Scientific Brief

A New, Innovative Process for Clarifying and Sterile Filtering Cells for Protein Purification Workflows

Introduction

Centrifugation and filtration have been widely accepted as techniques required for clarifying complex cell cultures to recover extracellular proteins such as monoclonal antibodies (mAbs). However, these steps can be time consuming and costly for labs growing their cultures in 24-well plates. This scientific brief offers an alternative to the use of centrifugation/filtration/flocculation to clarify and sterilize mammalian cell cultures. It describes an assessment of a new 24-well clarification and sterile filtration plate and a 24-well sterilization-only filter plate for the recovery of proteins present in the supernatant.

The Problem

In recent years, biopharmaceutical research processes have demonstrated major improvements in the quality and recovery of mAbs, which to an extent have been associated with culturing the expressing cell lines at high cell densities. This work has generated a great challenge in cell clarification, sterilization, and further downstream processing. These processes must remove large amounts of biomass and increased levels of contaminating cell debris generated during cell culture and harvesting.[1]

Traditionally, centrifugation and a combination of filtration methods have been widely accepted as techniques required for clarifying complex suspension cell cultures. Following cell culture, laboratory users manually move their samples to a centrifuge for clarification. The centrifugation process typically requires about 20 minutes. After centrifugation, the user has to recover the clarified supernatant from each sample and filter the protein product of interest through use of a sterile 0.2 µm syringe filter. In addition, because centrifugation will not always fully clarify a sample, some laboratories will process the samples through a 0.45 µm syringe filter first to ensure the 0.2 µm sterilizing-grade membrane does not clog.

In all, this time consuming and tedious manual workflow often requires more than 1 hour to process a single 24-well plate. In addition, it adds significant variability to the process. Each additional step in the clarification and sterilization process costs time and leads to increased sample loss due to adding hold-up volumes and reductions in protein recovery. Additionally, every additional step increases the potential for mistakes, lost samples, and process error.

Regardless of the cell line development path taken, the use of 24-well culture plates for growth is often a pivotal part of the process. Attempts are now being made to reduce costs, processing times, and errors. This is achieved by continuous optimization of the cell clarification and sterilization steps to increase the yield of antibodies and proteins per volume of culture.

New Technology Combines Cell Clarification and Sterilization in One Step

Cell growth in a 24-well plate presents challenges for fast and efficient clarification and sterilization of the proteins of interest. To help streamline protein purification workflows, new technology has been developed that employs a 24-well, multi-layer filter plate combining the cell clarification and sterile filtration functions.

The plate incorporates a top layer with a depth filter that efficiently clarifies the culture through capture of whole cells and removal of large cellular debris. A lower layer consisting of a dual 0.65/0.2 µm Supor® EKV membrane provides high-performance sterile filtration. With either a vacuum manifold or a centrifuge, high-density cell cultures (such as CHO or HEK) can be quickly processed resulting in the capture of cells, cell debris, and other aggregates in the filter media. This filter combination effortlessly recovers proteins from whole cell cultures of up to 25M+ cells/mL. The filter plate consolidates two or more separate processes (clarification and sterilization) into one workflow step that can be completed in less than 20 minutes. Subsequently, the samples can be moved downstream for analysis or purification. As an additional benefit, the cell clarification and sterilization filtration plates use seven times less plastic consumables by weight than traditional forms of recovery -- significantly reducing disposal costs and environmental burden.

24-Well Filter Plate Designed for Workflows Requiring Only Sterile Filtration

A companion 24-well filter plate is now available for use in general sterile filtration workflows where only sterile filtrate is required. This filter plate is well suited for high-volume (up to 7 mL) plate-based sterile filtration needs such as media, reagent, serum, or proteins. This plate features an upstream 0.65 µm membrane integrated with a downstream highly asymmetric 0.2 µm membrane for fast and efficient sterile-grade filtration.

Further Information and Applications

Both of the previously discussed 24-well filter plates are gamma-irradiated, automation friendly, and compatible with centrifugation and vacuum manifold workflows. For laboratories that do not have a centrifuge adapter, a vacuum manifold can be substituted as a lower-cost option. Both plates come individually bagged and include a collection plate and lid.

These innovative filter plate technologies can be applied to a variety of cell culture and protein purification workflows, including clone selection and candidate analysis, cell expansion studies, recombinant protein isolation prior to analysis, cell clarification, process optimization, and sterile filtration.

Results and Discussion

Testing was conducted to determine the performance of the 24-well clarification and sterilization filter plate and the 24-well sterile filtration plate.

The data in Table 1 are the average of the data collected with the high-density CHO cell cultures.

Table 1

Parameters recorded for the CHO cell culture with a concentration of 2.6 x 10^7 CHO cells/mL and processing with the 24-well clarification and sterilization filter plate.

