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Application Note

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Lab-Scale Clarification of Mammalian Suspension Cultures Using Sartoclear Dynamics® Lab V Kits

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Abstract

This study evaluates a novel method for lab-scale clarification of mammalian cell culture supernatants. The filtration step is conducted prior to purification of monoclonal antibodies with protein A affinity chromatography. In a conventional clarification process, cells are removed by a time-consuming centrifugation step followed by filtration. We show that the Sartoclear Dynamics® Lab V Kit is not only a significant time-saving method compared with the conventional clarification process. It also yields an improved clearance rate that we observed in combination with high flow rate filtration. For clarification of large-volume cell cultures, Sartoclear Dynamics® Lab V Kits are an attractive addition to standard lab instrumentation to increase productivity and throughput prior to protein purification.

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Introduction

With the Sartoclear Dynamics® Lab V Kit, Sartorius offers a new lab-scale method for fast filtration of 0.45 L cell culture samples. The standard centrifugation step is eliminated by the addition of diatomaceous earth (DE) to the cell culture prior to filtration, thus circumventing related issues like centrifuge capacity and availability. Diatomaceous earth supports the formation of a porous filter cake and prevents blockage of the filter membrane by rapid removal of cells from the cell culture supernatant. In this study, we tested the Sartoclear Dynamics® Lab V Kit for harvesting of mammalian cells and recovery of the expressed recombinant antibody.

Based on our study, we established a simple protocol for straight forward clarification of suspension cell cultures expressing recombinant antibodies. Monoclonal antibodies play an ever increasing role in advanced targeted therapies [1], and fast, time-saving and high-titer production of these recombinant proteins is a challenge for the biopharmaceutical industry as well as for lab-scale production facilities in industry and academia [1–3].

At Tubulis, a spin-off project at the Ludwig-Maximilian University (LMU), monoclonal antibodies are generated for the purpose of follow-up functionalization with a cytotoxic drug (antibody drug conjugate; ADC) using a novel chemoenzymatic conjugation strategy, termed Tub-tag® (Figure 1). Tub-tag® conjugation allows for a defined drug-to-antibody ratio (DAR), highly stable conjugates, specific antigen binding and a low aggregation risk [4–5].

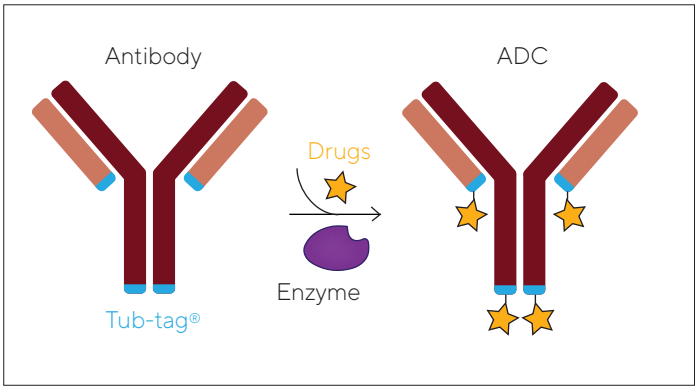

Figure 1: Tub-tag® technology for chemoenzymatic generation of highly stable antibody drug conjugates (ADCs).

Prior to purification and functionalization of monoclonal antibodies, clarification of 0.45 L cell cultures is a time-consuming procedure. Besides requiring the removal of cells by centrifugation, the standard purification process includes a filtration step of the harvested cell culture fluid to eliminate cell debris and small particles. Limited centrifuge space leads to multiple runs and doubles the time for clarifying supernatant. Slow flow rates during filtration increase the time spent on clarification. Moreover, clogging of the filter membranes results in a higher need for expensive consumables.

Materials and Methods

Freestyle™ 293-F cells (ThermoFisher Scientific, R79007) for stable expression of a monoclonal antibody were cultured in HEK GM growth and production medium (Xell, 851). For preparation of an inoculum culture, the cells were routinely cultured according to the manufacturer's instructions in shake flasks at 37°C, 5% CO_2 and 120 rpm. A BIOSTAT® A 2 L bioreactor (Sartorius Stedim Biotech) was inoculated with 0.6 L of suspension culture (0.6 x 10^6 cells per milliliter).

