addition of the transfection complexes and, where indicated, the CHOgro® Titer Enhancer was added to the culture.

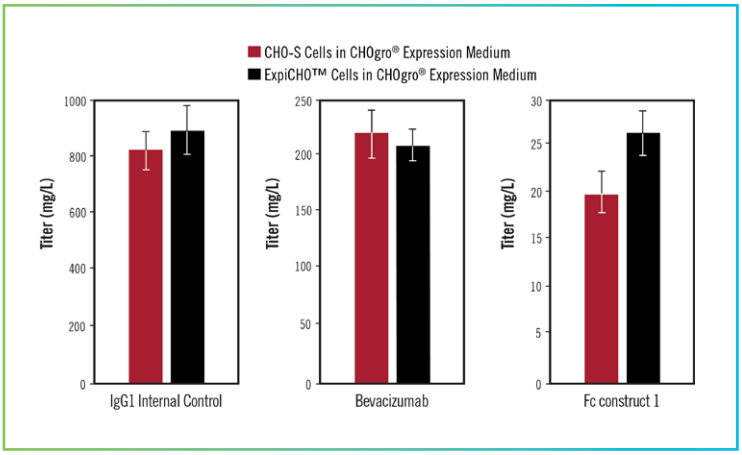


FIGURE 3: CHO-S or ExpiCHO™ cells yield similar titers using the CHOgro® High Yield Expression System. Both cell lines were transfected with plasmid encoding an IgG1 internal control antibody, Bevacizumab, or Fc-fusion construct. Day 14 supernatants were analyzed with an IgG ELISA.

Our results indicate that the attributes of the new and improved CHOgro® High Yield Expression System will help researchers obtain gram quantities of protein, simplify their workflow, and shorten their biotherapeutic development pipeline.

RESULTS

High Cell Growth and Viability Post-Transfection

To understand the effect of the CHOgro® Titer Enhancer on the health of high-density suspension CHO cells, we monitored cell viability and counts over seven days post-transfection using the CHOgro® High Yield Expression System. CHO-S cells were transfected on Day Zero with *Trans*IT-PRO® alone or *Trans*IT-PRO® and CHOgro® Titer Enhancer at a density of 4 x 10^6 cells/ml. Cell growth and viability were not significantly affected by the presence of the enhancer (Figure 2). We concluded that the increase in antibody titers observed using the CHOgro® Titer Enhancer is not due to changes in cell viability or gene delivery efficiency (data not shown), but instead appear to be the result of alterations to cellular pathways that control recombinant protein expression.

Comparable Titers with CHO-S and ExpiCHO™ Cells

The CHOgro® High Yield Expression System was developed for use in both CHO-S and ExpiCHO™ cells. Figure 3 illustrates that with multiple protein constructs, comparable titers are obtained using either suspension CHO cell line.

Transfection Complex Formation Time Is a Key Factor

Among the parameters assessed, the time the transfection reagent is allowed to incubate with DNA before addition of transfection complexes to the cultures (i.e., transfection complex formation time) was identified as a critical factor to optimize for achieving high titers using the CHOgro® High Yield Expression System. As shown in Figure 4, optimal complex formation time is less than five minutes for both CHO-S and ExpiCHO™ cells transfected with *Trans*IT-PRO® and CHOgro® Titer Enhancer. Using either cell line, the CHOgro® High Yield Expression System out-performs Competitor B. Importantly, optimal complex formation times are system specific and should not be used interchangeably between protocols with different transfection reagents.

