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Application Note

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From Mammalian Cell Cultures to Pure Proteins: Sartoclear Dynamics® Lab Significantly Reduces Cell Harvest Time

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Abstract

In this study, the novel Sartoclear Dynamics® Lab V Kit was evaluated for the removal of transiently IgG expressing mammalian MEXi-293E (HEK293) cells from cell cultures. The method was directly compared to the present standard method that required two centrifugation steps. After clarification, recombinant IgG harboring a Twin-Strep-tag® was purified from all samples in parallel by a one-step Strep-Tactin®XT Superflow® high capacity affinity purification process.

Overall, the use of Sartoclear Dynamics® Lab significantly reduced the time for sample clarification by up to 3.6-fold while maintaining total protein yield and quality. Moreover, Sartoclear Dynamics® Lab can be integrated easily into already existing lab processes, substantially decreasing hands-on time and thereby simplifying sample preparation.

Introduction

The relevance of recombinant proteins produced in mammalian cell lines has increased over the past few years, especially for therapeutic applications. One of the major goals in this field is efficient production of highly pure proteins. Key factors driving this efficiency are the expression system, the purification platform and the purification protocol itself. A transient expression system enables rapid production of proteins in milligram quantities and is the system of choice for obtaining recombinant proteins for research purposes.

Affinity tags are a commonly used and powerful tool for the purification of recombinant proteins. Genetically attached to the protein of interest, they simplify the purification process considerably by using the same strategy simultaneously for many different proteins. The high binding affinity of the Twin-Strep-tag® to Strep-Tactin®XT Superflow® high capacity makes it a powerful tool for nearly all downstream applications, particularly in combination with efficient, one-step purification. This facilitates the overall protein production process and makes the Strep-tag® system a highly attractive platform.

Sample preparation can be a time-consuming procedure. In particular, centrifugation for separating mammalian cells is a tedious step. Therefore, we compared a different method using the novel Sartoclear Dynamics® system with a standard centrifugation procedure as part of preparation of a transiently mammalian expressed Twin-Strep-tagged IgG.

Sartoclear Dynamics® Lab products are based on the principle of dynamic body feed filtration: Diatomaceous earth (DE) used as a filter aid is added to cell culture broth to sieve out cells and cell debris, creating a permeable filter cake that prevents blockage of the final filter. Sartoclear Dynamics® Lab eliminates the need for the individual steps of centrifugation and filtration, thus saving significant time and resources.

Materials and Methods

A Twin-Strep-tag® fused IgG was transiently expressed in a mammalian HEK-293 expression system. Three liters of MEXi-293E suspension cell culture (IBA GmbH, 2-6001-010) were transfected with the IgG encoding plasmid according to the manufacturer's protocol. Cells were cultured at 5% CO_2, 37°C and 125 rpm. Two days after transfection, the temperature was reduced to 32°C. After 16 days, the cell suspension was divided into three identical 1,000 mL aliquots. In order to challenge the clarification and purification process, cells were cultivated until significant cell death occurred.

One aliquot was subjected to the standard method for cell removal in two consecutive centrifugation steps. The first centrifugation was conducted at 300 × g for 10 min. at 4°C to remove cells gently, without any risk of impairment due to shear forces. The supernatant was centrifuged again at 10,000 × g for 20 min. at 4°C to remove cell debris and the remaining cells for subsequent affinity column purification.

The remaining two aliquots were processed using the Sartoclear Dynamics® system (Sartorius, SDLV-1000-40C-E). Diatomaceous earth in a quantity of 40 g or 60 g, respectively, was added, and each solution was mixed to obtain a homogenous suspension before being passed through the 0.22 µm PES sterile filter.

Buffer W (IBA GmbH, 2-1003-100) and BioLock solution (IBA GmbH, 2-0205-050) were added as recommended by the manufacturer in order to prepare samples for protein purification. IgG was purified using a Strep-Tactin®XT Superflow® high capacity via gravity flow (IBA GmbH, 2-4030-010). Samples were loaded on the columns using Wet Fred devices (IBA GmbH, 2-0910-001), which allow semi-automated parallel purification of large sample volumes. After the samples were applied, columns were washed with five column volumes of Buffer W (IBA GmbH, 2-1003-100). The protein was eluted under physiological conditions by adding three column volumes of Buffer BXT (IBA GmbH, 2-1042-025). Protein concentration was measured on a NanoDrop™ photometer at 280 nm.

