Enhancing monoclonal antibody production: advanced downstream purification strategies

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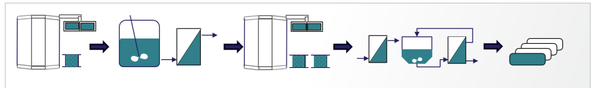
Abstract

This poster demonstrates the potential of implementing downstream process optimization strategies to lower labor, footprint, and consumables costs while maintaining product quality and yield. The Thermo Fisher Scientific technologies highlighted in this study can potentially decrease downstream costs by $2M.

Introduction

Monoclonal antibodies (mAbs) are a breakthrough in pharmaceuticals, offering targeted treatments for various serious health conditions. The manufacturing process, divided into upstream (cell culture) and downstream (purification and refinement) stages, requires precision and efficiency to enable high-quality products. Downstream processes, including chromatography, filtration, and formulation, are critical for achieving the desired product quality and safety. However, these processes face several bottlenecks such as low binding capacity, long processing times, high operational costs, contamination risks, scalability issues, and environmental impact. To address these challenges, process optimization strategies like high-capacity chromatography resins, buffer concentrates, single-use systems, automation, and sustainable practices can significantly enhance efficiency and productivity. These improvements aim to increase yields, reduce processing times, lower costs, and improve product quality, in an effort to accelerate the time-to-market and availability of mAb therapies.

Figure 1. Thermo Fisher Scientific optimized downstream process.



Materials and methods

Standard process: Protein A, AEX, and CEX purification

The standard downstream process represents a conventional three-column batch purification train. It includes Protein A capture, anion exchange (AEX) intermediate purification, and cation exchange (CEX) polishing. Clarified harvest is captured using a high-capacity Protein A resin operated in bind/elute mode to isolate the target monoclonal antibody. The Protein A eluate is adjusted for conductivity and pH and depth filtered before loading onto Thermo Scientific™ POROS™ XQ Strong Anion Exchange (AEX) resin operated in flow-through mode for impurity removal. A final polishing step uses Thermo Scientific™ POROS™ XS Strong Cation Exchange (CEX) resin in bind/elute mode to remove aggregates and product-related variants. Each chromatography step operates as an independent batch unit operation, requiring separate buffer preparation and manual transfer. This workflow serves as the modeled baseline for comparison to the intensified process.

Intensified process: Protein A, AEX, and HIC with inline dilution

The intensified downstream workflow builds upon the standard process while integrating automation and buffer efficiency strategies. Capture is performed using Thermo Scientific™ MabCaptureC™ High Capacity Protein A Resin, followed by AEX purification and final polishing with Thermo Scientific™ POROS™ Benzyl Ultra Hydrophobic Interaction Chromatography (HIC) Resin operated in flow-through mode. All chromatography steps are run on the Thermo Scientific™ DynaChrom™ Single-Use Chromatography System, which includes inline dilution for automated buffer blending with up to 10X concentrates. Gibco™ Process Liquid and Buffer Solutions were used to leverage pre-made and concentrated buffers for consistent and efficient preparation.

Economic modeling data

The economic modeling data were generated using BioSolve Process™ modeling software and were utilized to compare the intensified downstream workflow with a standard downstream workflow. Model inputs are based on experimentally verified parameters from bench and pilot studies at a 500 L bioreactor scale. Equipment, labor, and consumables assumptions are consistent between both cases to isolate the effects of automation, inline dilution, and buffer concentrate strategies. The analysis evaluates the relative impact of process simplification on operational efficiency, facility utilization, and resource requirements. Assumptions of standard upstream process remained the same for all economic modeling calculations.

Results

Improved efficiency and sustainability with high-capacity capture chromatography resins

Optimizing recovery and aggregate removal is crucial for maximizing yield and productivity. High recovery percentages enable more of the produced mAb to be retained, helping to increase yield and reduce raw material costs, thereby enhancing cost-efficiency. The first purification step, capture chromatography, utilized MabCaptureC Protein A Resin to capture the the mAb molecule from clarified harvest or harvested cell culture fluid. The process consisted of three wash steps to remove process- and product-related impurities, followed by an elution step with acetate buffer. Inline dilution, which eliminates separate buffer preparation steps and reduces processing time, was performed with the DynaChrom Single-Use (SU) Chromatography System to enhance downstream processing efficiency. The use of inline dilution with buffer concentrates supports consistent buffer composition and minimizes buffer consumption, enabling cost savings and a reduced manufacturing footprint.

Table 1. Impact of MabCaptureC resin and inline dilution on mAb production efficiency.

	Standard process	MabCaptureC with inline dilution	% improvement
Resin cost/batch*	$43K	$29K	33% ($14K)
Process liquids footprint (m^2)**	267	206	23% ($400K)

* Compared to commercially available Protein A resins (standard process).
** Process area cost based on Class D cleanroom at price of $5,900 per m^2.

Utilization of MabCaptureC resin and inline dilution with the DynaChrom SU Chromatography System recovered 95% of the target mab from the column. This high yield indicates efficient purification, with minimal loss of desired product. In addition, this combination reduced the resin cost/batch by 34% from $43K to $29K and decreased the process liquids footprint by 23%, saving $400K.

