

Corning® CELLine™ Disposable Bioreactor and Corning hybrigro™ SF Medium Offer a Complete Solution for Serum-free Hybridoma Scale-up and Protein Production

CORNING

Application Note

Hilary A. Sherman and Hannah J. Gitschier
Corning Incorporated, Life Sciences
Kennebunk, Maine

Introduction

Monoclonal antibodies (mAb) are widely used for biochemistry, molecular biology, and as potential therapeutic candidates. The ability to generate large quantities of mAb to meet these basic research and other large-scale manufacturing needs can be challenging. The Corning CELLine disposable bioreactor enables the *in vitro* generation of high yields of mAb in an easy-to-use, disposable vessel. In addition to producing higher product yields when compared to traditional static vessels¹⁻², the final product is also more concentrated. This is due to a two chamber design separated by a 10 kDa molecular weight cut-off membrane that allows nutrients and growth factors to diffuse into the production chamber, while retaining the mAb or cell product (e.g., recombinant protein) in a separate low volume compartment. This effectively reduces the downstream processing time that would be necessary to concentrate the final product. The reduced processing, in addition to higher overall production yields, can result in a 50% cost reduction per milligram of product produced². To demonstrate increased protein production and the concentration of product generated using this system, hybridoma cells were cultured under serum-free conditions using Corning hybrigro SF medium in the Corning CELLine disposable bioreactor and traditional cell culture flasks. Corning hybrigro SF is a complete,

animal component-free, defined medium specifically developed for serum-free growth and antibody production. This medium has been shown to support higher protein production compared to serum-containing culture medium³.

Methods and Materials

MH677 cells (proprietary murine hybridoma cell line) were cultured in standard tissue culture flasks in hybrigro SF medium (Corning Cat. No. 40-215-CV). Cell densities were maintained between 2.5×10^4 and 1.5×10^6 cells/mL by passaging cells twice per week using the Vi-CELL® cell viability Analyzer (Beckman Coulter) for viable cell enumeration. MH677 cells were expanded to seed 15 mL at 2.5×10^4 cells/mL into two T-75 flasks (Corning Cat. No. 430641U) and two CELLine disposable bioreactor vessels (Corning Cat. No. 353137) following the protocol provided (Corning Literature Code CLS-DL-CELLine-13)⁴, which includes filling the nutrient compartment with 1L of medium. T-75 vessels were harvested every 3 to 4 days, retaining a sample for viable cell enumeration, as well as 1 mL of medium to be frozen and stored for future analysis of IgG2a production. Cells collected from T-75 flasks were then re-seeded into new flasks with 15 mL of medium using 2.5×10^4 cells/mL. CELLine disposable bioreactors were sampled, and passaged every 7 days without changing the 1L of medium contained in the nutrient compartment. Cells were maintained in a humidified incubator at 37°C and 5% CO₂ for the duration of the 21-day culture period. To assess protein production, mouse IgG2a ELISA kits (Innovative Research Cat. No. IRKTAH1156) were

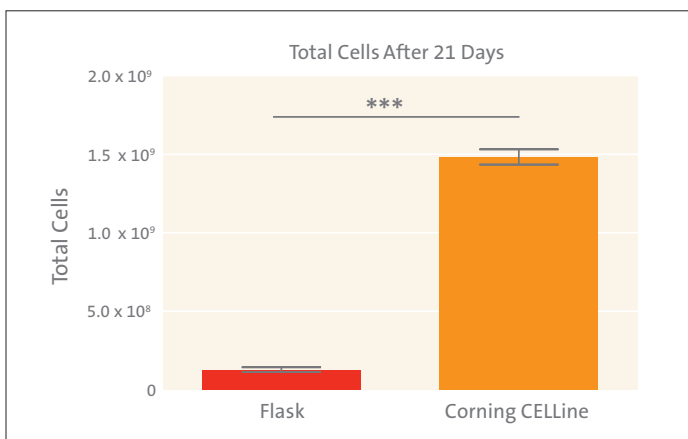


Figure 1. Total MH677 cell yields. Significantly more MH677 cells were recovered from Corning CELLine bioreactors after 21 days of culture compared to cells grown in T-75 flasks. Data shown with standard deviation. Unpaired T-test *** $p < 0.001$. $n = 6$ vessels from 3 independent studies.

