

## Removal Of Host Cell Proteins By Zeta Plus™ Filters During Monoclonal Antibody Purification

### Introduction

Protein A chromatography is a widely used and highly successful method of purifying recombinant monoclonal antibodies. However, since Protein A has high affinity for antibodies, harsh conditions such as low pH are often required to elute monoclonal antibodies from Protein A affinity columns. As a result, many proteins will denature and aggregate during this step, leading to lowered production yields, protein purity, and increased production costs.

This CUNO Application Brief details a solution that will significantly reduce protein precipitation during Protein A column elution step. Multiple stages of depth filtration using Zeta Plus™ depth filters are recommended before Protein A affinity columns. The functions of Zeta Plus depth filters are two-fold:

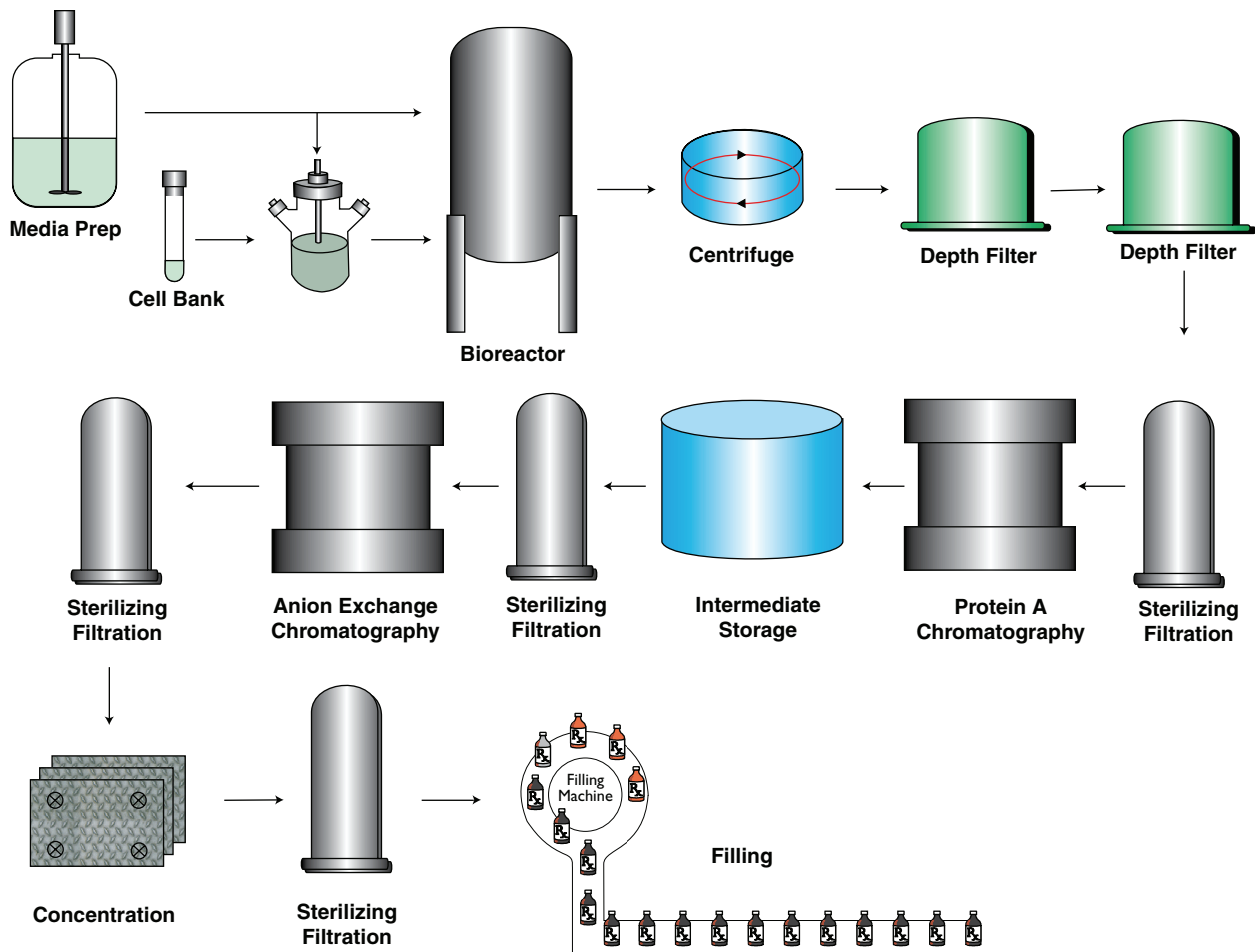
- Removing cell debris and other particulates that escape the upstream separation steps such as centrifugation;
- Capturing host cell proteins (HCP) that are released during fermentation process or upstream purification process.

