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Application Note

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Centrifuge-Free Clarification of Antibodies from Cell Cultures Using Sartoclear Dynamics® Lab Decimates Working Time: A Comparative Study

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Abstract

This study focuses on the clarification of 50 mL suspension adapted HEK-293-6E cells, transiently expressing human IgG4 by comparing a conventional harvest method and the new Sartoclear Dynamics® Lab method. While the conventional method comprises a centrifugation step and a filtration step by using Steriflip®, the Sartoclear Dynamics® Lab method using diatomaceous earth as a filter aid in combination with either a 150 mL or a 250 mL bottle-top filter.

The time to clarify and sterile filter 50 mL of mammalian cell culture is reduced 16-fold using the new Sartoclear Dynamics® Lab method compared with conventional clarification methods. In addition, the IgG4 yield is 10% greater using the new Sartoclear Dynamics® Lab method compared with conventional clarification methods. The purity of the purified IgG4 antibody is comparable between the Sartoclear Dynamics® Lab method and conventional clarification methods as observed on an SDS-PAGE gel.

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Introduction

Monoclonal antibodies and secreted proteins are typically harvested by centrifugation and downstream filtration in a time-consuming, multi-step procedure[1]. At BioServUK Ltd, the Standard Operating Procedure for harvesting, clarifying and filtering of 50 mL mammalian cell cultures (HEK-293-6E) takes on average 30 minutes.

Sartoclear Dynamics® Lab products utilized for clarification of cell culture broth are based on principles of dynamic body feed filtration: The filter aid diatomaceous earth (DE) is added to cell culture broth to sieve out cells, cell debris, and sub-micron particles, creating a permeable filter cake which prevents blockage of the dead-end filter[2]. Sartoclear Dynamics® Lab eliminates the need for the individual steps of centrifugation and filtration even when large volumes of mammalian cell culture are processed, thus saving significant time and resources over existing methods.

Diatomaceous earth is a naturally occurring sedimentary rock with a typical particle size in a range of 1 – 100 µm. The porous and inert nature of the material have seen it also become an integral feature in the biotechnology industry for clarifying large volumes (600 – 2,000 L) of high density mammalian cell cultures[1,3].

In this comparative study, we describe clarification and filtration of 50 mL HEK cell cultures, transiently expressing human IgG4 using conventional methods, and the new Sartoclear Dynamic® Lab method at BioServUK Ltd (Figure 1).

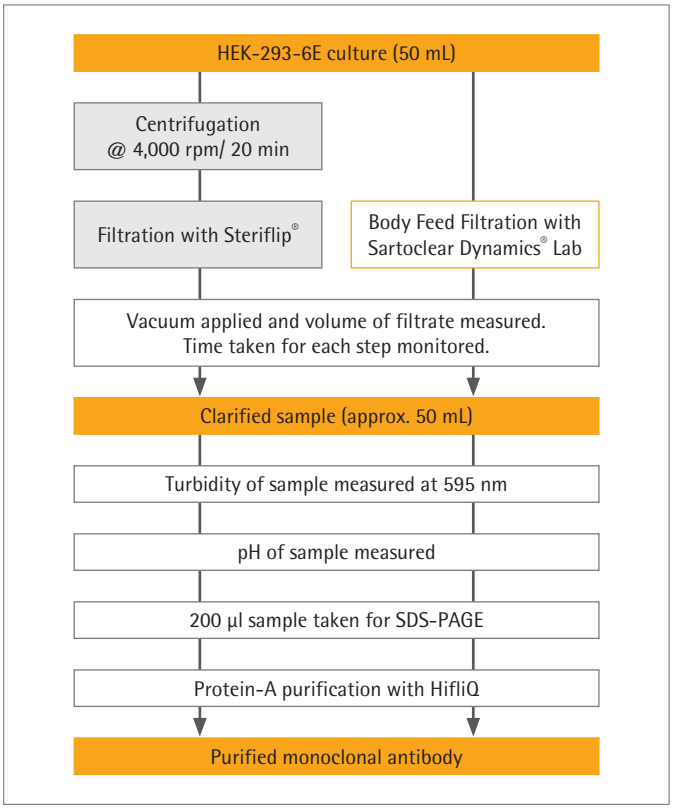

Figure 1: Downstream processing workflows of the conventional and Sartoclear Dynamics® Lab methods extending from clarification of cell culture to purification at BioServUK Ltd. Dark orange boxes: sample status in the process. Grey boxes: conventional process steps. Box with yellow outline: Sartoclear Dynamics® Lab method. White boxes: steps in the process flow in which measurements for the study were performed.

