

IgG purification

Intensification of plasma-derived IgG manufacturing through an affinity-based purification platform

Abstract

The conventional production of IgG from human plasma primarily relies on ethanol-based fractionation techniques. Although well-established, these methods often involve compromises between yield and purity, have a high level of process complexity, and require the use of large amounts of organic solvents and energy, leading to environmental concerns. This study presents a novel purification strategy using Thermo Scientific™ CaptureSelect™ FcXP Affinity Matrix as an efficient and scalable alternative for IgG isolation from human plasma. This affinity chromatography-based platform is a flexible and efficient alternative to plasma fractionation that eliminates the need for multistep cooled-ethanol precipitation cascades, resulting in a more environmentally friendly process, and most importantly, delivering yields that exceed those obtained through conventional fractionation.

Introduction

The global market for intravenous IgG (IVIG) continues to show strong growth, expanding at an estimated annual rate of approximately 7%. This increase is driven by the growing awareness and diagnosis of primary and secondary immunodeficiencies, the expansion of clinical uses, and an aging population with increased immunological needs [1]. As a result, the demand for plasma-derived therapies has increased, putting significant pressure on the plasma supply chain and prompting manufacturers to review and improve their purification technologies. The future of therapy using plasma-derived products depends on the global plasma supply. Currently, over 65% of all collected plasma comes from the United States, creating an imbalance in the global supply chain. Consequently, every liter of plasma has become strategically valuable, and production processes are under close review to optimize IgG recovery without sacrificing safety, quality, or cost [2].

Developed in the 1940s, the ethanol-based Cohn fractionation process remains the basis of commercial manufacturing processes for extracting therapeutic proteins, such as IgG, from pooled plasma. By alteration of pH, ethanol concentration, and temperature, proteins are separated into fractions via precipitation [3]. In most processes, Cohn fractions are used as crude starting materials that are purified through chromatography. These processes are well-established and ensure the quality and safety of plasma-derived therapeutic products. However, there are significant limitations in target recovery and environmental challenges due to the large volumes of ethanol used during production.

Large-scale chromatography has played a crucial role in enhancing both the purity and yield of proteins extracted from plasma. This technology has also supported the expansion of therapeutic plasma product offerings and improved the overall cost efficiency of plasma fractionation processes [4]. Since the previous century, affinity chromatography has been introduced in industrial plasma purification processes to purify plasma proteins such as albumin, von Willebrand factor, and factor VIII. However, capturing IgG directly from plasma or initial fractions remains a challenge [5].

Here we present a novel affinity chromatography-based purification method utilizing CaptureSelect FcXP Affinity Matrix as an efficient and scalable alternative for isolating IgG from human plasma. CaptureSelect FcXP Affinity Matrix effectively delivers high-purity IgG in higher yields than traditional fractionation methods. It also enables recovery from side fractions where IgG is usually lost during fractionation, resulting in a significant increase in overall yield. This affinity-based platform is a flexible and efficient alternative to multistep ethanol precipitation cascades for intensified plasma-derived manufacturing of therapeutics.

CaptureSelect FcXP Affinity Matrix: a novel solution for improved plasma-derived IgG purification

Thermo Scientific™ CaptureSelect™ affinity resins are designed to simplify workflows and reduce time and cost in biopharmaceutical drug development. These resins are based on single-domain antibody fragments (V_HH), which provide full functionality in antigen-specific recognition and high-affinity binding. These small ligands (~15 kDa) are the platform solution for many biopharmaceutical purification challenges, enabling higher purity and yield in a single purification step. CaptureSelect affinity resins are manufactured in an animal origin-free production process.

CaptureSelect FcXP Affinity Matrix has been designed to specifically bind the Fc region of IgG molecules (Figure 1). It is highly selective for the C_{H3} region of the IgG heavy chain and recognizes all IgG subclasses (IgG1–4). Elution can be performed under mild conditions (pH 4.0–5.0). The CaptureSelect FcXP ligand is available on a cross-linked agarose support or on rigid Thermo Scientific™ POROS™ beads, providing flexibility to customers according to their process needs.