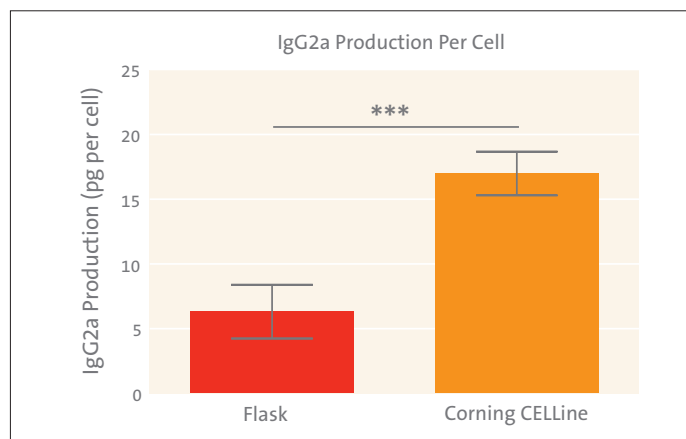


Figure 2. IgG2a production per cell. MH677 cells grown in the Corning CELLine bioreactors produced more IgG2a on a per cell basis compared to cells grown in T-75 flasks. Data shown with standard deviation. Unpaired T-test *** $p < 0.001$. $n = 6$ vessels from 3 independent studies.

used according to the manufacturer's protocol. Cell counts and IgG2a production from each harvest (3 for Corning® CELLLine™ disposable bioreactors, and 6 for T-75 vessels) were combined to achieve the total yield after 21 days in culture.

Results and Discussion

The unique design of the CELLLine disposable bioreactor combined with Corning hybrigro™ SF medium is ideal for users that require high yields of concentrated mAb that are produced under defined, serum-free culture conditions. The total yield of MH677 cells from each CELLLine disposable bioreactor was approximately 12-fold greater than the cell yield derived from the T-75 vessels after 21 days of culture (Figure 1). In addition to the generation of greater cell yields, MH677 cells produced approximately 3X greater IgG2a on a per cell basis when cultured in the CELLLine disposable bioreactor compared to the T-75 vessels (Figure 2). By combining greater cell yields with higher mAb production efficiency, the cumulative production of mAb was 30-fold greater in the CELLLine disposable bioreactor when compared to a standard T-75 flask (Figure 3). In addition to the benefit of attaining more protein with fewer vessels and reduced cell maintenance, there is also a time and cost savings associated with the reduced need for post-processing steps to concentrate mAb produced in the CELLLine disposable bioreactor². Based on the total volume of supernatant collected after 21 days in culture, the IgG2a collected from CELLLine disposable bioreactors was 60 times more concentrated compared to the more dilute product collected from the T-75 vessels (Figure 4). Although a higher volume of cell culture medium is required in the CELLLine disposable bioreactor, the mAb production efficiency per volume of medium is significantly higher when compared to the production per volume of medium used in a T-75 vessel (Figure 5).

