Downstream processing

Enhancing monoclonal antibody production: advanced downstream purification strategies

Background

Monoclonal antibodies (mAbs) represent a pivotal advancement in pharmaceuticals, providing targeted and highly effective treatments for a wide range of serious health conditions. The manufacturing of mAbs involves complex processes that require precision and efficiency to enable high-quality products. While upstream processes focus on the production of mAbs through cell culture, downstream processes are crucial for the purification and refinement of these proteins. These downstream steps, including chromatography, filtration, and formulation, are important for achieving the desired product quality and safety.

Despite advancements in technology, downstream mAb manufacturing faces several bottlenecks that can hinder productivity and increase costs. Common challenges include low binding capacity, long processing times, high operational costs, contamination risks, scalability issues, limited flexibility, and environmental impact. These bottlenecks can significantly affect the efficiency and cost-effectiveness of the manufacturing process, ultimately impacting the time-to-market and availability of mAb therapies. Process optimization offers a promising solution to these challenges. By implementing technologies such as high-capacity chromatography resins, continuous processing, buffer concentrates, single-use systems, automation, and sustainable practices, manufacturers can significantly enhance the efficiency and productivity of downstream processes. These strategies aim to increase yields, reduce processing times, lower costs, and improve product quality, while minimizing resource usage and waste.

This paper illustrates how implementing process optimization strategies can maintain product quality and yield while also lowering labor, footprint, and consumable costs for downstream manufacturing. Additionally, efforts are focused on enhancing the sustainability of the entire production process. All economic modeling data presented were generated using BioSolve Process™ software, with assumptions detailed in Appendix I.

Maximizing mAb production efficiency and sustainability: the advantage of high-capacity capture chromatography resins

In downstream mAb production, optimizing recovery percentage and aggregate removal is important for maximizing yield and productivity. High recovery percentages allow more of the produced mAb to be retained, directly increasing yield and reducing raw material costs, thereby enhancing cost efficiency. Effective aggregate removal is vital for maintaining product quality and safety, as aggregates can affect the efficacy and stability of the final product. Efficient removal techniques minimize loss of the target mAb, helping to facilitate high yields and reducing the need for additional purification steps, which can streamline the process and increase throughput. Together, these factors contribute to a more efficient, cost-effective, and compliant production process, ultimately impacting the delivery of high-quality mAb products.

The first step in the purification train, affinity chromatography, consists of a specific ligand being immobilized on a chromatography resin to selectively bind and purify the target mAb from the complex mixture of cell culture supernatant. Chromatography resins and columns offered by Thermo Fisher are designed to facilitate high purity and yield of mAbs, enabling efficient capture and minimal loss of the target molecule. The Thermo Scientific™ MabCaptureC™ Protein A Resin enables high specific binding capacity and enhanced alkaline stability characteristics for consistent purification and resin regeneration. MabCaptureC Protein A Resin combined with the Thermo Scientific™ DynaChrom™ Single-Use Chromatography System helps provide robust and fast purification. The DynaChrom single-use (SU) chromatography system brings innovation, scalability, and robust automation to single-use downstream bioprocessing. It offers modular, single-use fluid transfer assemblies, a configurable system design with expandable features, and well-developed automation software enabling performance, flexibility, and speed in bioprocessing. Using the DynaChrom SU chromatography system in capture chromatography with MabCaptureC Protein A Resin enhances efficiency and throughput while maintaining high purity and yield.

In our study, MabCaptureC Protein A Resin was used in the first purification step to capture the example mAb molecule from harvested cell culture fluid (Figure 1). The process consisted of three wash steps to help reduce process and product-related impurities, followed by an elution step with acetate buffer. Inline dilution, which can mitigate separate buffer preparation steps and reduce processing time, was performed with the DynaChrom system to enhance downstream processing efficiency.

In the context of downstream mAb production, inline dilution is used to adjust the concentration of process streams, such as buffers or product solutions, directly within the production line. Using inline dilution with the DynaChrom system offers several benefits. First, it supports increased efficiency and throughput by enabling continuous processing, which streamlines the purification process and allows handling of higher volumes of product in a shorter time. Second, it reduces buffer consumption through precise control of the dilution process, allowing for more efficient use of buffers, lowering operational costs, and minimizing environmental impact. Last, it improves process control and consistency by helping to ensure accurate dilution ratios, resulting in consistent product quality, enhanced robustness of the purification process, and better compliance with regulatory standards.