1000-Fold Scalability

Scalability of transient transfection was assessed in culture sizes

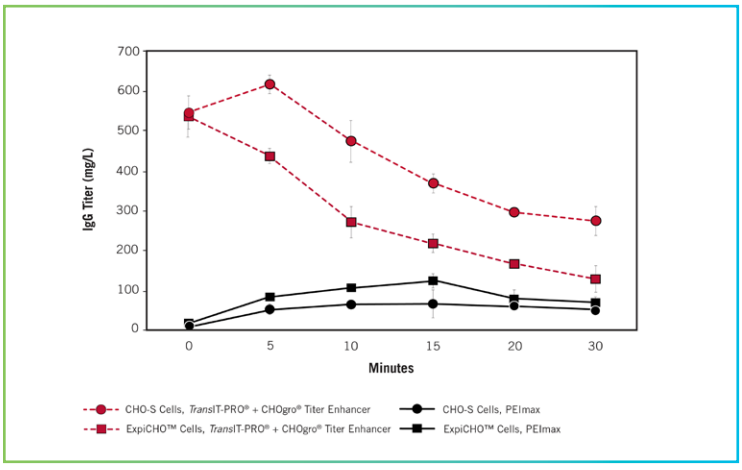


FIGURE 4: Complex formation time is a key parameter for achieving high titers with the CHOgro® High Yield Expression System. Transfection complexes, formed with either TransIT-PRO® or Competitor B, were incubated at the indicated times before addition to cultures of CHO-S or ExpiCHO™ cells. Day 7 supernatants were analyzed with an IgG ELISA.

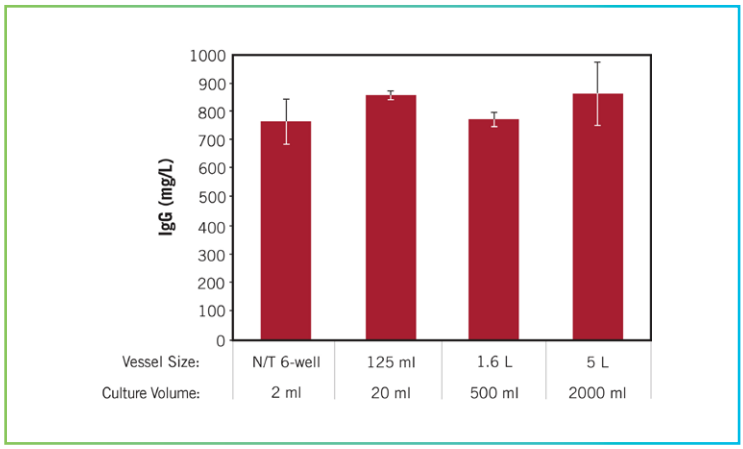


FIGURE 5: CHOgro® High Yield Expression System enables broad scalability, 1000-fold. Human IgG1 was produced by transient transfection in the following volumes/culture vessels: 2ml/non-tissue culture treated 6-well dish, 20mL/125mL Thomson flask, 500mL/1.6L Thomson flask, 2000mL/5L Thomson flask. Day 14 supernatants were analyzed with an IgG ELISA.

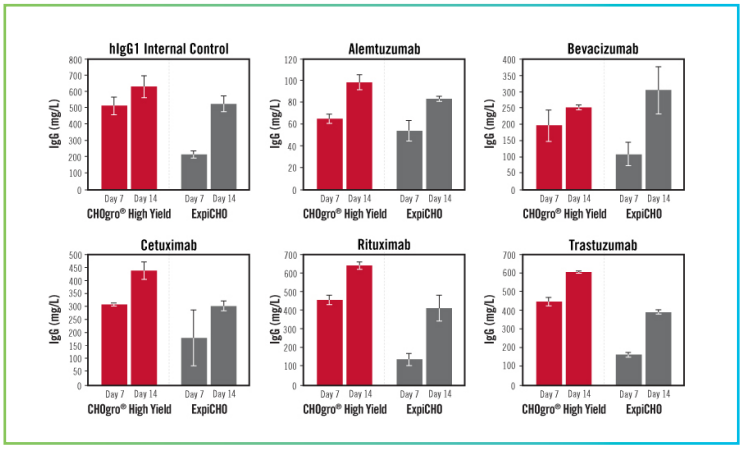


FIGURE 6: The CHOgro® High Yield Expression System outperforms Competitor A's Expression System in production of multiple antibody constructs.

ranging from 2mL up to 2L in shake flasks. Comparable titer concentrations were obtained from the smallest to the largest volume formats (Figure 5), which suggests the CHOgro® High Yield Expression System can be integrated into diverse research and manufacturing workflows. We typically perform screens in 2ml of culture medium per well in 6-well, non-tissue culture treated plates. These small-scale experiments accurately depict larger volumes and increase experimental throughput.