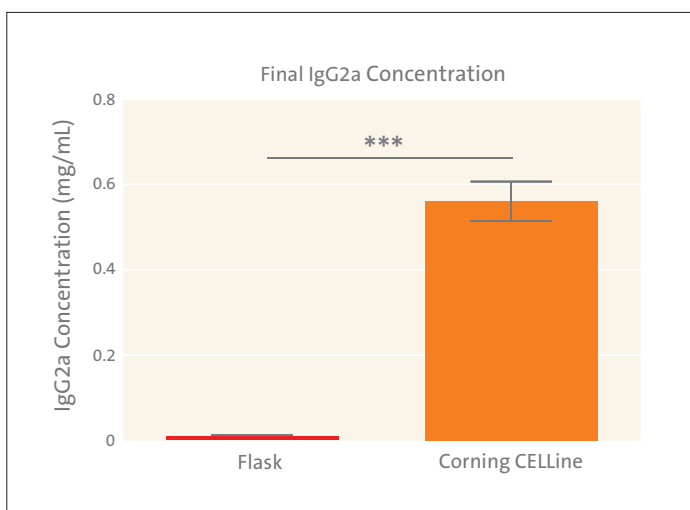


Figure 4. Final IgG2a concentration. When pooled, IgG2a collected from Corning CELLLine bioreactors was 60 times more concentrated than IgG2a collected from T-75 flasks. Data shown with standard deviation. Unpaired T-test *** $p < 0.001$. $n = 6$ vessels from 3 independent studies.

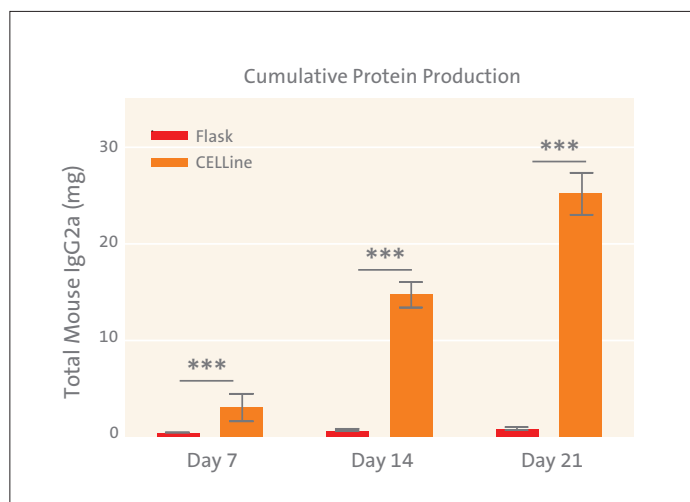


Figure 3. Cumulative IgG2a production. Significantly more IgG2a was produced in the Corning CELLLine bioreactors when compared to production from T-75 flasks. Data shown with standard deviation. Unpaired T-test *** $p < 0.001$. $n = 6$ vessels from 3 independent studies.

Conclusions

- ▶ The Corning CELLLine disposable bioreactor sustained 21 days of hybridoma culture, resulting in greater viable cell yield than that achieved in T-75 flasks.
- ▶ Significantly more mAb was produced on a per cell basis in the CELLLine disposable bioreactors than produced in T-75 flasks.
- ▶ Use of the CELLLine disposable bioreactor results in higher concentrated protein product yields, which may reduce costs due to decreased handling required to passage cells, change medium, and concentrate the desired final product.

