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Rapid Mammalian Cell Harvest without Centrifugation for Antibody Purification Using the Sartoclear Dynamics® Lab Filtration System

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Abstract

Monoclonal antibody expression systems typically utilise a signal peptide to ensure secretion of the antibody into the cell culture media. Although this reduces the complexity of purification and avoids the need for cell disruption, it does require the use of expensive and/or time-consuming techniques to separate cells from antibody-containing cell culture fluid. In this study, we describe our tests of Sartoclear Dynamics® Lab V, a novel system for rapid clarification of cell culture media without the need for centrifugation or any other costly equipment.

Introduction

Monoclonal antibodies are used in a wide range of applications including biopharmaceutical development, basic research and in vitro diagnostics. The production of antibodies from mammalian cells, whether it be standard production using hybridomas or recombinant production in Chinese Hamster Ovary (CHO) or Human Embryonic Kidney (HEK) cell lines, requires a process to separate the cells from the antibody-containing cell culture fluid. At bioreactor scale, typically above 50 litres, this is usually done by processes such as continuous centrifugation and depth filtration.[1,2]

However, at research scale, which is typically less than 10 litres of cell culture per antibody, these processes become both impractical and expensive. The most widely cited method for research scale clarification is an initial centrifugation step followed by filtration of the cleared supernatant through a 0.2, 0.22 or 0.45 µm filter, either using a hand-operated syringe or a vacuum driven bottle-top filter. These are inexpensive and easy to use with standard equipment present in most biotechnology laboratories. However, due to the shallow design of these filters, they are prone to clogging due to the presence of submicron particles remaining in suspension.[2]

At Absolute Antibody, we transiently express recombinant antibodies in HEK293 and CHO-K1 cells, typically working with approximately 50 antibodies per week from a scale of 30 mL up to 20 litres with a total weekly capacity of approximately 100 litres of cells. For the last six years, we have been using centrifugation followed by bottle-top filtration for all our antibodies. As our capacity has grown, so has the need for an increased number of centrifuges. Over this time, we have looked at a number of alternative options, including the use of filters designed for home brewing and flocculants such as Chitosan.[3] These approaches proved to be slow, prone to clogging and low throughput and to be of low quality (e.g. endotoxin contaminated).

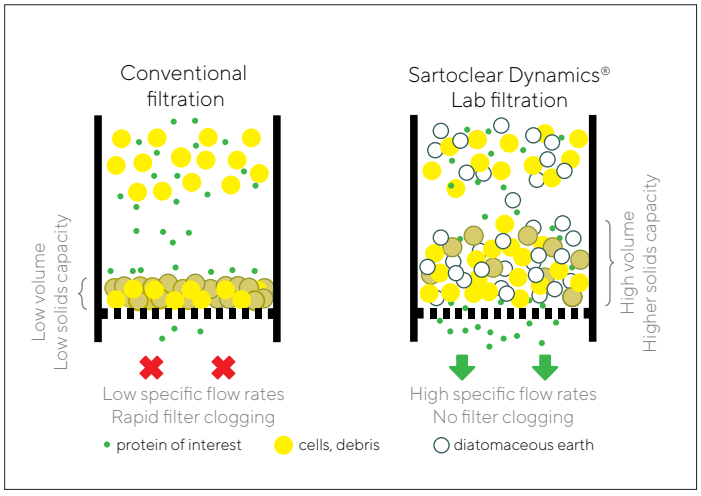

Figure 1: Principles of clarifying cell cultures using conventional filtration and Sartoclear Dynamics® Lab filtration.

The Sartoclear Dynamics® Lab V filtration system was designed to reduce the time and effort involved in clarifying mammalian cell cultures. The addition of diatomaceous earth (DE) to cultures supports the formation of a porous filter cake to prevent blockage of the filter, allowing rapid removal of cells and cell debris from the sample (Figure 1). This avoids the need for a centrifugation step, circumventing issues around centrifuge capacity and availability as well as preventing filters from clogging. We tested the Sartoclear Dynamics® Lab V filtration system and compared it with our standard process.

Materials and Methods

Suspension adapted HEK293 or CHO cells are grown in serum-free media and transfected with DNA plasmids encoding the heavy chain and light chain of a monoclonal antibody. The cells are harvested for purification 6 to 14 days post-transfection. For a typical expression batch of 1 litre, our original clarification process would involve centrifugation at 3,500 g in a bench top centrifuge (2 × 500 mL) for 45 minutes followed by filtration using one or more vacuum-driven PES 0.45 µm bottle-top filters. Filtered supernatant is then loaded onto an ÄKTA purifier with a 5 mL Protein A column for antibody capture and elution on low pH buffer. Antibody is then neutralised and proceeds either to additional purification steps (e.g. cation exchange or size exclusion chromatography) or directly to quality control depending on the requirements for the particular antibody batch. Quality control is performed by SDS-PAGE under non-reducing and reducing conditions, SEC-HPLC, endotoxin testing and, where required, an ELISA to measure binding activity (Figure 2).

