WHEATON®

Analysis of Dialyzed Flask Technology for Antibody Production

CELLine™ Bioreactor Flasks

Experiment Overview

Abstract

Efficient and cost effective hybridoma culture is essential to small and large scale monoclonal antibody production for research purposes. This study evaluates multiple aspects of two existing production methods and a new dialyzed cell culture flask method for culturing hybridoma cells. The flask separates the cell cultivation compartment from the cell culture media via a 10kDa cut off limit membrane. This method allows for multiple harvests, longer run times and a super-concentrated supernatant.

To determine the advantages of the dialyzed flask technology, an anti-6X histidine epitope tag secreting hybridoma cell line and an anti-AKT3 isoform secreting hybridoma cell line were selected. The current production methods for the anti-6X histidine epitope tag and anti-AKT3 isoform producing clones include an animal method (ascites) and a proprietary suspension culture method. Several criteria were measured including direct labor cost, reagent cost and yield of antibodies. The purified monoclonal antibodies were analyzed by SDS-PAGE 4-20% under denatured conditions and characterized by Western Blot, direct ELISA titration, and immunohistochemistry to assess performance.

Monoclonal anti-6X histidine epitope tag and anti-AKT3 isoform from the three methods were affinity purified by protein A. SDS-PAGE analysis showed that all antibody samples had a purity higher than 95%. As indicated by the study, production of monoclonal antibodies in ascites often leads to contamination by endogenous host proteins. Western Blot analysis demonstrated no decrease in performance from the antibodies produced in the dialyzed flask. Immunohistochemistry on prostate cancer slides exhibited similar results from all production methods. An economic analysis comparing the three methods was completed. A significant yield increase and a cost savings were realized from the new production method. This increase in performance was due to the reusable nature of the dialyzed flask and its ability to concentrate the antibodies.

How Do CELLine Bioreactor Flasks Work?

More cells mean a higher yield of recombinant protein or antibody. Due to the bottom gas permeable membrane, the CELLine Bioreactor Flask can sustain 20 times more cells than a conventional T-Flask. Importantly, no mechanical devices are required for this proprietary high-density culture method. In addition, the cells are also separated from the bulk media compartment, via a dialysis membrane. This allows for long-term bulk storage of media in the media compartment during operation, and a highly concentrated harvest from the cell compartment. Metabolites are freely exchanged between the two compartments, allowing the cells to grow in an optimal environment. Furthermore, a flask can be harvested multiple times, reducing the amount of consumables required by your lab.

In this study, one flask was harvested approximately every five days for each antibody of interest. They were re-seeded and placed back into service until the desired amount of antibody was collected. At this point, the productivity of the CELLine Bioreactor Flask was compared to two current antibody production methods, ascites and a proprietary suspension culture. The functional activity of the antibodies produced via the three methods were compared.

Media Compartment — The media compartment allows for bulk storage of cell culture growth medium. This reduces the media refreshing requirement significantly as the media compartment is fifty times the size of the cell compartment.

Metabolite Regulating Upper Membrane — The upper dialysis membrane has a 10 kDa cut off limit. This regulates the flow of metabolites to and from the cell compartment and retains all proteins in the cell compartment.

Cell Compartment — The cell compartment provides the ideal area to inoculate and achieve high density cultures. The compartment concentrates cells, their products, and limits the requirement for any exogenous growth factors.

Gas Permeable Lower Membrane — With static cultures, gas transfer rates can be the limiting factor in high density cultures. The CELLine flask places the cells directly against the gas permeable membrane to achieve optimal levels of oxygen and carbon dioxide.