CHOgro® High Yield Versus ExpiCHO™

Head-to-head comparisons of the CHOgro® High Yield Expression System to Competitor A's Expression System were performed using six different therapeutically relevant antibody constructs (Table 1). For these constructs, a higher or comparable protein titer was obtained using the CHOgro® High Yield Expression System at both Day 7 and 14 post-transfection (Figure 6).

METHODS

Cell Culture

FreeStyle™ CHO-S cells or ExpiCHO-S cells were cultured in CHOgro® Expression Medium supplemented with 4 mM L-Glutamine and 0.3% Poloxamer 188 (Mirus Bio). Cells were cultivated at 37°C in a humidified incubator with 8% CO_2 and shaking. Cell counts and viability (via propidium iodine staining) were measured using a Guava easyCyte™ 5HT flow cytometer (EMD Millipore).

Transient Transfection

CHO cells were transfected at 4 x 10^6 cells/mL in CHOgro® Expression Medium with 1 µg/mL plasmid DNA using either the TransIT-PRO® Transfection Reagent (Mirus Bio) at a 1:1 (vol:wt) reagent-to-DNA ratio, or with Competitor B at a 4:1

TABLE 1: Representative Antibody Targets Used in Figure 6

Molecule Name	Target	Company
hIgG1 Internal Control	Confidential	Mirus Bio, Ilex Oncology;
Alemtuzumab	CD52	Millennium and Berlex Genentech
Bevacizumab	VEGF	and BioOncology Bristol-Myers Squibb
Cetuximab	EGFR	and ImClone
Rituximab	CD20	Genentech and IDEC
Trastuzumab	HER2	Genentech

reagent-to-DNA ratio. CHOgro® Titer Enhancer was added to the *Trans*IT-PRO® Transfection Reagent at 20µl per 1mL of culture. Cultures were shifted to 32°C immediately post-addition of the transfection complexes to the culture. The transfections conducted with Competitor A's Expression System (Figure 6) followed the Max Titer Protocol: 6 x 10^6 cells/ml ExpiCHO™ cells cultured in Competitor A's Expression were transfected using Competitor A's CHO Transfection Kit at a 3.2:1 reagent-to-DNA

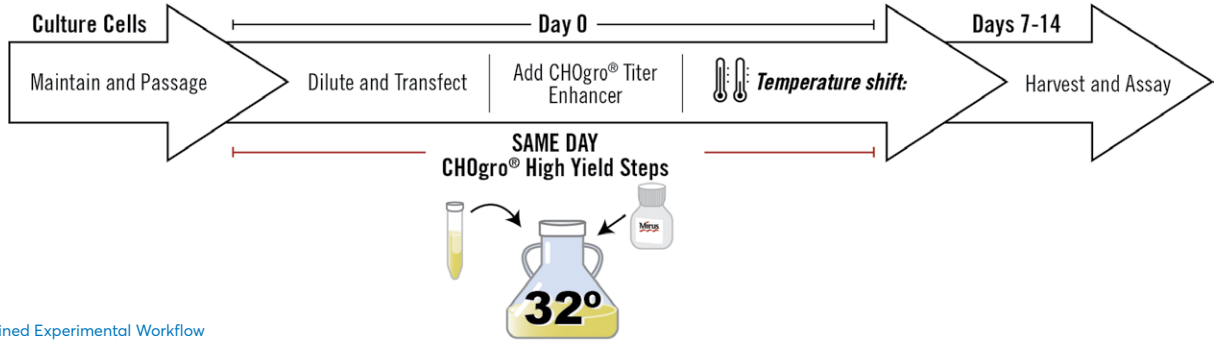


FIGURE 7: Streamlined Experimental Workflow

and 1µg plasmid DNA/ml of culture; Competitor A's CHO Enhancer and Feed were added at 24 hours post-transfection and cultures were shifted to 32°C, and at Day 5 a second volume of Competitor A's Feed was added to the appropriate flasks.