Removing host cell proteins by depth filtration will significantly reduce the amount of proteins precipitated during the elution step from Protein A columns.

### Monoclonal Antibody Production Challenges

The production of monoclonal antibodies was first described by Dr. Cesar Milstein and Dr. Georges Kohler at the MRC Laboratory of Molecular Biology in the U.K. in 1973. The world's first therapeutic monoclonal antibody was approved for sale in 1986. This antibody, Orthoclone OKT3, is manufactured by Ortho Pharmaceutical (a subsidiary of Johnson & Johnson) and is used for the treatment of acute allograft rejection in renal transplant patients. Since then, a total of 18 therapeutic antibodies targeting a variety of diseases such as cancers, autoimmune diseases, cardiovascular diseases and infectious diseases, have been approved by the FDA. Today, the therapeutic monoclonal antibody market is a \$10 billion business and is expected to grow at double-digit growth rate over the next 5 years. In addition to their therapeutic value, monoclonal antibodies are also important tools in the diagnostic field. Numerous monoclonal antibodies have been developed and used in the diagnosis of many diseases, pregnancy, and in drug testing.

Figure 1 illustrates a typical process of making recombinant monoclonal antibodies from mammalian cell cultures. At the optimal time for cell culture harvest, centrifugation is frequently employed for primary separation to remove solid cell mass from liquid phase that contains the secreted antibodies. Alternative technologies that can be used at this step are cross-flow filtration and depth filtration. After primary separation, depth filters are commonly used to trap the residual cell debris that escapes centrifugation. For specific capture and purification of monoclonal antibodies from the complex cell culture harvest fluid streams, Protein A chromatography is widely employed owing to its high degree of specificity for IgG (majority of monoclonal antibodies are IgG). Protein A can deliver purities in excess of 99% starting from cell culture harvest supernatants.



**Figure 1 — Flow Chart Of A Typical Monoclonal Antibody Production Process**

Following this step, usually only polishing type chromatographic steps are required to clear residual levels of host cell protein contaminants, leached Protein A, and high molecular weight aggregates. Protein A chromatography is by far the most costly step in monoclonal antibody purification. Therefore the Protein A chromatography resins and columns must be carefully protected.

One major obstacle of using Protein A columns is that these columns typically bind antibodies at neutral pH but are eluted at low pH (between pH 3 and 4). Since low pH tends to denature proteins, the denatured proteins can aggregate and precipitate, fouling costly Protein A columns and complicating downstream purification.

### The CUNO Solution

Recently several papers have been published regarding host cell proteins (Ref. 1, 2). One of these papers describes that adsorptive depth filters with positive charge were capable of removing particulates such as cell debris and capturing host cell proteins during monoclonal antibody purification (Ref. 2). The paper further demonstrates that removing host cell proteins prior to loading Protein A columns resulted in significant reduction of protein precipitation during Protein A elution step. Lastly, CUNO 90ZA depth filters were shown to outperform Millipore™ A1HC depth filters in removing host cell proteins and reducing protein precipitation.