Optimized efficiency with high-producing polish chromatography resins in flow-through mode

The polish chromatography stage in mAb manufacturing is crucial for removing host-cell proteins, residual DNA, aggregates, viral contaminants, and other impurities. Efficient and consistent mAb production can be accomplished using Thermo Scientific™ POROS™ Chromatography Resins in flow-through mode combined with inline dilution using the DynaChrom SU Chromatography System. Flow-through mode in the polish chromatography step allows the product to pass through the resin without binding, focusing on removing impurities such as host-cell proteins, DNA, and aggregates. This approach offers increased throughput, cost efficiency, and a simplified process by reducing required wash volumes and increasing capacity per cycle steps. The polish chromatography stage utilized POROS XQ Strong AEX Resin in flow-through mode to remove negatively charged impurities, such as endotoxin, DNA, host-cell proteins, and viruses, capturing 95% of the target mAb. This was followed by a second polishing step using POROS Benzyl Ultra HIC Resin in flow-through mode to remove high molecular weight species. The AEX flow-through product was adjusted to pH ~6 with acetic acid and diluted for HIC loading. Choosing HIC resin in flow-through mode over the traditional POROS XS Strong CEX Resin in bind/elute mode depends on the impurity profile and process challenges. HIC resin in flow-through mode reduces buffer usage, speeds up manufacturing, and lowers reagent costs, while achieving comparable impurity reduction to the standard CEX resin.

Table 2. Impact of POROS HIC resin on mAb production yield and purity.

Modality	CEX bind/elute mode	HIC flow-through mode
Resin	POROS XS	POROS Benzyl Ultra
Loading	45 g/L	300 g/L
Yield	96.4%	94.4%
Monomer purity by SEC	97.6%	97.9%

Utilization of POROS HIC resin in flow-through mode reduces buffer usage and supports a more economical and efficient purification train process. The use of inline dilution during this step maintains consistent buffer composition and reduces buffer consumption throughout the downstream purification process. The polish chromatography stage resulted in a yield of 94.4% compared to the 96.4% obtained by the standard POROS CEX resin, along with a purity of 97.9% compared to the 97.6% obtained by the CEX resin.

Table 3. Impact of MabCaptureC + inline dilution + HIC resin on mAb production efficiency and COGS.

	Standard process	MabCaptureC + inline dilution + HIC resin	% difference
Downstream footprint (m^2)*	205	143	30% ($356K)
Total FTE**	176	164	7% ($1.2M)**
Resin cost/batch	$51.3K	$32.1K	37% ($19.2K/batch)

* Downstream footprint cost based on a Class D cleanroom at a price of $5,900 per m^2.
** FTE labor cost based on an average FTE annual compensation of $93K.
Note: Downstream footprint, total FTEs, and resin cost per batch are reduced, and therefore the cost of goods sold (COGS) associated with the campaign are reduced accordingly.

The use of MabCaptureC, POROS AEX, and POROS HIC resins and inline dilution with the DynaChrom SU Chromatography System reduces buffer usage and therefore the COGS associated with the campaign (Table 3). This process optimization resulted in a more economical and efficient purification train process.

Enhanced resource utilization and efficiency with inline dilution and buffer strategies

Reducing buffer and process liquid usage can significantly enhance the efficiency and cost-effectiveness of downstream mAb manufacturing. Gibco Process Liquid and Buffer Solutions offer pre-made buffers, buffer concentrates, and custom solutions to streamline operations. Economic modeling with BioSolve Process software demonstrated significant cost savings from outsourcing buffer production or using buffer concentrates. By simulating various scenarios with detailed process parameters and cost data, the software quantified benefits such as reduced labor, minimized contamination risks, and optimized storage requirements.

Table 4. Impact of buffer outsourcing on mAb production efficiency and expense.

Cost type	Savings/batch*
Capital expenses	$21K
Labor expenses	$39K
Consumables expenses	$16K
Other	$40K
Materials	($82K)
Total savings/batch	$34K
Total savings/year	**$2.1M**
One-time CapEx new facility saving	**$8.5M**

- Data were generated using BioSolve Process software, and assumptions of generic in-house buffer preparation process (5,000 L process, 4 bioreactor facility, 63 batches/year operation) contributed to the economical modeling calculations. Buffer outsourcing data inputs are based on Thermo Fisher Scientific buffer preparation expense calculations.

Note: Utilization of Gibco Process Liquid Buffers and Solutions can potentially save $2.1M/year compared to in-house solution preparation and storage.

Conclusions

Optimizing downstream processes in mAb production is a strategic approach to bringing therapeutics to market quickly and safely. Implementing advanced purification techniques, such as high-capacity chromatography resins, inline dilution, and sustainable buffer strategies, can support enhanced efficiency, cost-effectiveness, and sustainability. Leveraging high-capacity MabCaptureC Protein A Resin and the DynaChrom SU Chromatography System supports strong yield and purity while reducing operational costs and labor. Inline dilution streamlines processes, maintains consistent buffer composition, and minimizes resource usage. Utilizing POROS resins in flow-through mode during polish chromatography reduces buffer consumption and campaign costs, maintaining high product quality and throughput.

Adopting these optimization strategies enables manufacturers to deliver superior mAb products efficiently addressing critical bottlenecks.

The combined strategies of high-capacity chromatography resins, inline dilution and sustainable buffer strategies shown in this poster can result in:

- **Reduced downstream and process liquid prep footprint by 30%, saving nearly $400K on process area costs**
- **Reduced FTE by up to 10%, saving nearly $1.5M in labor**
- **Reduced resin costs by 37%, saving nearly $20K per batch**
- **Outsourced process liquid production reduced costs by over $2M per year**

The adoption of these downstream optimization strategies can offer manufacturers the ability to better deliver quality mAb products that can be more efficient and sustainable. In an attempt to address some of the critical bottlenecks in purification and buffer management with different technologies and systems, manufacturers can potentially lower costs, increase productivity and better achieve regulatory standards. These optimization strategies can help support the development of life-saving mAb therapies that have the potential to reach patients sooner and more reliably.

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