Determination of IgG Titer

Post-transfection, supernatants were analyzed using a standard sandwich human IgG ELISA. In the Figures, the error bars represent the standard deviation of triplicate technical replicates, or in Figure 4, the range of duplicate samples.

CONCLUSION

The CHOgro® High Yield Expression System was engineered to maximize transient protein production in suspension CHO cells, while still maintaining a simple and cost-effective workflow (Figure 7). The ability to add expression enhancers at the time of transfection and immediately shift cell cultures to hypothermic conditions provides researchers with more flexibility in the timing of their experiments, which saves time and reduces the risk of contamination caused by repeated handling of the culture.

Eliminate endotoxin contamination

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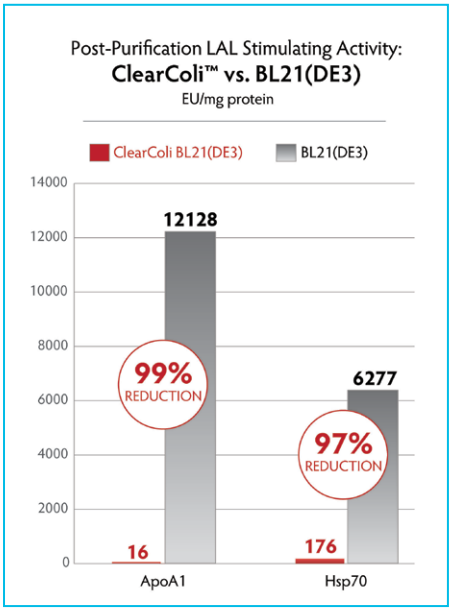
Protein Expression Levels: The relative production efficiency of endotoxin free ClearColi™ BL21(DE3) competent cells has been compared to normal BL21(DE3) cells using recombinant proteins. Although overall growth rates of the ClearColi are slower, final protein production levels are very similar when measured from equal cell densities.

Size	Cat. no.
12 reactions	89428-536
24 reactions	89428-538



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- **Fastest:** Simple 20 minute Midi/Maxi preps
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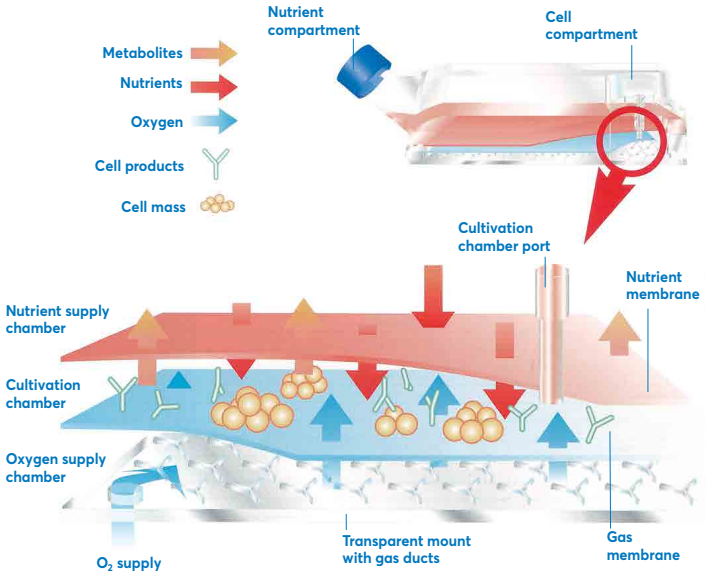
- **Ultra-Pure:** EndoZero™, vaccine grade and transfection ready