The monoclonal antibody used in this study was a human IgG2 with an isoelectric point (pI) at about 8.8 and a molecular weight of about 150.875 kD. The antibody was expressed in Chinese Hamster Ovary (CHO) cells and secreted into the cell culture media in a bioreactor. At harvest, cell mass were initially separated from the media by centrifugation. The supernatant was then passed through depth filters or a Fractogel TMAE (an anion exchange) column before being loaded on a Protein A column. In a key

experiment, the cell culture harvest samples were pretreated in the following ways prior to being loaded on the Protein A column: (i) control (no pretreatment); (ii) Millistak A1HC depth filter at 150 L/m<sup>2</sup> loading, 50 LMH flux; (iii) CUNO 90ZA depth filter at 150 L/m<sup>2</sup> loading, 50 LMH flux; (iv) flow through an anion-exchange column (a Fractogel TMAE column) at 50 mg/mL. After several washes, Protein A column was eluted with 100 mM acetate buffer at pH of 3.6. The turbidity and the protein content before and after Protein A column were measured by a spectrophotometer at O.D. 410 nm and by a CHOP ELISA assay respectively. The anion-exchange column (Fractogel TMAE) was demonstrated to be the most efficient in reducing turbidity of the Protein A eluates, followed by CUNO 90ZA depth filters and Millipore A1HC depth filters. Since depth filtration is a well-established component of monoclonal antibody production process, the paper concluded that depth filtration is the system of choice in removing host cell proteins prior to Protein A chromatography.



The Zeta Plus™ Family of Filter Products

Two observations made by Yigzaw et. al. are worth mentioning. (i) CUNO 90ZA depth filter provided > 98% protein recovery in the filtration step. This demonstrated that CUNO Zeta Plus™ filters have low affinity for monoclonal antibody. (ii) Both the number of passes through the depth filter and the amount of the centrifuge concentrate loaded on the filter were important operational parameters governing the adsorptive removal of host cell protein contaminants. In other words, multiple stages of depth filtration are preferred (two stages are recommended).

## Conclusions

Depth filtration has been established as a critical component in the monoclonal antibody production process because it can efficiently remove cell debris at low costs. The findings in the referenced papers show that depth filters can be effectively employed in an adsorptive capacity for the capture of soluble impurities in addition to their conventional role in clarifying particle rich fluid streams. Depth filters can effectively capture host cell proteins generated during the cell propagation and/or centrifugation process, thus preventing or reducing their precipitation and fouling of Protein A columns and contaminating monoclonal antibody eluates. In light of these findings, we recommend evaluating the following:

1. Two stages of depth filtration after the centrifugation step and upstream of the Protein A chromatography
2. Zeta Plus EXT Series filter with ZA media as the depth filter of choice for the capture of host cell proteins
3. To maximize the flow rate and throughput, we recommend the use of an open grade Zeta Plus EXT Series filter as the first stage depth filter followed by a tighter grade Zeta Plus EXT Series filter as the second stage depth filter

**Table 1 — Recommended Grades of Zeta Plus™ EXT Series for Host Cell Proteins Removal**

Host Cell Proteins Removal Stages	Zeta Plus EXT Series Grades and Types
1	60ZA05A, 90ZA05A
2	90ZA08A, 120ZA05A, 120ZA08A, 120ZA10A

## References

1. Strategies to address aggregation during protein A chromatography. Abhinav A. Shukla, Peter J. Hinckley, Priyanka Gupta, Yinges Yigzaw, and Brian Hubbard. *Bioprocess International* 2005, Vol. 3, Number 5: pp 36-44.
2. Exploitation of the adsorptive properties of depth filters for host cell protein removal during monoclonal antibody purification. Yinges Yigzaw, Robert Piper, Minh Tran, and Abhinav A. Shukla. *Biotechnol. Prog.* 2006, 22: pp 288-296.