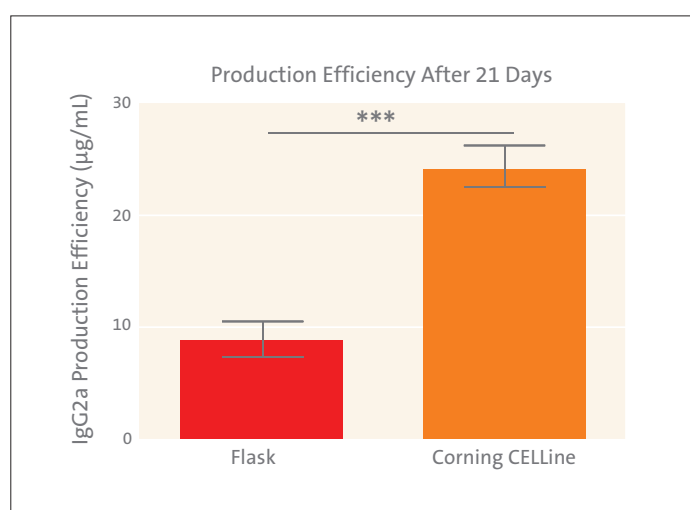


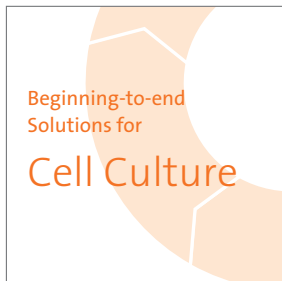
Figure 5. Production efficiency. Taking into account the higher volumes of medium required to fill the Corning CELLLine bioreactor, the IgG2a production efficiency per volume of medium used was significantly greater than the T-75 flasks. Data shown with standard deviation. Unpaired T-test *** $p < 0.001$. $n = 6$ vessels from 3 independent studies.

References

1. Vincent Dewar, Pierre Voet, Françoise Denamur, and Jean Smal. Industrial implementation of in vitro production of monoclonal antibodies. *ILAR Journal* (2005) 46(3):307-313 doi:10.1093/ilar.46.3.307.
2. Mohamed Trebak, Jae Min Chong, Dorothee Herlyn, and David W Speicher. Efficient laboratory-scale production of monoclonal antibodies using membrane-based high-density cell culture technology. *Journal of Immunological Methods* (1999) 230:59-70.
3. Hilary Sherman and Mark E Rothenberg. Corning hybrigro SF medium for high density hybridoma culture and increased production (2014). Corning Literature Code CLS-CG-AN-004.
4. Hilary Sherman and Mark E Rothenberg. Quick guide for antibody production in a Corning CELLline disposable bioreactor (2014). Corning Literature Code CLS-DL-CELLline-13.

For more specific information on claims, visit the Certificates page at www.corning.com/lifesciences.

Warranty/Disclaimer: Unless otherwise specified, all products are for research use only. Not intended for use in diagnostic or therapeutic procedures. Not for use in humans. Corning Life Sciences makes no claims regarding the performance of these products for clinical or diagnostic applications.



www.corning.com/lifesciences/solutions

At Corning, cells are in our culture. In our continuous efforts to improve efficiencies and develop new tools and technologies for life science researchers, we have scientists working in Corning R&D labs across the globe, doing what you do every day. From seeding starter cultures to expanding cells for assays, our technical experts understand your challenges and your increased need for more reliable cells and cellular material.

It is this expertise, plus a 160-year history of Corning innovation and manufacturing excellence, that puts us in a unique position to offer a beginning-to-end portfolio of high-quality, reliable cell culture consumables.

For additional product or technical information, please visit www.corning.com/lifesciences or call 800.492.1110. Customers outside the United States, please call +1.978.442.2200 or contact your local Corning sales office.

Corning Incorporated
Life Sciences
836 North St.
Building 300, Suite 3401
Tewksbury, MA 01876
t 800.492.1110
t 978.442.2200
f 978.442.2476
www.corning.com/lifesciences

**Worldwide
Support Offices**

ASIA/PACIFIC
Australia/New Zealand
t 61 427286832
China
t 86 21 3338 4338
f 86 21 3338 4300
India
t 91 124 4604000
f 91 124 4604099

Japan
t 81 3-3586 1996
f 81 3-3586 1291

Korea
t 82 2-796-9500
f 82 2-796-9300

Singapore
t 65 6572-9740
f 65 6861-2913

Taiwan
t 886 2-2716-0338
f 886 2-2516-7500

EUROPE

France
t 0800 916 882
f 0800 918 636

Germany
t 0800 101 1153
f 0800 101 2427

The Netherlands
t 020 655 79 28
f 020 659 76 73

United Kingdom
t 0800 376 8660
f 0800 279 1117

**All Other European
Countries**
t 31 (0) 20 659 60 51
f 31 (0) 20 659 76 73

LATIN AMERICA
grupoLA@corning.com
Brasil
t (55-11) 3089-7400
Mexico
t (52-81) 8158-8400



For a listing of trademarks, visit www.corning.com/clstrademarks. All other trademarks are the property of their respective owners.