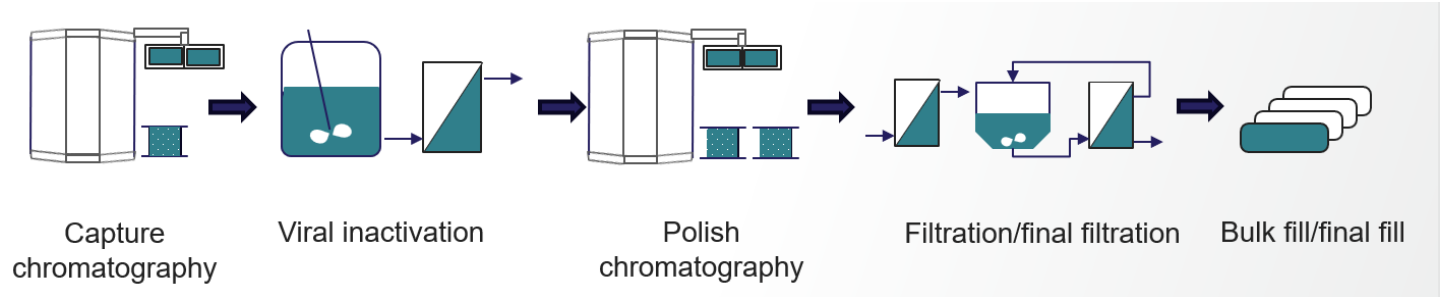

Figure 1. Overview of downstream process workflow for mAb production. The first purification step, capture chromatography, is followed by viral inactivation and a polish chromatography step. The polish chromatography stage consists of two polishing steps followed by filtration/final filtration and bulk fill/final fill steps.

The use of inline dilution with buffer concentrates helps to facilitate consistent buffer composition and minimize buffer consumption, leading to significant cost savings and reduced manufacturing footprint. The capture chromatography stage using inline dilution generated a yield of 95.5% of the target mAb captured from the column (data not shown). This high yield indicates efficient purification, with minimal loss of desired product.

Table 1. Impact of MabCaptureC Protein A Resin and inline dilution on mAb production efficiency.

	Standard process	MabCaptureC resin with inline dilution	% Improvement
Resin cost/ batch*	$43K	$29K	33% ($14K)*
Process liquids foot-print (m^2)†	267 ($1.6M)	206 ($1.2M)	23% ($400K)

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

The use of the high-capacity MabCaptureC Protein A Resin and inline dilution with the DynaChrom system helps to reduce the cost of the capture chromatography stage in the downstream mAb manufacturing process, as detailed in Table 1. This combination reduces the resin campaign cost by 33% from $43K to $29K and decreases the process liquids footprint by $400K. This optimization resulted in a more economical and efficient purification process.

Key takeaways:

- MabCaptureC Protein A Resin is a cost-effective resin that can support highly efficient purification with minimal loss of product. MabCaptureC Protein A Resin can help to reduce resin campaign costs by 33%.
- In addition, adoption of MabCaptureC Protein A Resin can help reduce the process liquids footprint by $400K.

Following the capture chromatography stage, a viral inactivation step was conducted to remove virus particles, followed by depth filtration to remove process-related impurities and particulates.

Boosting mAb efficiency: the impact of high-producing polish chromatography resins in flow-through mode

The polish chromatography stage in mAb manufacturing is necessary for removing host cell proteins, residual DNA, aggregates, viral contaminants, and other impurities. Although a critical step, resin chromatography does face a few bottlenecks, such as capacity limitations, flow rate constraints, buffer preparation challenges, process optimization complexities, and scalability issues. Overcoming these challenges is important for efficient and consistent mAb production and can be accomplished using Thermo Scientific™ POROS™ chromatography resins in flow-through mode combined with inline dilution using the DynaChrom single-use 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 the need for elution steps. In contrast, bind/elute mode involves binding the target molecule to the resin and then eluting it, which can be more time-consuming and complex. The combination of the POROS resins in flow-through mode with inline dilution using the DynaChrom system simplifies the downstream process, increases efficiency, reduces costs, and permits high-quality outcomes in mAb production.