Description	Format	Culture volume	Yield	Elution Volume	Cat. no.
ZymoPURE™ II Plasmid Midiprep Kit	Spin Column	≤50 ml	≤400 µg	≥100 µl	77001-396
ZymoPURE™ II Plasmid Midiprep Kit	Spin Column	≤50 ml	≤400 µg	≥100 µl	77001-466
ZymoPURE™ II Plasmid Maxiprep Kit	Spin Column	≤150 ml	≤1.2 mg	≥200 µl	77001-468
ZymoPURE™ II Plasmid Maxiprep Kit	Spin Column	≤150 ml	≤1.2 mg	≥200 µl	77001-470
ZymoPURE™ II Plasmid Gigaprep Kit	Spin Column	≤2.5 l	≤10 mg	≥2 ml	77001-472

Corning® CELLine™ Disposable Bioreactor for scale-up and protein production



The Corning CELLine disposable bioreactor is a two-chamber cell cultivation vessel in which the chambers are separated by a 10 kD molecular weight cut-off membrane. This arrangement allows for high density cell culture and high-yield monoclonal antibody (mAb) production while maintaining a concentrated product.

BENEFITS

- Two-chamber design yields highly concentrated mAb product
- Large nutrient compartment allows for extended culture period without media changes
- Silicone bottom offers large surface area for gas exchange
- Clear bottom allows for cell visualization

APPLICATION

Large quantities of mAb are often necessary for biochemistry, molecular biology, and cell culture applications. Traditional flask cultures often result in lower yields or a diluted final product that requires downstream processing. Here we show higher protein production efficiency (Figure 1) and cell yields (Figure 2) of mAb produced in the CELLine disposable bioreactor compared to traditional flask vessels.

CELLine disposable bioreactors or flasks were seeded with equal numbers of MH677 cells. Samples for IgG2a production were collected at every cell passage for 21 days.

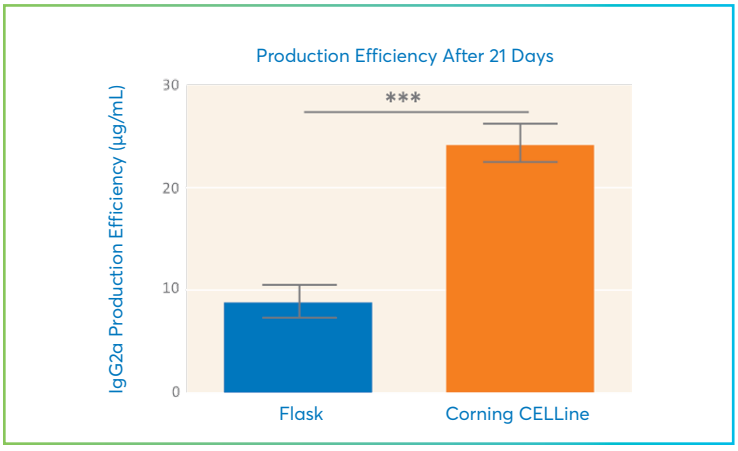


FIGURE 1: Production efficiency. Taking into account the higher volumes of medium required to fill the Corning CELLine bioreactor, the IgG2a production efficiency per volume of medium used was significantly greater than the T-75 flasks. Data shown with standard deviation. Unpaired T-test ***p<0.001. n=6 vessels from 3 independent studies.

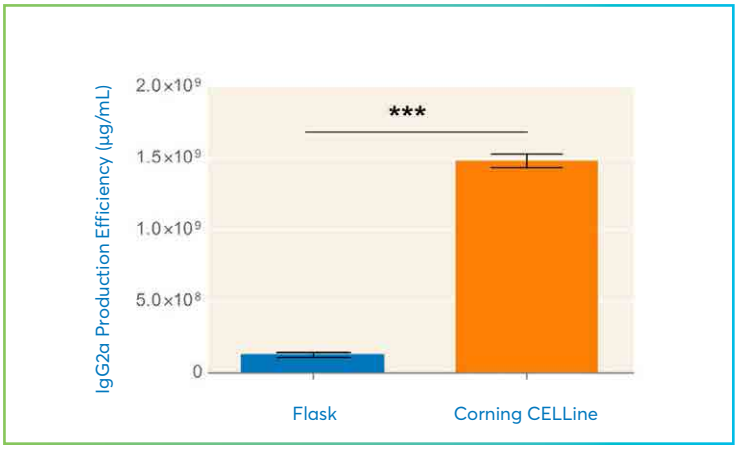


FIGURE 2. Total cells after 21 days.