After purification, the samples (centrifuged supernatant, filtered supernatant, supernatant with Buffer W and BioLock solution, elution fractions) were analyzed under reducing conditions on an SDS-PAGE gel. In addition, the samples were blotted onto a nitrocellulose membrane, and the Twin-Strep-tagged IgG heavy chain was detected with Strep-Tactin®-HRP conjugate (IBA GmbH, 2-1502-001) according to the manufacturer's protocol.

Finally, elution fractions from each cell removal experiment were analyzed by size exclusion chromatography (SEC) on an Äkta™ purifier system using a Superdex 200 Increase 3.2/300 column (GE Healthcare, 28990946).

Results and Discussion

Viable peak cell density was 1.5 × 10^7 cells/mL, and viability was 95% after 13 days of cultivation. Sixteen days after transfection, viable cell density and viability decreased to 6.8 × 10^6 cells/mL and 63%, respectively. Hence, many dead cells and substantial cell debris were in the culture, hampering the cell removal step.

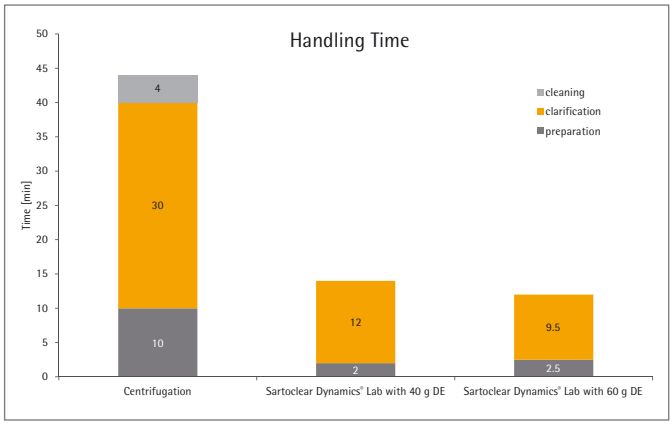


Figure 1: Comparison of clarification methods by handling time. One liter of HEK293 cell culture each was clarified by the standard process (centrifugation) and by Sartoclear Dynamics® Lab with 40 g and 60 g filter aid, resp. Sartoclear Dynamics® Lab significantly reduces the time needed for clarification of HEK293 cell culture media.

Handling times for sample preparation and cell removal for both methods were directly compared (Figure 1). In the case of the standard centrifugation-based method, the sample preparation time included balancing the weight of the centrifuge tubes and removal of the supernatant. The sample preparation time for the Sartoclear Dynamics® system included the addition of DE to the cell suspension.

Hands-on time for cell removal by centrifugation was up to 5-fold higher compared with the sample preparation time needed for the Sartoclear Dynamics® system. In addition, Sartoclear Dynamics® Lab decreased the time required for cell removal by 2.5-fold (with 40 g DE) and 3.1-fold (with 60 g DE). The complete Sartoclear Dynamics® Lab procedure took only 12 min. and 14 min., respectively. In contrast, the full sample preparation and cell removal time for centrifugation culminated in 44 min. of overall processing time. In summary, by using Sartoclear Dynamics® Lab with DE for sample preparation, the total processing time was reduced by up to 3.6-fold.