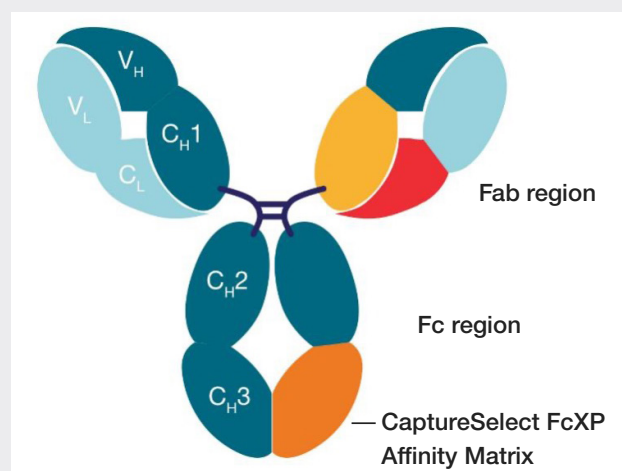


Figure 1. Selectivity of CaptureSelect FcXP Affinity Matrix for the C_{H3} region. Other CaptureSelect products are available with selectivity for the C_{H1} region (yellow) or C_L region (red).

Study methods

General purification setup

The CaptureSelect FcXP resin cycling study was conducted using the following purification setup (Figure 2). The process began with column equilibration using PBS buffer (pH 7.4), followed by sample loading with cryo-poor plasma (CPP).* Two intermediate washes were performed, first with PBS and then with 0.5 M NaCl in PBS, to remove nonspecific binding. Elution was performed using 0.02 M acetic acid (pH 4.0), and a final strip step was performed with 0.1 M glycine (pH 2.0) to clean the column. The column was then re-equilibrated with PBS. A clean-in-place (CIP) step using 0.1 M NaOH for 20 minutes was performed every five cycles.

*CPP is obtained from fresh-frozen plasma (FFP), which is collected from whole blood or plasma donations and frozen within 8 hours of collection. Thawed plasma is centrifuged to separate the liquid component from the cryoprecipitate. Individual cryoprecipitate units are connected to a pooling harness bag set and combined to create a pool. This pooling process produces a single, therapeutically effective blood product.

Column details

The column used was a 5 mL Thermo Scientific™ CaptureSelect™ FcXP MiniChrom™ Pre-packed Column (agarose support) with a dynamic binding capacity of 39.1 mg/mL IgG at 10% breakthrough. IgG samples were processed as indicated in Table 1.

Table 1. Purification parameters.

Parameter	Value
Load concentration	7.3 g/L IgG
Load volume	21.4 mL
Sample flow rate	1 mL/min
Sample residence time	5 min

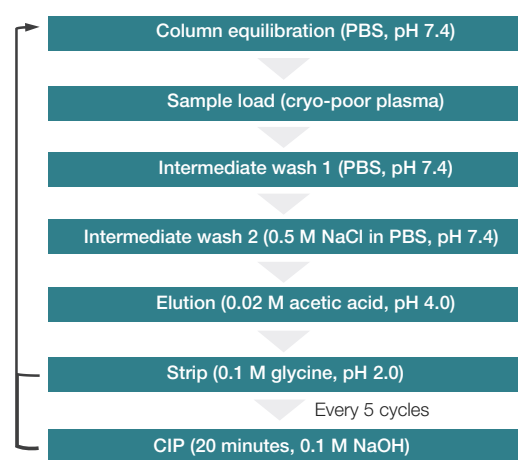


Figure 2. Summary of the purification setup for the cycling study.

Analytical testing

Analytical tests were performed on the elution pools of each run to evaluate different process parameters (Table 2).

Table 2. Summary of analytical tests.