The polish chromatography step used the Thermo Scientific™ POROS™ XQ Strong Anion Exchange (AEX) Resin in flow-through mode to remove negatively charged impurities such as endotoxins, DNA, host cell proteins, and viruses. This step resulted in 95% of the target mAb being captured from the column. This first AEX polishing step was followed by a second polishing step that used the Thermo Scientific™ POROS™ Benzyl Ultra Hydrophobic Interaction Chromatography (HIC) Resin in flow-through mode to remove high molecular weight species. The AEX flow-through product was adjusted to pH ~6 using acetic acid, and diluted to become HIC load material. The decision to use HIC resin in flow-through mode rather than the more traditional Thermo Scientific™ POROS™ XS Strong Cation Exchange (CEX) Resin in bind/elute mode depends on the impurity profile and process challenges of the individual production process. The HIC resin used in flow-through mode reduces buffer usage, which speeds up the manufacturing process and lowers reagent costs while still achieving comparable impurity reduction compared to the standard CEX resin.

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

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

* 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.

Similar to the capture chromatography step, the polish chromatography stage utilized inline dilution with the DynaChrom system for both the AEX and HIC purification steps. The use of inline dilution during this step continues to maintain consistent buffer composition and reduce buffer consumption throughout the downstream purification process. The polish chromatography stage generated a yield of 81% of the target mAb, which is comparable to an anticipated yield of 82% with the standard POROS CEX resin.

Table 3. Impact of combining MabCaptureC Protein A Resin, inline dilution, and HIC resin on mAb production efficiency and COGS.

	Standard	MabCaptureC resin, inline dilution, and HIC resin	% Difference
Downstream footprint (m^2)*	205 ($1.2M)	143 ($844K)	30% ($356K)
Total FTEs**	176 ($16.4M)	164 ($15.2M)	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 Protein A Resin, POROS AEX and HIC resins, and inline dilution with the DynaChrom chromatography system reduces downstream footprint, total FTEs, and resin cost per batch (Table 3), resulting in a more economical and efficient purification process.

Key takeaway:

- Combination of POROS AEX and HIC resins in flow-through mode enables robust impurity removal while reducing downstream footprint and total FTE expenses.

Following the polish chromatography steps, the eluate was processed through a final TFF step for concentration, buffer-exchange, and final formulation.

Enhancing mAb production with inline dilution and buffer strategies: resource utilization and efficiency gains

Since downstream mAb manufacturing processes utilize such large volumes of specific buffers and process liquids, minor reductions in individual buffer and process liquid usage can have a significant impact on the overall efficiency and cost of the entire downstream process. Process liquid and buffer usage can be reduced by using pre-made buffers or buffer concentrates. Gibco™ Process Liquid and Buffer Solutions are designed to enhance mAb manufacturing by offering pre-made buffers, buffer concentrates, and custom solutions. These products help enable consistent quality and composition, reducing preparation time, labor costs, and contamination risks. Manufactured under stringent quality control conditions, they facilitate reliable and reproducible results, supporting regulatory compliance and process verification. Available in various volumes and concentrations, Gibco™ solutions expediate easy scaling from development to large-scale production, ultimately enhancing efficiency, consistency, and cost-effectiveness in biopharmaceutical processes.

Outsourcing buffer preparation or using buffer concentrates can be highly beneficial when aiming to streamline operations and reduce labor costs. These solutions are particularly advantageous in scenarios where consistent buffer quality and composition are critical for reproducible results. Additionally, they are ideal for facilities looking to minimize the risk of contamination and reduce the time and resources spent on in-house buffer preparation. Buffer concentrates are especially useful when storage space is limited, as they require less volume and can be easily diluted to the desired concentration on-site. Overall, these approaches enhance efficiency, consistency, and cost-effectiveness in biopharmaceutical manufacturing.

To illustrate the potential cost savings from outsourcing buffer production or using buffer concentrates, economic modeling with BioSolve Process software was performed. By inputting detailed process parameters and cost data, the software simulated various scenarios and provided insight into cost savings, efficiency gains, and potential risks. This modeling helped quantify the benefits of 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 saving (new facility)	$8.5M

* Data were generated using BioSolve Process software, and assumptions of a generic in-house buffer preparation process (5,000 L process, 4 bioreactor facility, 63 batches/year operation) contributed to the economic 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.