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CORNING CELLINE DISPOSABLE BIOREACTOR, STERILE, CORNING®

CORNING CELLINE DISPOSABLE BIOREACTOR FOR ANTIBODY AND RECOMBINANT PROTEIN PRODUCTION

Description	Cat. no.
CELLine 1000 System	47735-592

Eliminate the risk of cross-contamination

CORNING® HYBRIGRO™ SF MEDIUM

Size	Cat. no.
Corning 500 mL hybrigro SF Medium (6/cs)	11010-977

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Sf9 cells are cultured in non-humidified, non-CO_2 incubators at 27°C (room temperature) and display both monolayer and suspension culture qualities. With their fast doubling times of 10-22 hours, Sf9 cells are easily scaled up to large cultures using bioreators.

Description	Cat. no.
Corning® insectagro® Sf9 Serum-Free/Protein-Free Medium, 1X	45001-054

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PEPROGROW™ HEK293 MEDIA, PEPROTECH, INC.

AN ANIMAL COMPONENT-FREE, PROTEIN-FREE, SERUM-FREE, CHEMICALLY-DEFINED, COMPLETE MEDIUM FORMULATION FOR THE *IN VITRO* CULTIVATION OF HEK293 CELLS

- Complete media
- Animal component-free
- Serum-free
- Protein-free
- Chemically-defined

PeproGrow™ HEK293 is an animal component-free, protein-free, serum-free, chemically-defined, complete medium formulation for the *in vitro* cultivation of HEK293 cells (Thermo Fisher Scientific FreeStyle™ 293-F cells, catalog number R790-07). This medium is intended for recombinant protein expression in suspension culture, which is recommended for a 5-day batch culture with a seeding density of 0.6 x10^6 cells/mL. This ready-to-use medium contains L-alanyl-L-Glutamine, amino acids, vitamins, and salts.

Size	Cat. no.
1 L	**10778-850**

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The SpectraMax® i3x offers an expanded dynamic range. It is engineered for performance with Spectral Fusion™ illumination for increased sensitivity across the entire excitation range. The SpectraMax® i3x also features a cooled photomultiplier tube (PMT) for improved detection in extremely low light. These features enable users to generate more data points without the need to dilute.

Description	Power	Weight	Cat. no.
SpectraMax® i3x Multi-mode detection platform	100 - 240 VAC, 2 A, 50/60 Hz	68.3 lbs. (31.0 kg)	**10014-924**

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- Excellent linearity, low variability, stable flourescence signal
- Compatible with reducing agent and other additives
- **Ex/Em:** 480/598 nm

Description	Size	Cat. No.
AccuOrange Protein Quantitation Kit	200 assays	**89493-574**
AccuOrange Protein Quantitation Kit	2000 assays	**89493-572**

Colorimetric cytotoxicity detection

LDH CYTOTOXICITY KIT II (COLORIMETRIC), PROMOCELL

PROVIDES A FAST, SENSITIVE, AND PRECISE ALTERNATIVE TO THE RADIOACTIVE [3H]-THYMIDINE AND [51CR] RELEASE ASSAYS

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- Can be analyzed spectrophotometrically
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Based on the activity of lactate dehydrogenase (LDH), our colorimetric and fluorometric LDH Cytotoxicity Kits provides a fast, sensitive, and precise alternative to the radioactive [3H]-thymidine and [51Cr] release assays. LDH is a stable cytoplasmic enzyme present in all cells and is rapidly released into the surrounding medium when cells are damaged. In this assay, LDH activity is determined by a coupled enzymatic reaction. LDH catalyses the conversion of lactate to pyruvate, triggering an enzymatic reaction that produces a water-soluble red formazan dye or an intense fluorescent product. The increase in the amount of formazan produced in culture medium correlates directly to the increase in the number of lysed cells and can be analyzed spectrophotometrically.