Process parameter	Analytical test
Load concentration	Based on total protein concentration using A_{280} , and IgG concentration using nephelometry
IgG subclass distribution	Nephelometry
IgA, IgM	Nephelometry and ELISA
Factor XIa activity	Coagulation test: activity calculated on STA Coag Expert™ Software
Ligand leaching	Commercially available CaptureSelect FcXP Leakage ELISA
Monomer purity	Size-exclusion chromatography and SDS-PAGE
Overall productivity	Calculation based on yield and time

Results

IgG mass, yield, and recovery

The IgG masses and yields (percentage of IgG in the eluate) obtained are presented in Figure 3. The amount of IgG present in the eluate after each cycle remained stable at approximately 143–145 mg (dark blue bars), and the yield was consistently above 90% in most cycles (red dots). Resin recovery (percentage recovered from all fractions) was >94% during all cycles. These results indicate robust resin performance.

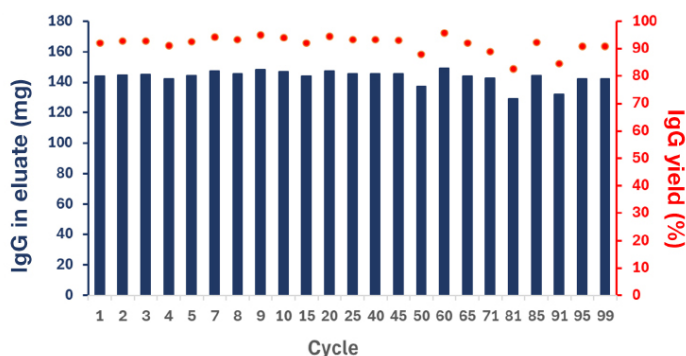


Figure 3. Minimal changes observed in IgG mass (mg) and yield (%) in the eluates in all 100 cycles. A selection of representative runs is shown.

IgG subclass distribution

To maintain biological function and ensure patient safety, it is essential that the IgG subclass distribution in the final therapeutic product remain consistent and not be impacted by resin reuse. Subclass distribution during the cycling study was analyzed using nephelometry. Figure 4 shows the subclass distribution in one out of every 10 runs, compared to the CPP pool. IgG subclass distribution after affinity purification was well-maintained over time and remained comparable to the distribution pattern in the starting material.

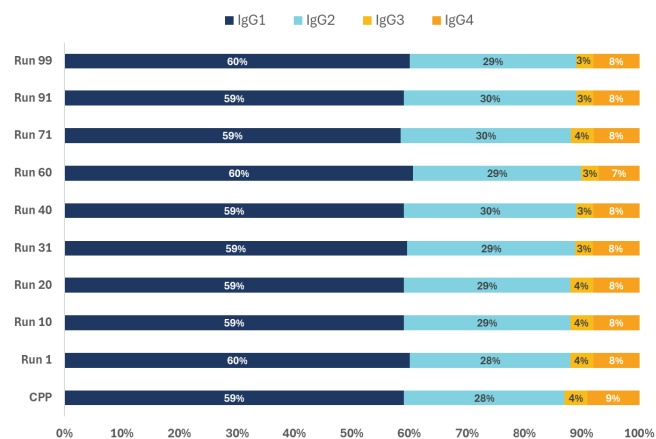


Figure 4. IgG subclass distribution measured during the cycling, compared to the starting material (CPP). Results show a well-maintained subclass distribution. Some runs do not sum to 100% due to rounding error.

Impurity depletion

To reduce the risk of adverse effects, such as unwanted immune activation or coagulation issues, the IgA, IgM, and factor XIa (FXIa) levels in the final therapeutic product should be kept within the limits set by regulatory agencies. Thorough impurity depletion during the capture step helps to improve overall process yield and reduces the burden on subsequent downstream purification steps. Resin reuse and cleaning should not lead to a decrease in the impurity removal capability over time. During this study, IgA and IgM removal and remaining FXIa activity were measured over all cycles. Figures 5A and 5B show the levels of IgA and IgM depletion (red lines) and the impurity percentage in the pools (blue lines), for IgA and IgM, respectively. Figure 5C shows the levels of depletion (red line) and remaining FXIa activity (blue line). These results clearly demonstrate excellent and consistent impurity depletion that is maintained over time.