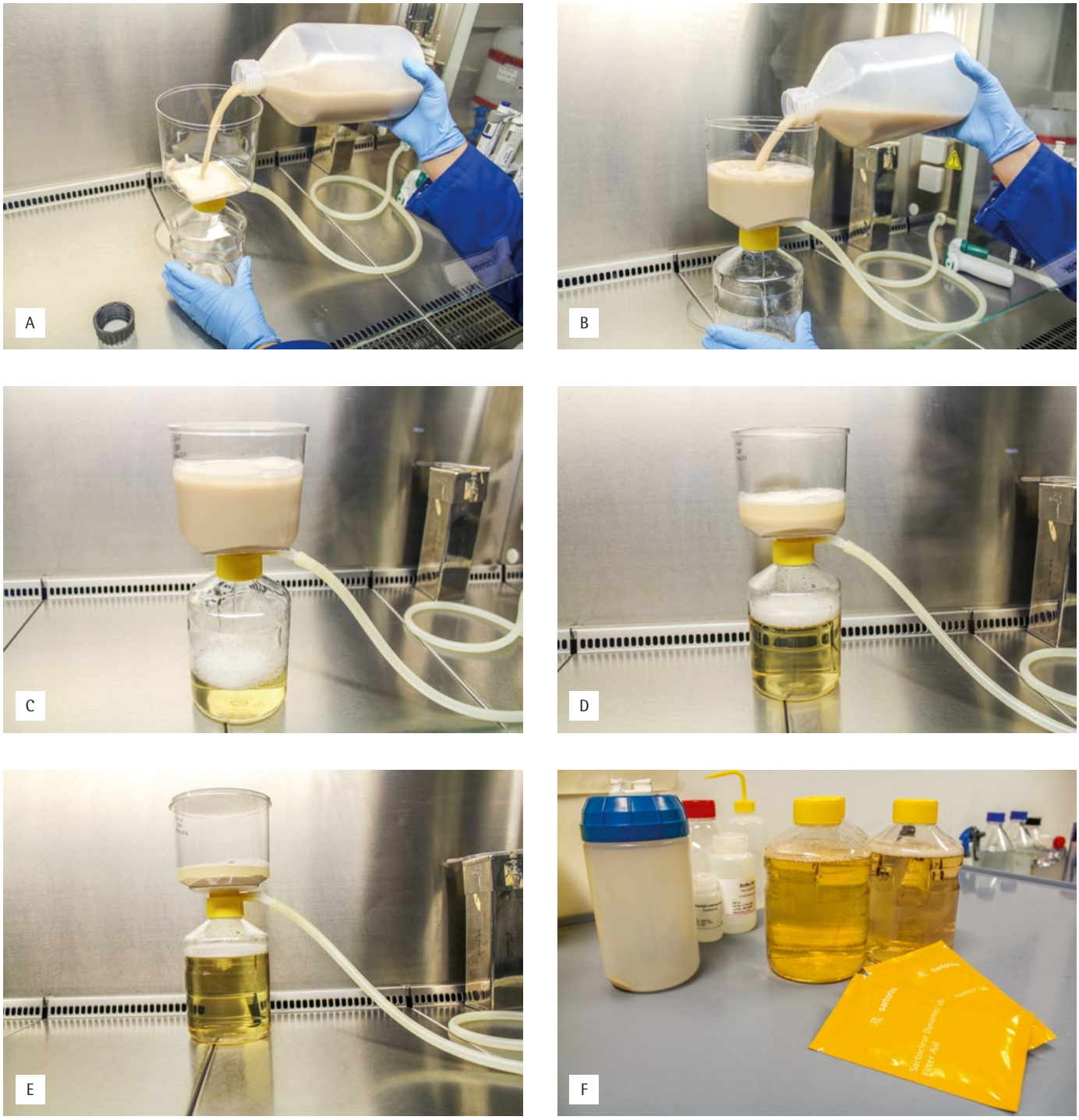


Figure 2: Pouring in and clarification of mammalian cell culture supernatant with 40 g of filter aid using the filter unit Sartolab® RF included in the Sartoclear Dynamics® Lab kit (A – F). A tube is connected to a vacuum pump.

It is worth mentioning that the centrifuged sample was not sterile. This was not an issue in the present study because column purification was started immediately after clarification under non-sterile conditions. However, if the harvested supernatant needs to be sterile, an additional filtration step must be performed after centrifugation. This is not the case for the Sartoclear Dynamics® Lab procedure as all samples are automatically sterile-filtered during clarification.

Besides reducing process time, the Sartoclear Dynamics® system is advantageous with regard to its scalability. There is no need for any instrumentation, except for a vacuum pump, to use the Sartoclear Dynamics® system (Figure 2). In contrast, the conventional centrifugation method requires a centrifuge and a rotor to accommodate tubes with a sufficient volume.

The protein yield was determined after affinity column purification to exclude the possibility that unspecific binding of IgG to the components might occur in the Sartoclear Dynamics® system. The resulting IgG yields of the centrifuged sample and Sartoclear Dynamics® Lab processed samples were comparable (Figure 3), ruling out any negative effect of Sartoclear Dynamics® Lab on the protein yield.