Description	Size	Cat. no.
LDH Cytotoxicity Kit II (colorimetric)	500 assays	**10183-712**

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- Smooth surface
- High-grade polystyrene construction
- HDPE screw cap construction with either standard seal or vented filter
- Free of RNase, DNase, and human DNA
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Dimensions	Max. volume	Cap type	Culture area	Packaging	Cat. no.
Long Form Cell Culture Roller Bottles					
497 L x 122 W, mm	4970 mL	Standard Seal	1700 cm²	12/Bag	**82051-020**
497 L x 122 W, mm	4970 mL	Vented Filter	1700 cm²	12/Bag	**82051-022**
497 L x 122 W, mm	4970 mL	Standard Seal	1700 cm²	12/Bag	**82051-024**
497 L x 122 W, mm	4970 mL	Standard Seal	1700 cm²	1/Bag	**82051-028**
497 L x 122 W, mm	4970 mL	Plug Seal	1700 cm²	12/Bag	**89129-504**
Short Form Cell Culture Roller Bottles					
269 L x 122 W, mm	2520 mL	Vented Filter	850 cm²	24/Bag	**82051-006**
269 L x 122 W, mm	2520 mL	Standard Seal	850 cm²	24/Bag	**82051-008**
269 L x 122 W, mm	2520 mL	Vented Filter	850 cm²	2/Bag	**82051-010**
269 L x 122 W, mm	2520 mL	Standard Seal	850 cm²	2/Bag	**82051-012**
269 L x 122 W, mm	2520 mL	Vented Filter	850 cm²	24/Bag	**82051-016**

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Size	Cat. no.
2 L	**89423-232**
10 L	**89423-230**
20 L	**89423-228**
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Versatile, self-contained capsules include all fittings and tubing to simplify installation into filtration setups and will work with any laboratory pump. Dual pressure gauges allow trans-membrane pressure (TMP) differential calculations.

Description	Electrical	Cat. no.
Tangential Flow Filtration Systems		
EVO Tangential Flow Filtration System	110 to 230 VAC	**76409-724**
Tangential Flow Filtration System	115 V	**16003-712**

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- Transformation efficiency: 1 x 10^7 cfu/µg pUC19 DNA
- Engineered *E. coli* B strain to promote disulfide bond formation in the cytoplasm
- Constitutively expresses a chromosomal copy of the disulfide bond isomerase DsbC
- Enhanced BL21 derivative
- Expresses a chromosomal copy of T7 RNAP

Protease deficient/B strain. Chemically competent *E. coli* cells suitable for T7 protein expression with enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm.

Description	Size	Cat. No.
Shuffle T7 Express Competent *E. coli*	12 x 0.5ml Tube	**103391-806**

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Polyester membrane support. Effective filtration area of VacuCap 60 PF filters is 30cm^2; VacuCap 90 PF filters, 60cm^2.

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VacuCap™ 60	60 mm	0.1 µm	**28143-330**
VacuCap™ 60	60 mm	0.2 µm	**28143-332**
VacuCap™ 60	60 mm	0.2 µm	**28143-340**
VacuCap™ 60	60 mm	0.45 µm	**28143-334**
VacuCap™ 60 PF	60 mm	0.8/0.2 µm	**28139-704**
VacuCap™ 90	90 mm	0.1 µm	**28143-316**
VacuCap™ 90	90 mm	0.2 µm	**28143-315**
VacuCap™ 90	90 mm	0.2 µm	**28143-338**
VacuCap™ 90	90 mm	0.45 µm	**28143-317**
VacuCap™ 90 PF	90 mm	0.8/0.2 µm	**28139-706**

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- Omega modified PES membranes come in a wide range of MWCOs, color-coded