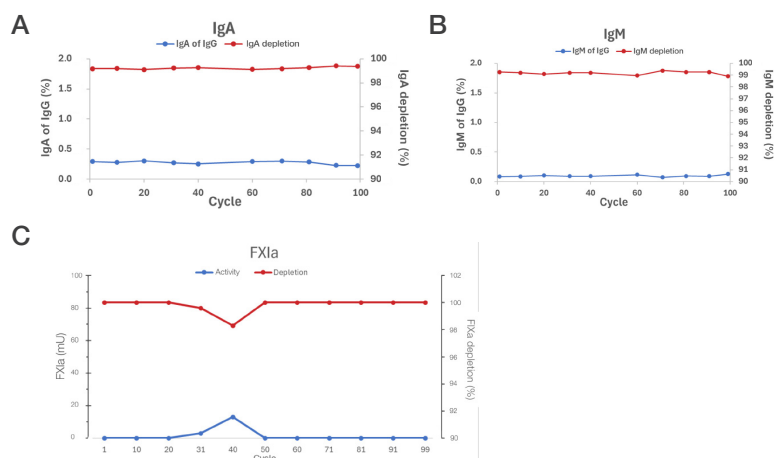


Figure 5. Depletion of impurities. (A) IgA and (B) IgM levels are depicted as percent depletion (red line) and percent of IgG content. (C) FXIa levels are depicted as percent depletion (red line) and remaining activity (blue line). The results show the high and consistent impurity-depleting capability of the resin.

Ligand leaching

When affinity chromatography is used in a biotherapeutic production process, monitoring of leached ligand is mandated by regulatory agencies. Ligand leaching was evaluated using the Thermo Scientific™ CaptureSelect™ FcXP Ligand Leakage ELISA. Results show low levels of leached ligand in the eluates and no increase in ligand leaching over time (Table 3).

Table 3. Monitoring of ligand leaching.

Cycle	Leached ligand concentration (ppm)
1	2.3 ± 0.5
20	1.0 ± 0.4
40	0.8 ± 0.3
60	1.0 ± 0.6
81	1.9 ± 0.5
91	1.4 ± 0.3
99	1.8 ± 0.5

Monomer purity

Monomer purity was determined by SDS-PAGE and size-exclusion chromatography (SEC) in the elution pools of cycles 1, 40, 60, 81, 91, and 99. The results reveal consistently high purity of >99% and no

impact of resin reuse or CIP. The SDS-PAGE results (Figure 6A) consistently show one band for the elution pools, and these same fractions return one clear peak in the SEC results (Figure 6B).

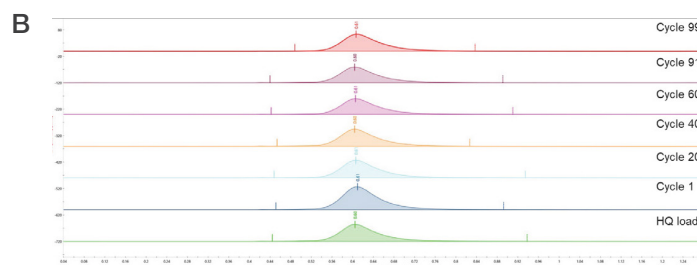
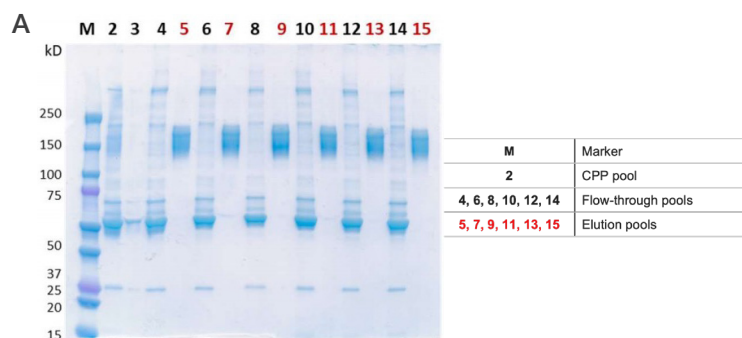


Figure 6. Evaluation of monomer purity. (A) Nonreduced SDS-PAGE of the CPP pool (lane 2), flow-through pools (lanes 4, 6, 8, 10, 12, 14), and the elution pools (lanes 5, 7, 9, 11, 13, 15) of 6 cycles evaluated during the cycling study. The lanes of the elution pools consistently show a single band. (B) The elution pools of cycles 1, 40, 60, 81, 91, and 99 were analyzed using SEC (column: Tosoh Bioscience G3000SW, 7.5 x 600 mm, 10 μm particle size). The chromatograms of all tested cycles reveal a single clear peak.

