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Application Note

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Reducing sample preparation time from Sf9 insect cultures by using Sartoclear Dynamics® Lab

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Abstract

In this study, I tested the Sartoclear Dynamics® Lab V500 Kit as a novel method for the clarification of cell culture media prior to purification of a recombinant protein expressed in Sf9 cells. The vector from which this protein is expressed encodes a signal sequence to ensure secretion of the protein from the cells following expression. It is therefore necessary to remove cells from the media before purifying the protein but using traditional methods to do this (centrifugation followed by filtration) can be time consuming. Here, I show that the Sartoclear Dynamics® Lab filter offers significant time savings over conventional methods with no noticeable effect on protein yield, making this product an ideal addition to any laboratory looking to increase productivity and throughput from their insect cell expression systems.

Introduction

Knowing the three dimensional structure of proteins is key to understanding their functions and, for those implicated in disease, this information can aid the design of novel drugs with high specificity for their targets.

Availability of pure protein is important for structure determination and it is now common practice in many laboratories to utilise several different protein expression systems. The choice of system is usually dependent on the species origin, modification(s) required and yield of the target protein. The use of signal sequences in expression vectors results in expressed proteins being secreted into the cell culture media at relatively high purity, avoiding the need for expensive cell disruption equipment and minimising the time involved in optimising purification protocols[1]. A major drawback of this approach, however, is the amount of time required to clarify large volumes of cell culture media prior to purification. Available centrifuge space can either limit the culture volumes that are chosen or increase the time spent clarifying media when having to use multiple runs. I have also noted slow flow rates when filtering insect cell culture media, even after removal of the cells by centrifugation. This probably corresponds to the presence of submicron particles, which, for mammalian cell cultures, have been shown to remain in suspension after centrifugation and cause clogging of filter membranes[2].

The Sartoclear Dynamics® series of filtration products was originally developed to significantly reduce the amount of time involved in harvesting mammalian cells from cultures. The addition of diatomaceous earth to cultures supports the formation of a porous filter cake to prevent blockage of the filter, allowing rapid removal of the cell culture media from the sample (figure 1). This avoids the need for a centrifugation step, circumventing issues surrounding centrifuge capacity and availability. For my research, the proteins of interest are expressed in insect cells and secreted from them, so must be purified from the culture media. I therefore tested the Sartoclear Dynamics® system for the clarification of insect cell culture media containing a target protein and compared the results with my standard method.

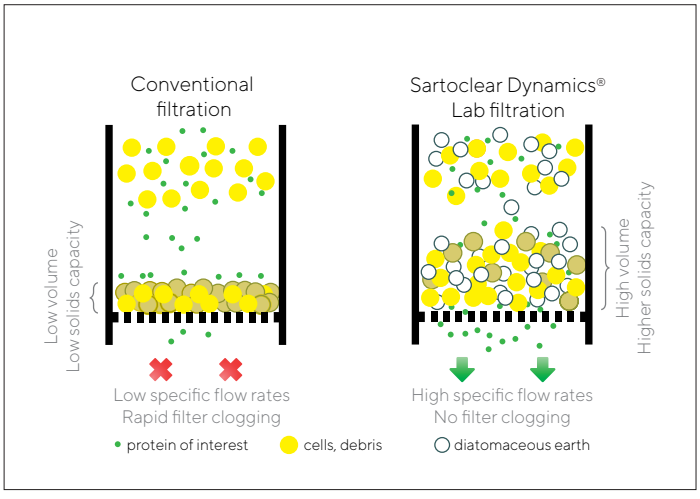

Figure 1: Principles of clarifying cell cultures by using the conventional and the body-feed-filtration method utilised by Sartoclear Dynamics® Lab.

Materials and Methods

The protein of interest was expressed using an insect baculovirus expression system. Sf9 cells (ThermoFisher Scientific, 11496015) were cultured in Sf-900™ II SFM (ThermoFisher Scientific, 10902088) as per the manufacturer's instructions. A 1.5 L suspension culture was infected with baculovirus encoding the gene for the protein of interest and incubated at 27°C with shaking at 240 rpm for 90 hours.

The culture was harvested and divided into three 470 mL aliquots for clarification and protein purification. One aliquot was clarified using a standard method. Cells were removed by centrifugation at 5,000 g for 15 minutes and the supernatant passed through a 0.22 µm PES filter. The second and third aliquots were clarified using the Sartoclear Dynamics® system. 5 or 10 g diatomaceous earth (DE) was added and the solutions mixed to homogeneous suspensions before passing the cultures through the included 0.22 µm PES filters.

Protein in each clarified sample of the cell culture media was purified by immobilised metal affinity chromatography (IMAC). Media was loaded onto 5 × 1 mL HisTrap excel columns (GE Healthcare Life Sciences, 17-3712-05), linked in series and pre-equilibrated with HTE buffer (50 mM HEPES, 500 mM sodium chloride, pH 7.4). The column was washed with 100 mL HTE buffer and 75 mL HTE buffer containing 25 mM imidazole to remove contaminants, and the protein of interest was eluted with 25 mL HTE buffer containing 500 mM imidazole.

Results and Discussion

Upon harvesting the cell culture, the density measured 1.5×10^6 total cells per millilitre. The time spent clarifying each cell culture sample was recorded (figure 2). This was broken down into preparation time (i.e. measuring culture volumes and balancing centrifuge tubes or adding the DE for the standard or Sartoclear Dynamics® methods, respectively), centrifugation time (for the standard method only), and filtration time (both methods).