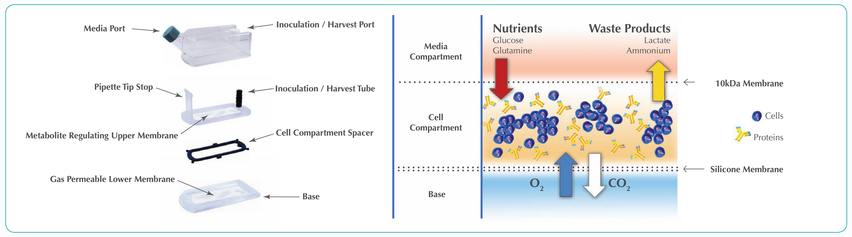


Figure 1A

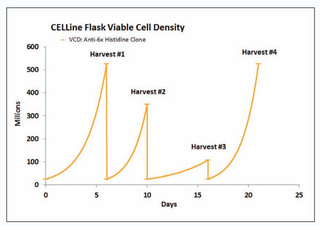


Figure 1B

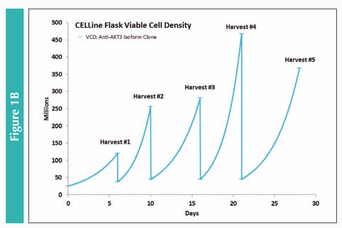
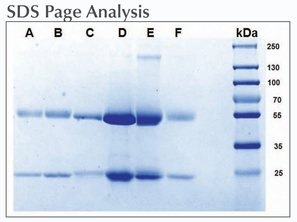


Figure 1A and 1B. The viable cell density (VCD) was measured each time a CELLine Bioreactor Flask was seeded and harvested. The flasks were harvested multiple times until the desired amount of antibody was obtained. Figure 1A indicates the harvesting schedule and the cell concentrations for the Anti-6x Histidine producing hybridoma. This flask was harvested four times and reached cell concentrations above 500 million. Figure 1B indicates the harvesting schedule and the cell concentrations for the Anti-AKT3 Isoform producing hybridoma. This flask was harvested five times and reached cell concentrations above 450 million. A simple batch method was employed for this study and more aggressive harvesting schedules as well as longer runtimes will lead to increased antibody production.

SDS Page Analysis

Figure 2

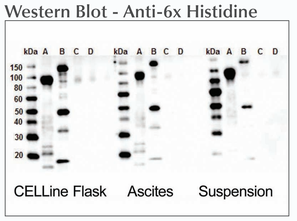


A = Antibody anti-6X histidine epitope tag - CELLine
B = Antibody anti-6X histidine epitope tag - Ascites
C = Antibody anti-6X histidine epitope tag - Suspension
D = Antibody anti-AKT3 isoform - CELLine
E = Antibody anti-AKT3 isoform - Ascites
F = Antibody anti-AKT3 isoform - Suspension

The purity of the anti-6x histidine epitope antibody and the anti-AKT3 isoform epitope antibody following SDS-PAGE 4-20% analysis following protein A affinity purification. The purity of all samples was greater than 95% by densitometry analysis. The lower bands indicate the light chain and the higher bands indicate the heavy chain of the denatured antibody. Note the contamination by endogenous host proteins (Figure 2, lane E) from the ascites production method.

Western Blot - Anti-6x Histidine

Figure 3

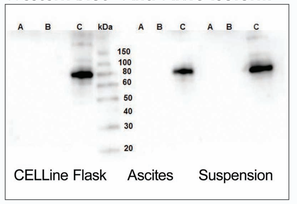


A = Purified histidine tagged recombinant protein (100ng)
B = E. coli cell lysate containing histidine tagged recombinant Protein (200ng)
C = Recombinant GST tagged protein (100ng), negative control 1
D = Recombinant FLAG tagged protein (100ng), negative control 2

Western Blot for mouse monoclonal anti-6X histidine epitope tag antibody (p/n: 200-301-382). The Western Blot for all three 6X histidine epitope tag antibody samples indicated that the antibody specifically recognized the tagged recombinant protein and cell lysate containing tagged recombinant protein. The negative control indicated no false positives for additional recombinant proteins.

Western Blot - Anti-AKT3 Isoform

Figure 4



A = Purified recombinant GST-AKT Isoform - 1 (50ng)
B = Purified recombinant GST-AKT Isoform - 2 (50ng)
C = Purified recombinant GST-AKT Isoform - 3 (50ng)

Western Blot for mouse monoclonal anti-AKT3 antibody (p/n: 200-301-E75). The Western Blot for all three anti-AKT3 antibody samples indicated that the antibody specifically recognized the AKT Isoform 3. The negative controls indicated no false positives for Isoforms 1 and 2.

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Direct ELISA

Titration for mouse monoclonal anti-6X histidine epitope tag anti-AKT3 antibodies.