In the modified downstream process, the centrifugation and filtration steps are replaced with the use of Sartoclear Dynamics® Lab V. In the case of a 1-litre culture, 20 g of DE filter aid were added to 1 litre of cells. The cells and DE are mixed vigorously and then added directly to a 1,000 mL PES 0.22 µm Sartolab® RF vacuum-driven filter included in the Sartoclear Dynamics® Lab kit. A vacuum is applied and the clarified cell culture fluid collected in the 1 litre bottle. The filtrate is then taken through the purification and QC process as described above.

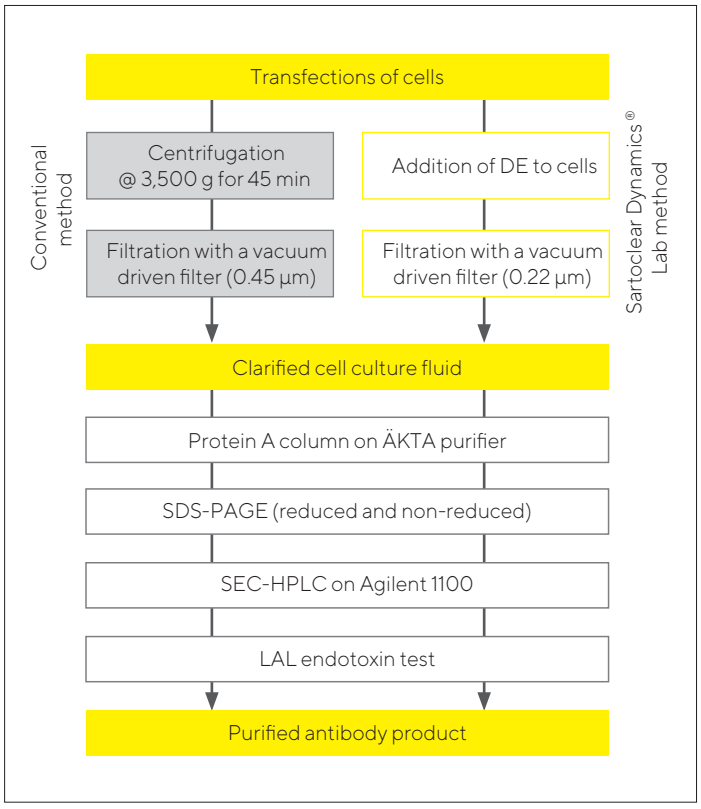

Figure 2: Downstream processing workflow of recombinant antibody production at Absolute Antibody. Grey boxes show the original process of clarification using centrifugation and filtration. The boxes with yellow outlines show the new work flow using Sartoclear Dynamics® Lab V.

Results and Discussion

To benchmark the Sartoclear Dynamics® Lab V against our standard process, a two-litre culture of a human IgG1 anti-EGFR antibody (Cetuximab; Absolute Antibody catalogue number Ab00279-10.0) was prepared in HEK293 cells. Six days post-transfection the culture was split into two equal volumes. At the point of harvesting, the cell density was 2.4×10^6 cells/mL with a viability of 65%.

The first litre was taken through our conventional process. This involved a 45-minute centrifugation step followed by filtration using three 0.45 µm PES 500 mL bottle-top filters. These filters typically block after about 400 mL of supernatant has been filtered, meaning on average three filters are required per litre of cell culture. Filters with a pore size of 0.45 µm are used rather than 0.22 µm to increase the volume of supernatant that can be filtered prior to blockage. Filtering of 1 litre of cells took 9 minutes, 48 seconds, of hands-on time, which added up to an overallprocess time of approximately 55 minutes.

The second litre was taken through the Sartoclear Dynamics® Lab V process. Two 10-gram pouches of DE were added to the cells, followed by vigorous mixing. The cells were then poured into a 1,000 mL 0.22 µm Sartolab® RF filter and a vacuum was applied. The filtration ran to completion with no blockage and took a total of 8 minutes, 27 seconds, from applying the DE to completion of filtering. This represents approximately 15% of the time taken by our conventional approach, as illustrated in Figure 3.