After 260 h, the culture was harvested and divided into four 0.45 L samples for different clarification approaches. Samples 1–3 were clarified using the Sartoclear Dynamics® Lab V Kits with different concentrations of diatomaceous earth (Table 1). The samples were homogeneously mixed with 5–20 g of DE/0.45 L and directly filtered using a Sartolab® RF Bottle Top filter. Sample 4 was clarified by centrifugation at 8,000 g for 30 minutes and subsequent filtration through a 0.22 µm PES Sartolab® Bottle Top filter (180C5).

Table 1: Experimental setup comparing different clarification methods. Samples 1-3 were clarified using Sartoclear Dynamics® Lab with DE concentrations of between 5 g and 20 g per 0.45 L cell culture broth. Sample 4 was clarified by a centrifugation step and subsequent filtration.

Sample #	1	2	3	4
Method	Sartoclear Dynamics® Lab	Sartoclear Dynamics® Lab	Sartoclear Dynamics® Lab	Centrifugation and filtration
Conditions	5 g DE / 0.45 L	10 g DE / 0.45 L	20 g DE / 0.45 L	8,000 x *g*, 30 min.; 0.22 µm PES Sartolab®

Before and after the clarification process, the turbidity was measured at OD600 with an Eppendorf BioPhotometer®. The IgG concentration in the clarified cell culture fluid was analyzed using a Vanquish Flex UHPLC system (ThermoFisher Scientific) with a MAbPac™ Protein A column (ThermoFisher Scientific, 082539). For preparative purification of the monoclonal antibody, 50 mL of the clarified cell culture fluid samples were each loaded onto a 1 mL HiTrap™ MabSelect SuRe™ pcc column (GE Healthcare Life Sciences, 17-5491-12) that was pre-equilibrated with sodium phosphate buffer. The antibody was eluted using an acidic pH and stored under neutral conditions. For detection of protein aggregates and fragments, the purified antibodies were analyzed in a Vanquish Flex UHPLC system (ThermoFisher Scientific) using a MAbPac™ SEC-1 column (ThermoFisher Scientific, 074696).

Results and Discussion

Upon harvesting, the cell density measured was 4.8 x 10^6 cells per milliliter, and the maximum viable cell density was 4.1 x 10^6 cells per milliliter (85%). The optical density of the cell culture broth was OD600 = 1.602 (Figure 3).

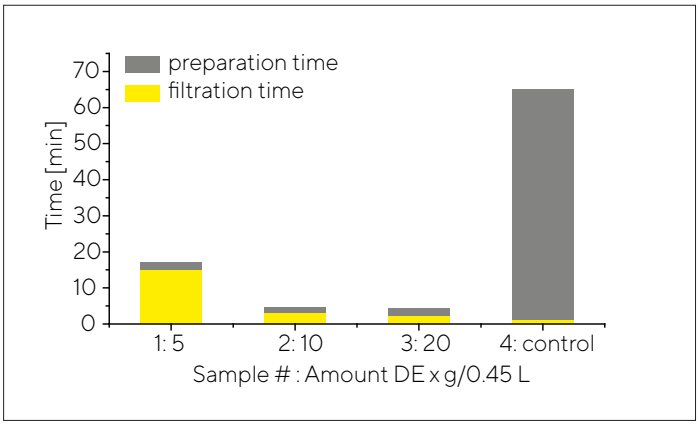

Figure 2: Preparation and filtration times of the clarification process using Sartoclear Dynamics® Lab with different quantities of DE compared with the control. For samples 1–3, preparation time includes setup of the filtration device, adding DE to flasks, mixing cell culture broth with DE and filtration time. For sample 4, filtration time includes the centrifugation step (measuring tubes, balancing tubes and centrifugation time) and the filtration step.

The time spent on the total clarification process (e.g., measuring volumes, balancing tubes, centrifugation and filtration of the control sample, adding DE to flasks, setup of filtration device and filtration time), as well as the time it took for filtration, was measured (Figure 2).

The standard centrifugation and filtration method took 65 minutes total, whereas clarification with Sartoclear Dynamics® Lab V Kits needs in total 17.7 minutes (5 g / 0.45 L), respectively 4.6 minutes (10 g / 0.45 L) and 5 minutes (20 g / 0.45 L). While filtration time with Sartoclear Dynamics® Lab V Kits was not reduced compared with that of the control sample, the absence of the centrifugation step lowered the total process time by more than 73%. The samples with 10 – 20 g/0.45 L DE resulted in a total process time of < 98%, compared with the control.