Figure 5A

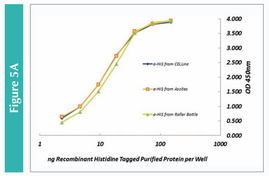


Figure 5B

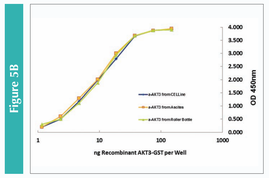
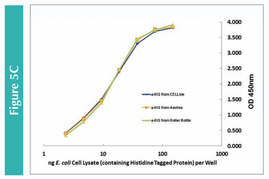


Figure 5C



Samples of the antibodies were evaluated with a recombinant histidine purified protein, a recombinant AKT3-GST and a histidine tagged E. Coli lysate. As indicated by the titrations all samples exhibited similar sensitivity across multiple concentrations.

Immunohistochemistry

Figure 6A

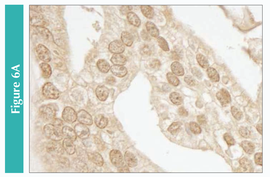


Figure 6B

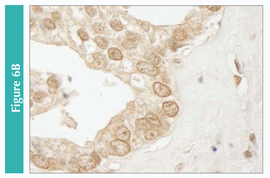


Figure 6C

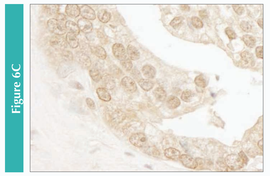


Figure 6. Detection of AKT expression in formalin-fixed, paraffin-embedded and sectioned human prostate cancer sections utilizing monoclonal antibody produced using the WHEATON CELLine (A), suspension (B) or ascites (C) methods. Notice similar staining pattern and intensity between all three methods.

Economic Analysis

Ascites (5 Mice)		Current 2 Liter Suspension Production Method		CELLine Bioreactor Flask	
Animals, Bedding and Food	$86.40	Consumables	$11.51	Flask	$212.00
Bioreactor Flasks (T75 / T150)	$2.56	Serum Free Media	$109.56	Serum Free Media	$273.90
DMEM	$1.17	Low IgG FBS	$78.00	Low IgG FBS	$195.00
Fetal Bovine Serum	$17.50	Flask (T75 / T150)	$6.83	Bioreactor Flasks (T75 / T150)	$6.83
Labor for Cleaning	$40.00	DMEM:	$1.17	DMEM	$1.17
Labor for Injections	$20.00	Fetal Bovine Serum:	$17.50	Fetal Bovine Serum	$17.50
Labor for Tapping	$30.00	Labor for Culturing	$30.00	Labor for Culturing	$70.00
Labor for Processing	$30.00	Labor for Counting	$10.00	Labor for Counting	$20.00
Labor for Purification	$60.00	Concentration Filter	$375.00	Labor for pH Adjustment	$10.00
0.22um Filter	n/a	0.22um Filter	$11.18	0.22um Filter	$5.78
Labor for Concentration	n/a	Labor for Concentration	$40.00	Labor for Concentration	n/a
Labor for Purification	n/a	Labor for Purification	$60.00	Labor for Purification	$60.00
Total Cost	**$287.63**	**Total Cost**	**$750.75**	**Total Cost**	**$872.18**
Total Hours	**9**	**Total Hours**	**7**	**Total Hours**	**8**
Anti-6x Histidine		**Anti-6x Histidine**		**Anti-6x Histidine**	
Yield (mg)	60	Yield (mg)	43	Yield (mg)	68
Cost per Milligram	$4.79	Cost per Milligram	$17.46	Cost per Milligram	$12.83
Anti-AKT3 Isoform		**Anti-AKT3 Isoform**		**Anti-AKT3 Isoform**	
Yield (mg)	38	Yield (mg)	20	Yield (mg)	28.9
Cost per Milligram	$7.57	Cost per Milligram	$37.54	Cost per Milligram	$30.18

Conclusion

Monoclonal anti-6X histidine epitope tag and anti-AKT3 isoform were affinity purified by protein A. SDS-PAGE analysis showed that all antibody samples had a purity higher than 95% (Figure 2). Production of monoclonal antibodies in ascites often leads to contamination by endogenous host proteins, as indicated in Figure 2, lane E. Western Blot analysis demonstrated no difference in antibody performance between the three production methods. The CELLine anti-histidine epitope tag could recognize specifically purified recombinant histidine tagged protein and cells lysates that contain expressed recombinant histidine tagged proteins (Figure 3, lane A and B). The CELLine anti-AKT3 isoform antibodies specifically recognized AKT3 isoform 3 (Figure 4, lane C). Direct ELISA titration corroborated the Western Blot results (Figure 5A, 5B and 5C). Immunohistochemistry using the three antibodies on prostate cancer slides indicated a similar result (Figure 6). Finally, an economic analysis comparing the three methods concluded that a significant yield increase and cost savings per mg is observed with the CELLine Bioreactor Flask method vs. the other two methods.