Materials and Methods

Fifty-millilitre shake flask cultures of HEK-293-6E cells grown in F17 media were co-transfected with human IgG4 heavy and light chain expression vectors. Based on previous experiments, transient IgG4 expression yields were expected to be approx. 50 mg/L. Cells were harvested six days post-transfection. At the time of harvest, cell viability was over 90%, with a typical cell density of approx. 6×10^6 cells/mL. Cell density was measured using a Neubauer haemacytometer and cell viability determined using a live dead staining with Trypan Blue.

For all 50 mL cell cultures clarified by the Sartoclear Dynamics® Lab method, 2 g DE were swirled gently by hand until all DE dispersed (approx. 1 min.), and the solution was transferred to either a 150 mL or 250 mL 0.22 µm bottle-top filter unit. For all 50 mL cell cultures clarified by the conventional method, these were centrifuged at 4,000 rpm for 20 minutes and filtered using a Steriflip® device after centrifugation. As a control regarding yield and antibody purity, 50 mL transfected cell cultures were clarified by centrifugation alone.

The time taken to handle, centrifuge and filter each sample was determined and used to compare the different clarification methods.

The turbidity of the unclarified and clarified samples was measured at an absorbance of 595 nm using a UV/Vis spectrophotometer. The pH of the unclarified and clarified samples was determined using a calibrated Jenway 3802 pH probe.

A sample from each purified sample was analysed by reducing and non-reducing SDS-PAGE on a 10–20% Tris glycine gel with Tricolour molecular weight markers (Expedeon Ltd). Ten microlitres of each purified sample were loaded on to the gel after denaturing for 10 minutes at 85°C.

Fifty millilitres of clarified cell cultures were purified by batch binding to Protein A resin (Protein Ark cat. no.: PA-A1) for 1 hour at ambient temperature (binding buffer: 1.5 M Glycine/NaOH buffer, 3 M NaCl, pH 9.0) and eluted using Proteus mini spin columns (Protein Ark cat. no.: MSF500, Elution Buffer: 0.2 M Glycine/HCl buffer pH 2.5).

Results and Discussion

The addition of DE to 50 mL cell cultures is rapid and technically simple, with 2 g DE dispersing within 1 minute. Processing time for a 50 mL cell culture is reduced by 16-fold using dynamic body feed filtration compared with Steriflip® filtration after centrifugation (Figure 2). The slight difference in filtration time between the Sartoclear Dynamic® Lab bottle-top filter of 150 mL (2 minutes) and that of 250 mL (1 minute) could be caused by the different filtration areas of the bottle-top filters (18 cm^2 for 150 mL bottle-top filter and 24 cm^2 for the 250 mL bottle-top filter).

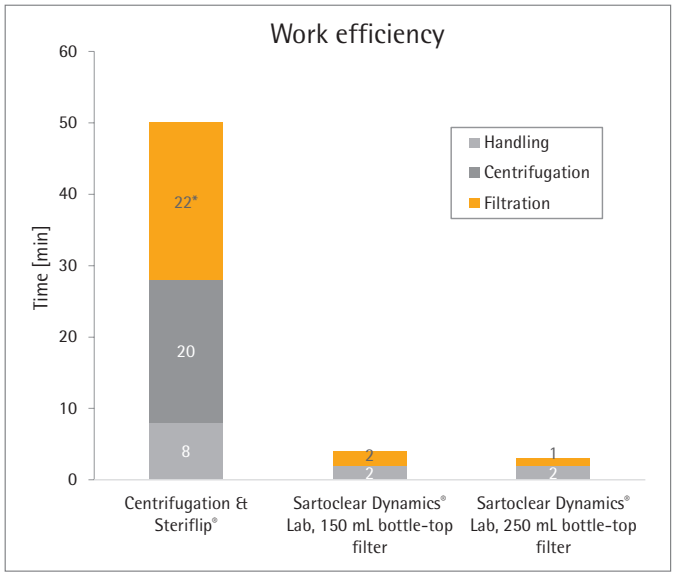

Figure 2: The handling, centrifugation and filtration time taken to clarify a 50 mL mammalian cell culture using the conventional method centrifugation combined with Steriflip® filtration and using the new Sartoclear Dynamic® Lab method. * Steriflip® filter clogged at a filtrate volume of 42 mL.