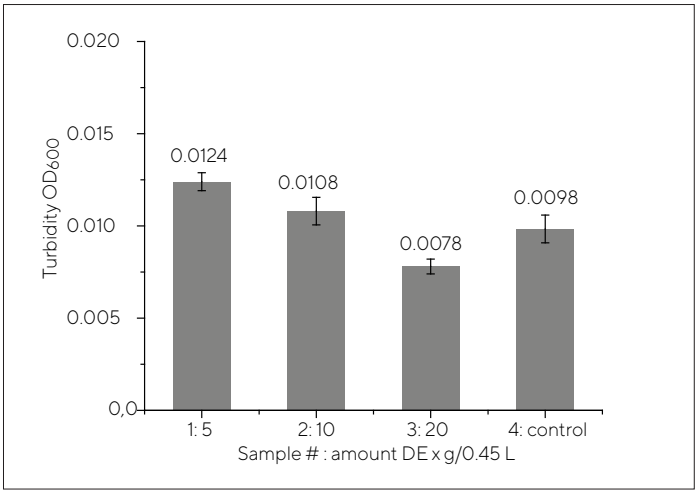

Figure 3: Comparison of clarification methods by turbidity measurements at OD600 after clarification. Error bars indicate the standard deviation.

Measurement of the optical density after clarification showed low turbidity in comparison with the measurement performed before the clarification step. The turbidity before clarification shows an OD600 of 1.602. Sample 1 with 5 g DE/0.45 L displayed the highest turbidity in comparison with sample 2, sample 3 and the control. This indicates residual cell debris present in the filtered supernatant. Sartoclear Dynamics® Lab V Kit 20 g of DE/0.45 L shows an OD600 of 7.8 x 10^{-3} and, therefore, the best clearance rate of 99.51% (Figure 3).

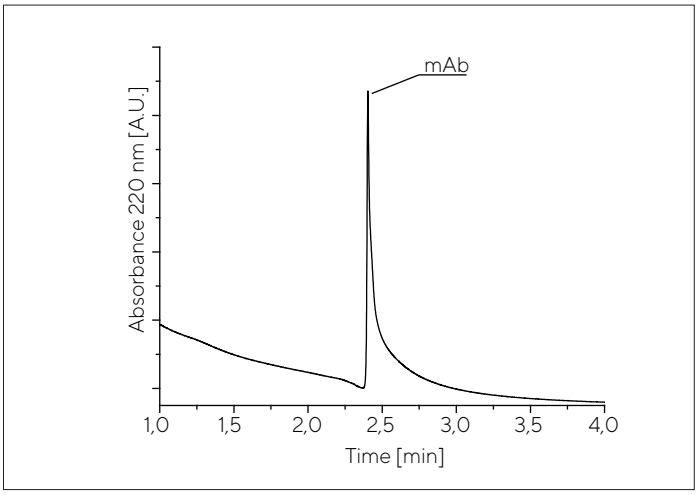

Figure 4: Characteristic chromatogram for IgG titer measurement using an analytical MAbPac™ Protein A column.

The slow flow rates and higher turbidity values observed using 5 g of DE/0.45 L can be explained by insufficient formation of a porous filter cake to separate supernatant from the cells and cell debris. For our application, the recommended use is ≥ 10 g of DE/0.45 L. Besides higher specific flow rates, higher clearance rates prevent an unnecessary load on the Protein A column and eliminate the need for CIP.

To test the effect of Sartoclear Dynamics® Lab V Kit on protein yield, the total IgG titer was measured with an analytical Protein A column. Figure 4 shows a characteristic chromatogram for determination of the IgG titer by integration of the peak area at 220 nm.

The IgG titer after clarification using Sartoclear Dynamics® Lab V Kits showed a higher mAb titer compared with the control sample. Sample 1 with 5 g of DE/0.45 L showed a higher mAb titer (33 mg/L) in comparison with the conventional clarification method (15 mg/L). The IgG titers using Sartoclear Dynamics® Lab V Kit with 10 g and 20 g of DE/0.45 L were also higher (29 mg/L and 25 mg /L) compared with that of the control sample (Figure 5).