By outsourcing buffer production, ~$2M/year can be saved compared to in-house buffer production (see assumptions in Table 4).

Reducing buffer quantities lowers material costs, helps reduce environmental impact, and enhances process efficiency by decreasing waste and storage requirements. Together, these strategies lead to higher output with fewer resources, lower operational expenses, and greater responsiveness to market demands, ultimately supporting an optimized overall mAb production process.

Key takeaways:

- Outsourcing buffer production and using buffer concentrates can potentially help save up to $2M/year.
- The DynaChrom single-use chromatography system can reduce manufacturing footprint by up to 25% (data not shown).

Conclusion

The optimization of downstream processes in mAb production is not merely a technical endeavor but a strategic means of getting a therapeutic drug to market more quickly while still maintaining safety and efficacy. As demonstrated, the implementation of advanced purification techniques such as high-capacity chromatography resins, inline dilution, and sustainable buffer strategies can enhance the efficiency, cost effectiveness, and sustainability of mAb manufacturing.

By leveraging high-capacity MabCaptureC Protein A Resin and the DynaChrom single-use chromatography system, manufacturers can achieve target yield and purity of the final product while reducing operational costs and labor requirements. The utilization of inline dilution further streamlines the process, helping ensure consistent buffer composition and minimizing resource usage. These advancements contribute to a more robust and scalable production framework, capable of meeting the growing demand for high-quality mAb therapies.

The polish chromatography stage, utilizing POROS™ resins in flow-through mode, exemplifies another opportunity for substantial cost savings and efficiency gains. This approach not only reduces buffer consumption and campaign costs but also maintains high product quality and throughput. The strategic use of buffer concentrates and outsourced buffer production further underscores the importance of resource optimization, offering significant reductions in labor, footprint, and environmental impact.

In conclusion, the adoption of these downstream optimization strategies helps manufacturers generate quality mAb products more efficiently. By addressing the critical bottlenecks in purification and buffer management, higher productivity, lower costs, and enhanced efficiency can be achieved. Ultimately, these advancements can help therapies to reach patients faster and more reliably, supporting the promise of precision medicine in the modern healthcare landscape.

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Appendix I

Assumptions for economic modeling data with BioSolve Process software:

- List price (no discount applied)
- Software used for labor and facility analysis
- 12-month campaign length, 80% facility efficiency
- 6 x 2,000 L bioreactor production process
- 18 batches per year or 80% utilization
- Standard process with seven seed train steps
- ~70% process yield
- 8% cost of capital
- Sustainability modeling assumes facility is in Massachusetts, USA
- mAb sale price of $300 per gram
- Process area cost based on a Class D cleanroom at a price of $5,900 m2
- FTE labor cost based on an average FTE annual compensation package of $93,000, unless otherwise noted

Appendix II

Disclaimer

The data and case studies presented in this document are provided for informational purposes only and are intended to illustrate potential process and economic outcomes associated with the use of bioproduction technologies offered by Thermo Fisher Scientific. The analyses include examples of standard fed-batch and intensified upstream processing, as well as traditional and intensified downstream protein purification workflows, modeled using BioSolve Process™ software by Biopharm Services.

All modeling and performance data are representative of actual running scenarios designed to demonstrate the possible value of process intensification strategies. These results are not intended to predict or guarantee actual process performance or financial outcomes. Process results, including yield, productivity, and cost efficiency, will vary based on multiple factors such as equipment configuration, process parameters, scale, and facility design.

The data illustrate how the combination of high-performing media, optimized feed strategies, and intensified bioreactor operation can enhance productivity and operational efficiency in upstream cell culture, while integration with intensified downstream purification approaches can further improve throughput, reduce the cost of consumables and labor, and enhance process sustainability.

The example comparison between standard and intensified workflows—such as potential yield improvements from an industry average of 4 g/L to approximately 8.4 g/L—is provided to demonstrate the potential benefits of integrated intensification strategies.

Thermo Fisher Scientific does not guarantee or warrant specific outcomes for any customer implementation. Each process must be evaluated and optimized based on individual operational and technical requirements.

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