Untransfected HEK-293-6E cells had a turbidity (absorbance at 595 nm) of 1.83 and a pH of 7.5, and transfected HEK-293-6E cells before clarification had a turbidity of 1.71 and a pH of 7.6. After clarification by the conventional method and the new Sartoclear Dynamic® Lab method, all samples had a turbidity of 0.00 and a pH of 7.7.

Importantly, use of the new Sartoclear Dynamics® Lab body feed filtration method does not significantly affect yield (Figure 3) or antibody purity (Figure 4) compared with conventional clarification methods.

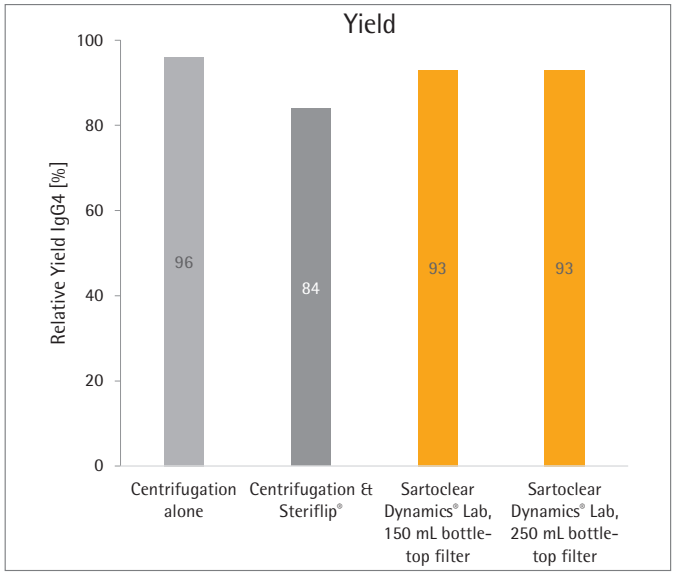

Figure 3: Relative human IgG4 recovery [%] after Protein-A purification using different clarification methods. The relative IgG4 recovery is based on an expected yield of 50 mg/L.

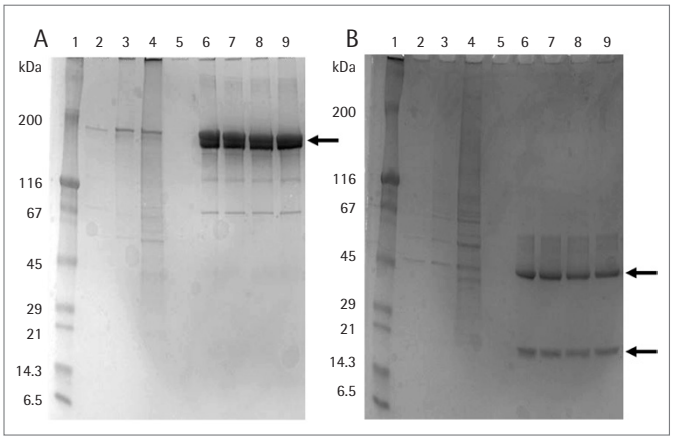

Figure 4: Non-reducing (A) and reducing (B) SDS-PAGE gel analysis of purified human IgG4 following Protein A affinity chromatography shows no effect on antibody purity by both clarification methods. Human IgG4 (approx. 150 kDa) and the heavy (approx. 45 kDa) and light (approx. 20 kDa) chains are highlighted with a black arrow. Samples: (1) prestained markers; (2) feedstock elution; (3) centrifugation alone: before purification; (3) centrifugation alone: wash step; (4) centrifugation alone: unbound material; (5) centrifugation alone: wash step; (6) centrifugation alone: elution (7) centrifugation + Steriflip®; (8) Sartoclear Dynamics® Lab, 150 mL bottle-top filter; (9) Sartoclear Dynamics® Lab, 250 mL bottle-top filter.

Conclusion

We conclude that the dynamic body feed filtration method utilized by Sartoclear Dynamics® Lab products is a unique clarification method, which replaces the need for expensive floor-standing centrifuges and is an indispensable tool for clarifying high cell density mammalian cultures without compromising protein yield or purity. This method saves significant time when performed at the 50 mL laboratory scale and is directly applicable to industrial-scale processing and manufacture.

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Abbreviations

DE	Diatomaceous earth
HEK	Human embryonic kidney
Tris	Tris(hydroxymethyl)aminomethane
SDS-PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis

Disclaimer

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