5 mL Concentrated CHO cell Culture at 26 Million Cells/mL Upstream	Upstream Culture	Downstream Filtrate (Vacuum: 15 inHg)	Downstream Filtrate (Centrifugation: 1,000 x g)
Processing Time	–	20.2 ± 6.3 min	15 min
Hold-up Volume (trapped in filter)	–	300 – 450 µL	400 – 450 µL
pH	7.2	7.3	6.8
Conductivity (µS/cm)	≈ 10,100	≈ 9,200	≈ 9,800
Turbidity (NTU)	≈ 1,900 - 2,600	≈ 1.8	≈ 2.4
Optical Density at 600 nm	≈ 18 - 19	0	0
Total Protein Recovery (%)	–	98.3 ± 8.2	95.4 ± 11.4
IgG Recovery (%)	–	91.3 ± 11	85.0 ± 6.9

Table 2 shows a summary of the data collected with the HEK293T cell cultures and the supernatant.

Table 2

Parameters recorded for the HEK293T cell cultures at 2 – 4 x 10^6 cells/mL and processing with the 24-well clarification and sterilization filter plate and the 0.2 µm sterile filtration plate, respectively.

Step	Initial Culture	Clarification	Filtration Supernatant (0.2 µm filter)	
Plate	–	24-well Depth + EKV	24-well EKV	
Material (HEK 293T cell culture/supernatant)	HEK293T at 2 – 4 million cells/mL (Upstream)	HEK293T cell culture at 2 million cells/mL (Upstream)	Supernatant of HEK293T cell culture at 4 million cells/mL (Upstream)	
Sample	Upstream culture	Downstream filtrate (Vacuum: 7 mL/15 inHg)	Downstream filtrate (Vacuum: 7 mL/15 inHg)	Downstream filtrate (Centrifugation: 6 mL, 1,000 x g/5 min)
Processing Time	–	4.3 ± 0.4 min	2.7 ± 0.7 min	5.0 min
pH	7.1 – 7.6	7.2		
Conductivity (µS/cm)	≈ 10, 836	≈ 10,906		
Turbidity (NTU)	≈ 81 – 226	≈ 0.91		
Optical Density at 600 nm	≈ 1.3 – 2.0	0.001		
Total Protein Recovery (%) (Max of 5.4 mg total protein)	–	101.2 ± 0.4	101.2 ± 1.0	99.4 ± 1.0

The data shows that the 24-well filter plates behaved similarly when used under vacuum or centrifugation, with respect to parameters such as media pH, conductivity, optical density, and protein recovery. The pH and conductivity of the samples were in a similar range before and after filtration through the 24-well plates for both the CHO cell culture (Table 1) and the HEK293T culture (Table 2), indicating that none or negligible amounts of filtering media material were released downstream to the filtered samples. The removal of cells can also influence the pH and conductivity, but the effect was not observed here.

The turbidity and optical density showed that after filtration of the mammalian cell cultures or supernatant of the HEK293T culture, the filtrates obtained with both 24-well filter plates contained clarified media with minimal breakthrough.

For the protein recovery, it was observed that > 95% of the overall total proteins from the CHO or HEK293T cell cultures were recovered when 24-well plates were used.

The IgG recovery was lower than for the total proteins when the CHO cell cultures were used. The most likely cause for this is differences in the methods of detection used, or potentially from damage to the CHO cells during the initial concentration of the cell culture.

Conclusion

This study's objective was to assess the efficiency of the 24-well, multi-layer clarification and sterile filtration plate and the 24-well sterile filtration plate when used with mammalian cell suspensions. High-density CHO cell suspension, artificially concentrated, and HEK293T cell cultures were used with the 24-well filter plates processed by vacuum or centrifugation.

The data indicates that both processes performed equivalently. The suitability of the 24-well plate for the clarification of mammalian cell cultures, in particular for high-density CHO cells (up to 2.6 x 10^7 cells/mL) and HEK293T cells (up to 4 x 10^6 cells/mL), was demonstrated. Total protein recovery (from ≈ 5 to 10 mg initial total proteins) was determined to be greater than 95% with the 24-well plates regardless of the plate type used.

These tests demonstrated that the new filter plates achieved high recovery rates and low hold-up volumes. Reductions in handling and filtration steps reduces the risk of protein loss and improves workflow efficiency. The proteins were filtered from cell cultures in less time and with fewer workflow steps than traditional protein purification workflows.

Our study indicated that the performance of these new 24-well filter plates is commensurate with the traditional cell clarification and sterilization technologies available on the market and can now be completed in less time and with less steps.

References

[1] Identification and tracking of problematic host cell proteins removed by a synthetic, highly functionalized nonwoven media in downstream bioprocessing of monoclonal antibodies. 2019. Journal of Chromatography A, 1595, pp 28-38.

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