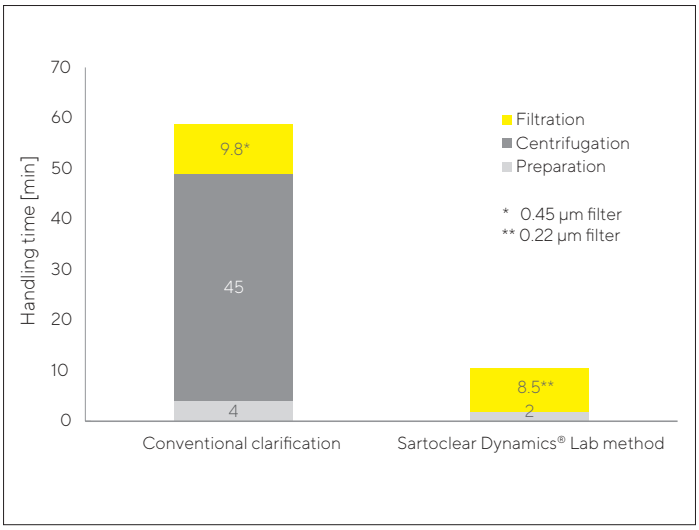

Figure 3: Comparison of clarification methods by handling time. Each 1 litre of cell culture with a density of 2.4×10^6 cells/mL was clarified with the conventional method and with the Sartoclear Dynamics® Lab method. Sartoclear Dynamics® Lab eliminates the need for a centrifugation step and substantially reduces the time required for clarification of mammalian cell cultures.

To confirm that the Sartoclear Dynamics® Lab V process had no effect on the quality of the antibody, the two samples proceeded through purification and quality control separately. The final purified antibodies showed no detectable differences in product quality as determined by SDS-PAGE (Figure 4) or SEC-HPLC (Figure 5). An endotoxin measurement was taken for each sample, with both giving a reading of < 0.05 EU/mg, which is the lower limit of detection of the testing kit we routinely use.

To confirm that the modified process had no effect on the function of the antibody, an indirect ELISA was performed to show binding to human EGFR-Fc (Absolute Antibody catalogue number Pr00117-10.9). As shown in Figure 6, the method of cell clarification had no impact on binding activity. Additionally, the final yields obtained by both processes were almost identical, showing that DE has no impact on both quality and quantity of antibody.

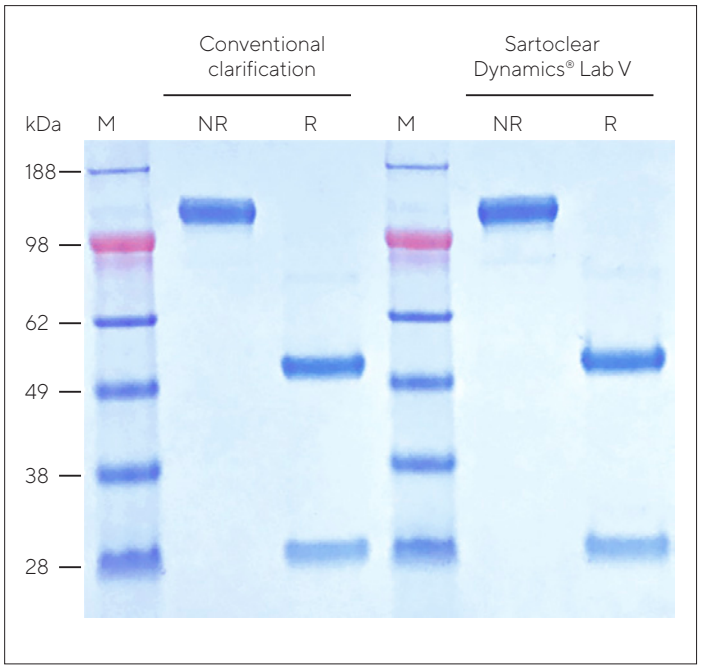

Figure 4: SDS-PAGE gel image (NR – non-reducing, R – reducing, M – markers) of Protein A purified anti-EGFR antibody (Cetuximab; Absolute Antibody catalogue number Ab00279-10.0) following conventional clarification by centrifugation and filtering and clarification using Sartoclear Dynamics® Lab V.