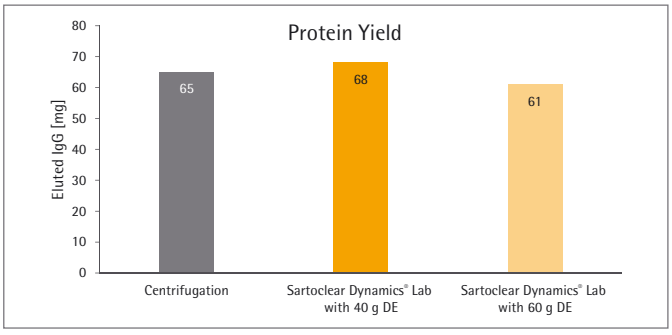


Figure 3: Comparison of clarification methods by protein yield. Comparable mAb yields were purified independently of the clarification method used.

To test the effect of the Sartoclear Dynamics® system on product quality, samples were analyzed on SDS-PAGE (Figure 4) and by SEC analysis (Figure 6). Bands corresponding to the heavy and light chains of the IgG were present in the elution fractions of all samples, and no difference was observed between the centrifuged sample and samples clarified by Sartoclear Dynamics® Lab. The Twin-Strep-tag® heavy chain was detected in all samples by Western blot analysis (Figure 5), confirming the identity of the protein of interest. Moreover, the results of the SDS-PAGE analysis showed the efficiency of the Twin-Strep-tag®:Strep-Tactin®XT Superflow® high capacity purification from mammalian cell supernatant in a simple, one-step purification process.

Results of SEC analysis revealed that regardless of the sample preparation method chosen, there was no effect on the aggregation rate (25%) of IgG. The main peak (75%) corresponds to monomeric IgG. It can be assumed that the high aggregation rate resulted from the low cell viability of the culture at the point of harvest. Manufacturers' protocols recommend harvesting cells before viability drops below 75%.

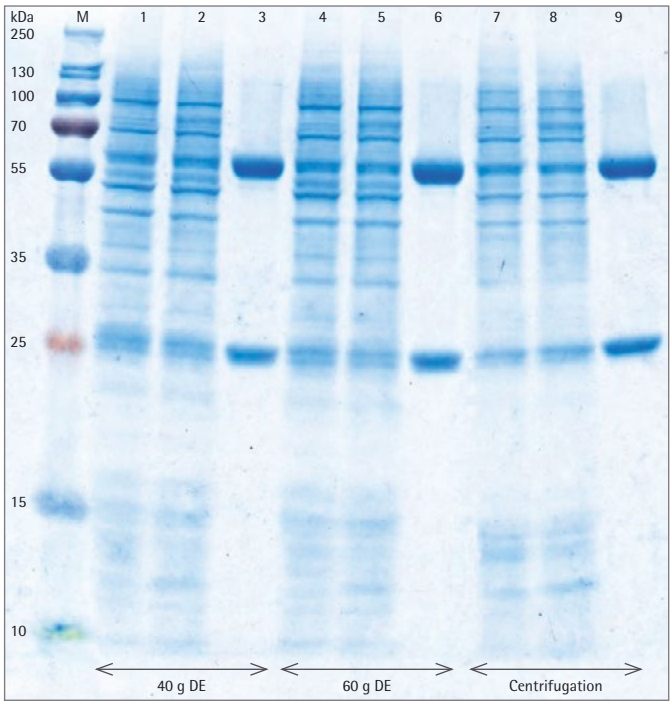


Figure 4: SDS-PAGE analysis of cell culture supernatants clarified by centrifugation and Sartoclear Dynamics® Lab, plus elution fractions after purification. Protein purity was 100% in all elution fractions, independently of the clarification method used. Samples 1, 4 and 7) supernatant; 2, 5 and 8) supernatant with BioLock and Buffer W; 3, 6 and 9) elution.