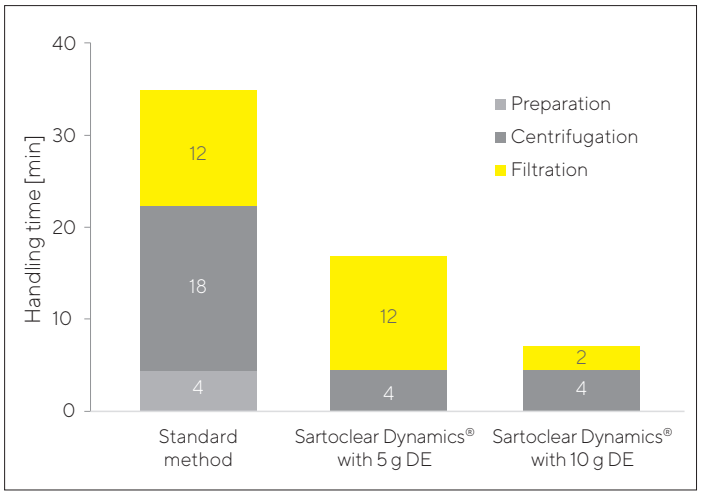

Figure 2: Comparison of clarification methods by handling time. Sartoclear Dynamics® Lab significantly reduces the amount of time required to clarify cell culture media.

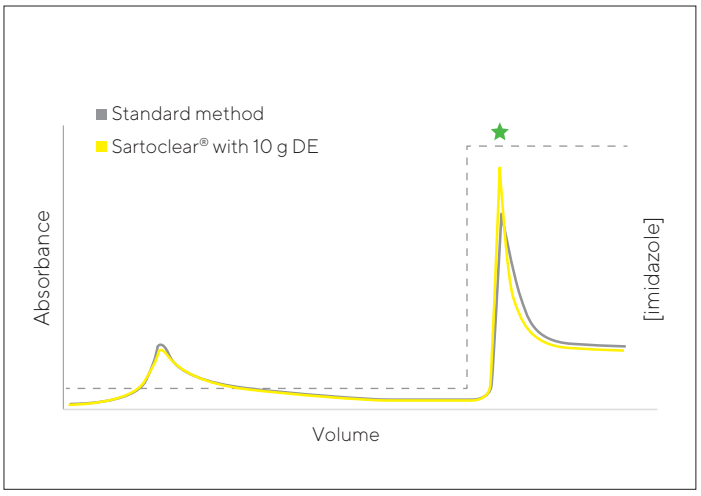

Figure 3: Protein elution peaks (★) were comparable between samples prepared using a standard centrifugation-filtration method and the Sartoclear Dynamics® Lab filtration system with 10 g DE.

Preparation of clarified cell culture media using the standard centrifugation-filtration method took approximately 34 minutes. Whilst filtration time was not reduced relative to the standard method when using a Sartoclear Dynamics® device with 5 g DE, the lack of a need for a centrifugation step meant the total preparation time was reduced by > 53 %. Increasing the quantity of DE to 10 g resulted in the total preparation time being reduced to 6 minutes, a time saving of > 82 %.

In terms of throughput, the filtration times for the standard, 5 g DE and 10 g DE methods equated to flow rates of 2.35, 2.35 and 14.1 L/h, respectively. In the sample prepared using 5 g DE, some settling of the diatomaceous earth and cells was observed part way through filtration. It is possible that early settlement of the DE caused some reduction in the flow rate. Although it is clear that most of the DE/cells mixture can be resuspended by gently swirling the filter unit to return the flow rate to its normal level when using lower quantities of DE, it might be prudent to use 10 g of DE for this method when filtering cultures with higher cell densities.

To test the effect of the Sartoclear Dynamics® system on protein yield, protein from each sample was purified by IMAC and the size of elution peaks compared (figure 3). The sample prepared using the Sartoclear Dynamics® system with 10 g DE actually produced an elution peak with a higher maximum absorbance than that from the sample prepared with the standard method, potentially indicative of more protein being present. However, the elution peak from the standard sample was slightly broader in comparison (i.e. in a slightly higher volume), so essentially the protein yield was unaffected by mixing the sample with DE.

Conclusion

Sartoclear Dynamics® Lab allows for more rapid clarification of cell culture media. The lack of a requirement for a centrifuge and significant time savings achieved using this filtration system can significantly increase productivity and throughput. There appears to be no negative effect on protein yield after mixing the sample with diatomaceous earth. The system is available in a wide variety of formats for the clarification of cell culture media in 15 mL to 1 L batches making it suitable for a wide range of requirements.

References

1 Berrow NS, Alderton D, Sainsbury S. A versatile ligationindependent cloning method suitable for high-throughput expression screening applications. Nucleic Acids Research. 2007;35(6):e45. doi:10.1093/nar/gkm047

2 Liu HF, Ma J, Winter C, Bayer R. Recovery and purification process development for monoclonal antibody production. mAbs. 2010;2(5):480-499. doi:10.4161/mabs.2.5.12645.

Abbreviations

DE diatomaceous earth
IMAC immobilized metal affinity chromatography
PES polyethersulfone

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