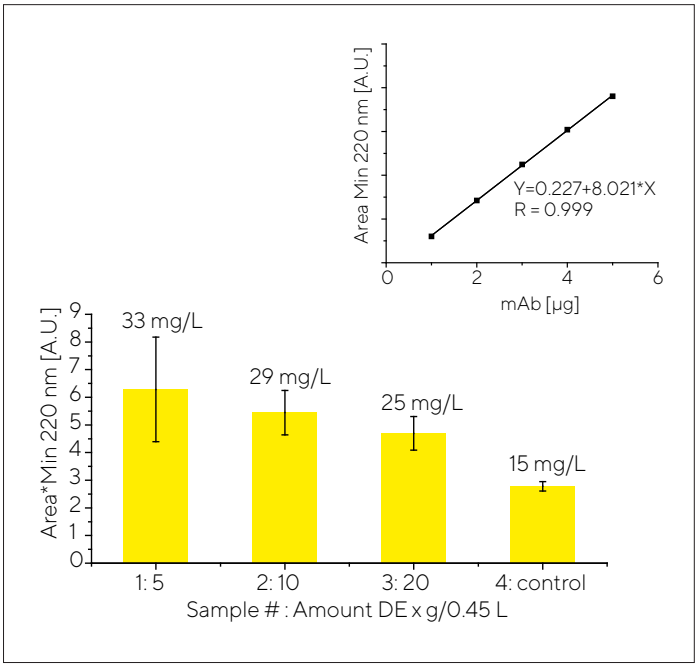

Figure 5: IgG titers after clarification using Sartoclear Dynamics® with different quantities of DE compared with the control. Error bars indicate the standard deviation. The linear calibration curve is shown in inset.

This could be explained by better recovery of mAb in the cell culture liquid due to elimination of the need for centrifugation and/or to the presence of DE during filtration. Furthermore, the effect of increased IgG titers in samples 1–3 was observed in the milligram range. Whether these effects also appear in the gram range of IgG titers should be investigated by further experiments.

The effect of the Sartoclear Dynamics® Lab V Kit on mAb aggregate content and fragment levels was measured by analytical size-exclusion chromatography (SEC). Figure 6 shows a characteristic size-exclusion chromatogram for the monoclonal antibody after Protein A purification.

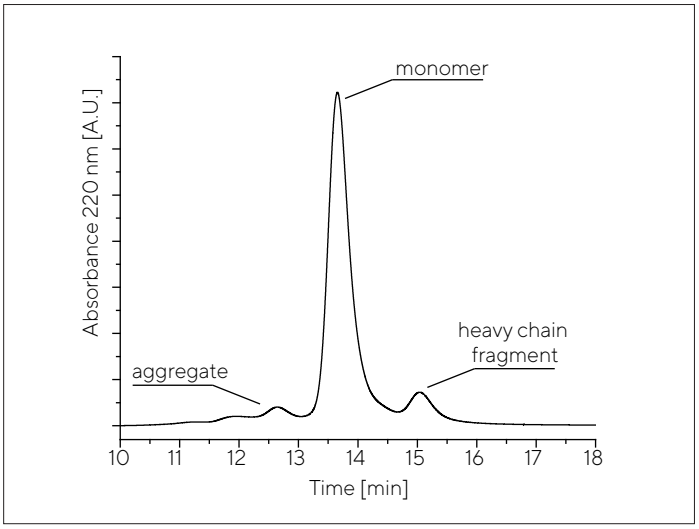

Figure 6: Example of an analytical size-exclusion chromatogram of a purified antibody after clarification using Sartoclear Dynamics® Lab V Kits and after Protein A purification.

Analysis of aggregate content and fragment levels by SEC indicates that all samples contained at least ≥ 76% of IgG monomer. For Sartoclear Dynamics® Lab V Kits, the analytical SEC measurements showed a similar aggregate content and a heavy chain fragment level, compared with the control (Figure 7). The conventional clarification process by centrifugation and filtration through a 0.22 µm Sartolab® Bottle Top filter showed 11% of mAb aggregates, whereas clarification of samples using Sartoclear Dynamics® Lab V Kits with different quantities of DE showed an aggregate content of between 4%–5%. The heavy chain fragment content for all samples was between 11%–20%.