Description	MWCO	Color	Membrane material	Pore size	Cat. no.
Nanosep Centrifugal Devices					
Nanosep®	3K	Gray	Omega		**29301-782**
Nanosep NAB Centrifugal Devices					
Nanosep® NAB		White	NAB with glass fiber		**76360-456**
Nanosep Centrifugal Devices					
Nanosep®	10K	Blue	Omega	–	**29300-620**
Nanosep®	30K	Red	Omega	–	**29300-622**
Nanosep®	100K	Clear	Omega	–	**29300-624**
Nanosep®	300K	Orange	Omega	–	**29300-626**
Nanosep MF Centrifugal Devices					
Nanosep® MF		Aqua	Bio-Inert	0.2 µm	**29300-642**
Nanosep® MF		Wildberry	Bio-Inert	0.45 µm	**29300-644**
Nanosep® MF		Clear	wwPTFE	0.2 µm	**76308-654**
Nanosep® MF		Clear	wwPTFE	0.45 µm	**76308-658**

Rapid method for gene expression analysis

PURExpress® *IN VITRO* PROTEIN SYNTHESIS KIT, NEW ENGLAND BIOLABS

A RAPID METHOD FOR GENE EXPRESSION ANALYSIS

PURExpress® is a novel cell-free transcription/translation system reconstituted from the purified components necessary for *E. coli* translation. The relative nuclease-free and protease-free nature of the PURExpress platform preserves the integrity of DNA and RNA templates and results in proteins that are free of modification and degradation. Transcription and translation are carried out in a one-step reaction, and require the mixing of only two tubes. With results available in a few hours, PURExpress saves valuable laboratory time and is ideal for high throughput technologies.

Description	Size	Cat. no.
PURExpress *In Vitro* Protein Synthesis Kit	10 Reactions (25 µL)	**101446-494**
PURExpress *In Vitro* Protein Synthesis Kit	100 Reactions	**102715-964**

Deliver research-grade results to any laboratory

EMAX® PLUS MICROPLATE READER, MOLECULAR DEVICES

ROBUST, HIGH VALUE MICROPLATE READER DESIGNED TO DELIVER RESEARCH-GRADE RESULTS TO ANY LABORATORY

- Research-grade results
- Pre-defined protocols
- Eight standard filter modes

EMax® Plus Microplate Reader benefits include: 8 filters for a variety of assays (covering visible range from 405–750nm), a compact instrument footprint, pre-defined protocols with SoftMax® Pro Software, and walk-up usability.

Description	Cat. no.
EMax® Plus Microplate Reader	**10119-370**

Designed to be easy-to-use lab workhorses

AZURE IMAGING SYSTEMS, AZURE BIOSYSTEMS

INNOVATIVE WESTERN BLOT AND GEL DOCUMENTATION SYSTEMS DESIGNED TO BE EASY-TO-USE LAB WORKHORSES

- Designed for flexible Western blot applications
- A wide range of applications with multiple light sources and filters

The 200 gel imaging workstation can image multiple types of gels. The darkroom replacer, model 300, features fast chemiluminescent detection with no film or darkroom needed. It can be upgraded to any of the ascending Azure Imager models (400, 500 or 600) with a simple field upgrade.

The visible fluorescent western imaging system, model 400, features a three-channel RGB excitation for Cy5/Cy3/CY2 Western applications. It also features fast chemiluminescent detection with no film or darkroom needed. It can be upgraded to an IR imaging system (model 600) with a simple field upgrade.

Description	Cat. no.
Systems	
Chemi and NIR Imaging, Azure 500	**76353-886**
Chemi and RGB Imaging, Azure 400	**76353-888**
Chemiluminescet Western Imaging, Azure 300	**76353-890**
Gel Doc, Azure 200	**76353-892**
The Ultimate Western Blot Imaging System, Azure 600	**76353-884**
Modules	
Module for Adding TPN Channel to Azure 300	**76353-896**
Module for Adding TPN Channel to Azure 500	**76353-894**

Description	Cat. no.
Black Tray	**76353-900**
Trans White Table	**76353-898**
IQOQPQ	**76353-902**

These exact models are coming soon to Canada. Please contact your VWR Sales Rep to learn about the Azure Biosystems models currently available in your region.

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