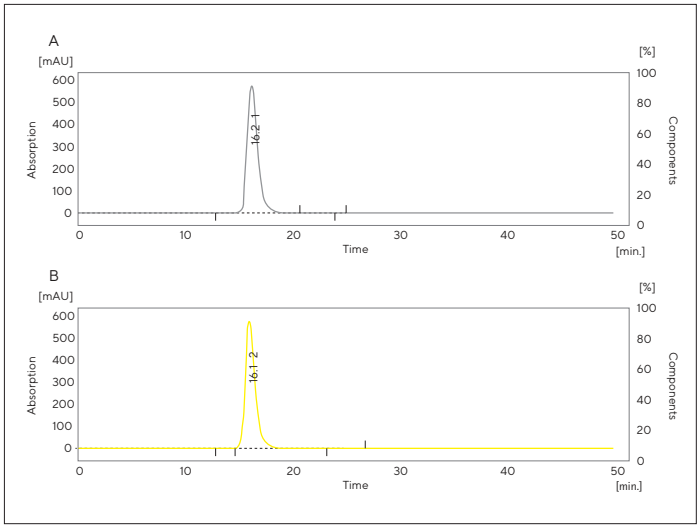

Figure 5: SEC-HPLC of Protein A purified anti-EGFR antibody (Cetuximab; Absolute Antibody catalogue number Ab00279-10.0) following conventional clarification by centrifugation and filtering (A) and following clarification using Sartoclear Dynamics® Lab V (B). Both samples show identical profiles.

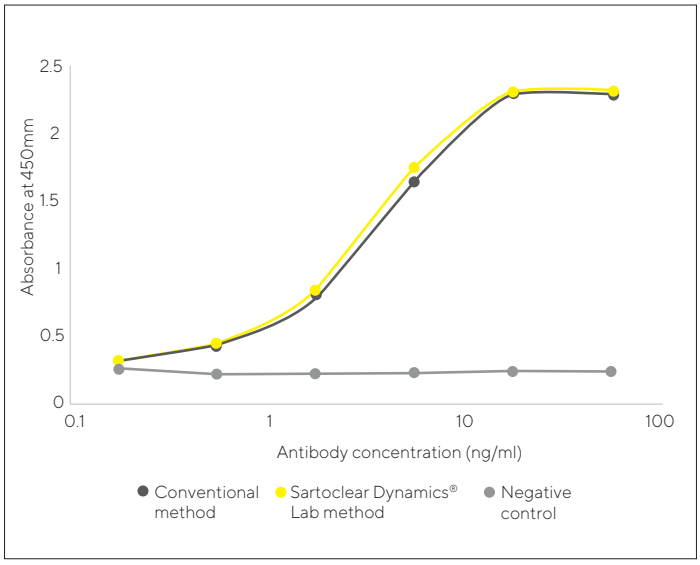

Figure 6: Indirect ELISA showing binding of antibodies to EGFR-Fc. Anti-EGFR antibodies (Cetuximab) were purified by our conventional process and utilising Sartoclear Dynamics® Lab V. There is essentially no difference in binding activity.

Conclusion

We have clearly demonstrated here that Sartoclear Dynamics® Lab V enables rapid clarification of mammalian cell cultures without the need for centrifugation. We have done this by taking two litres of cell culture transiently transfected with an antibody and comparing a standard centrifugation-based clarification process with the diatomaceous earth-based Sartoclear Dynamics® Lab V process. We were unable to detect any meaningful differences in the final product quantity or quality as determined by a host of measurements (SDS-PAGE, SEC-HPLC, ELISA and endotoxin), demonstrating that the diatomaceous earth-based system has no impact on product quality. Importantly, the Sartoclear Dynamics® Lab V process gave an impressive time saving of approximately 85%. This makes Sartoclear Dynamics® Lab V an attractive option to increase productivity and throughput for the clarification step of secreted protein expression and purification systems.

References

1 Kempken, R., Preissmann, A., and Berthold, W. (1995). Clarification of animal cell cultures on a large scale by continuous centrifugation. J. Ind. Microbiol. 14, 52–57.

2 Liu, H.F., Ma, J., Winter, C., and Bayer, R. (2010). Recovery and purification process development for monoclonal antibody production. MAbs 2, 480–499.

3 Riske, F., Schroeder, J., Belliveau, J., Kang, X., Kutzko, J., and Menon, M.K. (2007). The use of chitosan as a flocculant in mammalian cell culture dramatically improves clarification throughput without adversely impacting monoclonal antibody recovery. J. Biotechnol. 128, 813–823.

Abbreviations

DE	Diatomaceous Earth
PES	Polyethersulfone
SDS-PAGE	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
SEC-HPLC	Size Exclusion Chromatography-High Performance Liquid Chromatography
ELISA	Enzyme-linked Immunosorbent Assay
EGFR	Epidermal Growth Factor Receptor

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