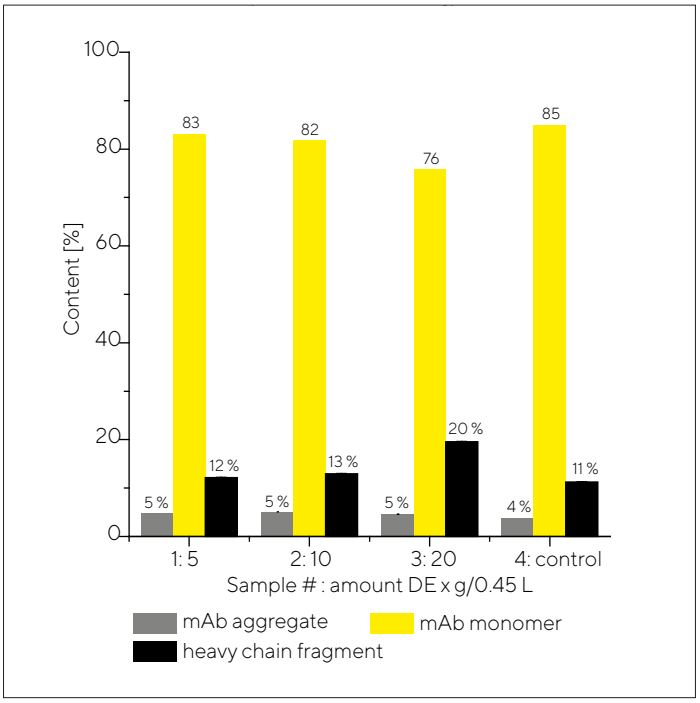

Figure 7: IgG titers after the clarification process using Sartoclear Dynamics® Lab with different amounts of DE compared with the control. Error bars indicate the standard deviation.

However, in addition to five-fold determination of IgG titers by analytical Protein A chromatography and three-fold measurement of the aggregate content by analytical SEC, it must be considered that the samples were taken from a single clarification setup under the respective sampling conditions and, consequently, the results using Sartoclear Dynamics® Lab V Kits clarification are from a single observation.

Conclusion

Sartoclear Dynamics® Lab V Kits are a convenient method for rapid clarification of mammalian cell culture broth. The novel lab-scale filtration system significantly increases productivity by eliminating the centrifugation step during the clarification process. In this study, the effects of diatomaceous earth on protein yield and mAb aggregation are neutrally assessed.

The increased IgG titers observed using Sartoclear Dynamics® Lab V Kits could likely prove to be a significant advantage over the conventional clarification method. Analysis by size-exclusion chromatography showed similar levels of mAb aggregates and heavy chain fragments in the Sartoclear Dynamics® Lab V Kits samples compared with the control.

Based on these results, clarification with the Sartoclear Dynamics® Lab V Kit with 10 g to 20 g of DE/0.45 L cell culture is a method well suited to clarifying large-volume cell cultures.

References

Weiner, L.M., R. Surana, and S. Wang, Monoclonal antibodies: versatile platforms for cancer immunotherapy. Nature Reviews Immunology, 2010. 10: p. 317.

Liu, H.F., et al., Recovery and purification process development for monoclonal antibody production. mAbs, 2010. 2(5): p. 480-499.

Birch, J.R. and A.J. Racher, Antibody production. Advanced Drug Delivery Reviews, 2006. 58(5): p. 671-685.

Schumacher, D., et al., Current Status: Site-Specific Antibody Drug Conjugates. Journal of Clinical Immunology, 2016. 36(1): p. 100-107.

Helma, J., et al., Tub-Tag Labeling; Chemoenzymatic Incorporation of Unnatural Amino Acids, in Noncanonical Amino Acids: Methods and Protocols, E.A. Lemke, Editor. 2018, Springer New York: New York, NY. p. 67-93.

Abbreviations

ADC	Antibody drug conjugates
CIP	Cleaning in place
DE	Diatomaceous earth
mAb	Monoclonal antibody
OD	Optical density
SEC	Size-exclusion chromatography
UHPLC	Ultra high-pressure liquid chromatography

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