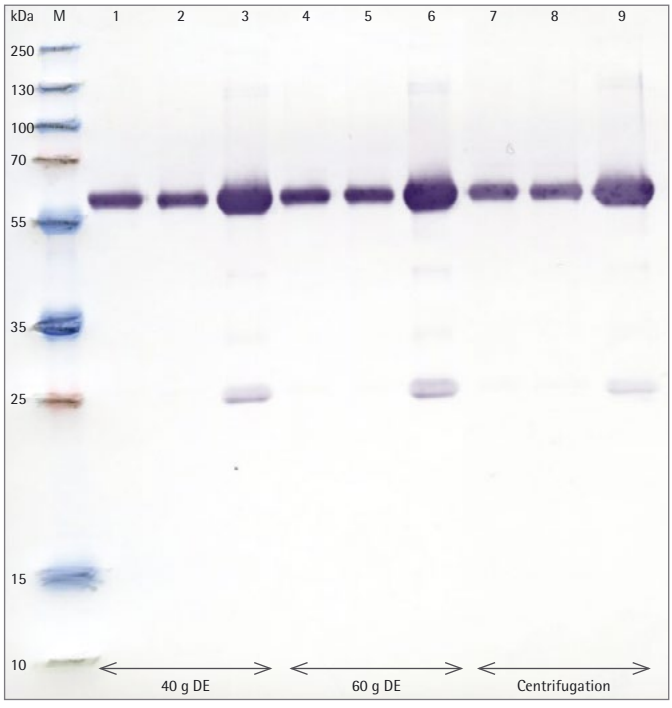
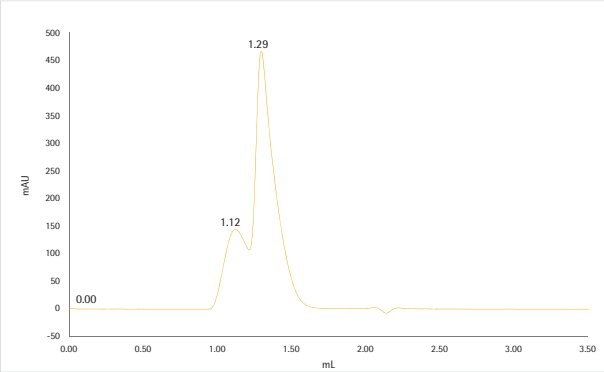
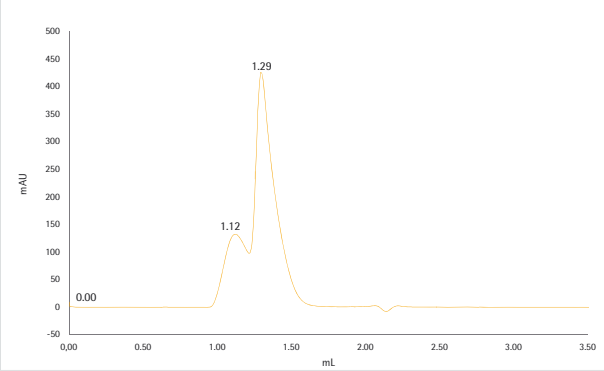


Figure 5: Western Blot analysis of cell culture supernatants clarified by centrifugation and Sartoclear Dynamics® Lab, plus elution fractions after purification. The Twin-Strep-tag® of the mAb's heavy chain was detected by the Strep-Tactin®-HRP conjugate. Samples 1, 4 and 7) supernatant; 2, 5 and 8) supernatant with BioLock and Buffer W; 3, 6 and 9) elution.

Centrifugation



40 g DE



60 g DE

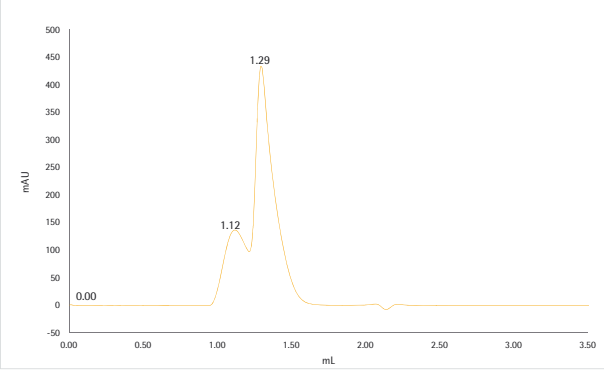


Figure 6: Size exclusion chromatograms of eluted mAb after purification from cell culture supernatants clarified by centrifugation, Sartoclear Dynamics® Lab with 40 g filter aid and 60 g filter aid, respectively. The clarification method did not have any influence on the mAb aggregation level. Peak 1.12 represents aggregated mAb (25%) and band peak 1.29 monomeric mAb (75%).

Conclusion

In summary, the efficient transient expression in the MEXi-293E (HEK293) cell line combined with simple, one-step Strep-Tactin®XT Superflow® high capacity purification generates more than 60 mg of highly pure IgG per liter of cell culture. The use of Sartoclear Dynamics® Lab significantly reduced the cell removal time compared with the standard centrifugation-based process, without affecting protein yield or product quality.

Abbreviations

HEK	Human embryonic kidney
PES	Polyethersulfone
DE	Diatomaceous earth
SEC	Size exclusion chromatography

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