Resin productivity

To facilitate sustained process efficiency, the productivity of the resin should remain stable, as demonstrated in Figure 7. The resin productivity of CaptureSelect FcXP Affinity Matrix was calculated with and without CIP as part of the process, and the results showed stable productivity over time.

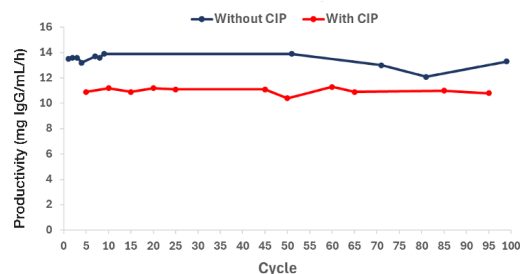


Figure 7. Resin productivity of CaptureSelect FcXP Affinity Matrix, calculated with and without CIP.

Conclusion

This study demonstrates that CaptureSelect FcXP Affinity Matrix enables highly efficient purification of plasma-derived human IgG in a single step. The results show consistently high yield and recovery. Monomer purity remains high across multiple cycles while maintaining IgG subclass distribution. The resin effectively removes unwanted impurities such as IgA, IgM, and procoagulant FXIa.

CaptureSelect FcXP resin is a scalable platform solution for purifying human IgG, helping to enhance the productivity of therapeutic IgG manufacturing processes. The reduction of ethanol use during the manufacturing process contributes positively to overall sustainability and operational safety. Additionally, this method can facilitate the recovery of IgG from side fractions, where it is typically lost during fractionation, resulting in a significant increase in overall yield.

Future recommendations

Even though the affinity elution pool already meets the purity requirements for plasma-derived therapeutics, some customers may prefer to add a polishing step to further remove residual traces of IgA, IgM, FXIa, and other impurities. For ultralow-IgA formulations, anion exchange (AEX) polishing with Thermo Scientific™ POROS™ HQ resin is particularly beneficial. Incorporating an AEX polishing step can also contribute to viral clearance. Optionally, other plasma proteins can be recovered from the flow-through fraction of the FcXP affinity column by using CaptureSelect affinity resins.

Considerations for choosing a resin support

CaptureSelect affinity resins can be produced on either agarose or Thermo Scientific™ POROS™ beads. POROS chromatography beads have several physical features that make resins based on this backbone very effective. The smaller bead size provides high resolution, and the large through-pores, which increase the surface area, help ensure very efficient purification. The beads are made of a rigid synthetic polymer that allows for the use of higher pressure and flow rates. The combination of these features makes POROS resins highly productive.

However, this may come with a trade-off in binding capacity. Agarose resins usually offer higher binding capacity but generally operate at lower flow rates. The CaptureSelect FcXP ligand is available on either agarose or POROS chromatography beads. The specificity of the ligand remains the same on both resins, but the final choice of the resin backbone will depend on the specific process requirements.

Summary of resin supports for CaptureSelect FcXP ligand

Agarose resins	POROS beads
Made of highly cross-linked agarose	Made of a rigid synthetic polymer
Tend to operate at lower flow rates	Large pore structure, allowing fast mass transfer
Typically provide higher binding capacities	High productivity
Commonly used in affinity chromatography	Designed for high-speed chromatography

Authors

Michael Andesner, Kevin Sleijpen, Gregor Richter, Frank Detmers, Thermo Fisher Scientific.

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Ordering information

Product	Quantity	Cat. No.
CaptureSelect FcXP Affinity Matrix	5 mL	1943712005
	10 mL	1943712010
	50 mL	1943712050
	250 mL	1943712250
	1 L	194371201L
	5 L	194371205L

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