Advantages

- No contamination of endogenous protein from host
- No animal handling or activities associated with animal use
- High yield of proteins/antibodies
- Recombinant proteins and antibodies produced in CELLine Bioreactor Flask retain their biological properties
- Lower cost per milligram and greater yield compared to alternative in vitro methods

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Materials and Methods

Western Blot

Samples were separated through SDS-PAGE 4-20% under denatured conditions and transferred onto 0.2 micron nitrocellulose membrane followed by incubation with blocking buffer (Rockland, p/n MB-070) at RT for 1h. The membrane was incubated with primary antibody (anti-his tag antibody or by anti-AKT3 isoform antibody) at a 1:5000 dilution in blocking buffer (Rockland, p/n MB-070) overnight at 4°C. After several washing steps, the membrane was incubated with rabbit secondary anti-mouse – HRP at a 1:20000 dilution in blocking buffer (MB-070), for 1h at RT. Binding was detected with Femtomax™ HRP substrate (Rockland, p/n FemtoMax™-110), with exposure for 15s.

Direct ELISA

Recombinant proteins or cell lysates were coated for 1h at 37°C in 1X PBS, titrated 1:2 starting from 150ng / well to 1.2ng / well final concentration followed by blocking at RT in 1% fish gel for 1h. Antibodies, anti-his tag and anti-AKT3 isoform (100 ng / well each), were applied for 1h at 37°C in 1% fish gel. Binding was detected with rabbit secondary antibody anti-mouse coupled with HRP at a 1:10000 dilution in stabilizer solution with TMB for 30 mins at RT in the dark. The reaction was stopped with 1N HCl and absorbance was measured at 450nm.

Immunohistochemistry

All reagents are from Rockland Immunochemicals (Gilbertsville, PA) unless otherwise noted and all incubations were at room temperature. Formalin-fixed, paraffin embedded, 4µm section human prostate cancer histology slides were purchased from US Biomax, Inc. Sections were blocked with Immunohistochemistry Blocking Buffer (MB-071-0100) for 5 mins then incubated with 1:100 anti-AKT antibody variants for 1h. Detection of AKT followed the standard protocol of the IHC MaxTag Histo Kit (KHA002). Specifically, sections were incubated in the presence of biotinylated anti-mouse antibody, followed by streptavidin peroxidase and developed with DAB. All sections were briefly counterstained with hematoxylin. Immunohistological images were taken at room temperature using a microscope with x20 objective. Tiff images were imported into Adobe® Photoshop® software for composite production.

WHEATON Company Profile

WHEATON® is dedicated to providing quality products and services for the laboratory research, diagnostic packaging, and specialty pharmaceutical industries. For over one hundred years, WHEATON® products have been present when the greatest discoveries and advances in science were accomplished. Whether it is scientific research or commercial packaging, the WHEATON® brand represents quality, reliability and trust. WHEATON® is proud to be there when the scientists and packagers of the world say, "Because it's my life's work ... I trust WHEATON™."

Rockland Immunochemicals Company Profile

Since 1962, Rockland has remained a global leader in development of products for biotechnical research. In addition to our antibody lines, we also produce a variety of blood products and cell cultures. Our reputation is based on attention to detail, customer service and continuous investment in the tools and technologies required to develop a world-class product line. We understand and appreciate how our products are helping solve significant global problems in life science research. We take great care to validate our products before they reach any research laboratory. Rockland products are widely used for research of cancer, neurology, cell biology and immunology.

We pride ourselves on working with leading researchers to identify bioreagent needs. From the creation of customized antibodies to the formation of novel in vitro assay systems, we offer both scientific skills and cGMP compliant manufacturing facilities required to support an international market demand.

Authors

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