Application note

Astrea Bioseparations
A Biotage Company

Polishing mAb derived host cell proteins with HCPure™ mixed-mode adsorbent

Protein A adsorbents are commonly used as the first chromatography step in downstream purification to capture monoclonal antibodies (mAbs) and fragments containing an Fc region. Intermediate and polishing steps are incorporated after the capture step to achieve the final purity required for the intended application. These chromatographic steps remove residual impurities, including host cell proteins (HCPs), host cell DNA, aggregates, leached Protein A, endotoxin, and a variety of viruses. This application note describes the optimal conditions for the use of HCPure™ mixed-mode adsorbent for the polishing of mAb derived host cell proteins.

The primary capture of mAbs and fragments within this application note, use the GORE® Protein Capture Device, a composite PTFE membrane with a native-Protein A ligand. Use of intermediate washes with high salt, low pH, or arginine were evaluated for optimizing HCP removal on the GORE® Protein Capture Device. Post-affinity mAbs and fragments were then polished using HCPure™, a novel adsorbent consisting of a proprietary mixed-mode ligand on beaded agarose.

HCPure™: A unique mixed-mode adsorbent

HCPure™ primarily utilizes hydrogen bonding and hydrophobic interaction chromatography to bind host cell impurities while the target protein remains unbound. Figure 1 visualizes the chemical diversity of mixed-mode adsorbers, including HCPure™ resin, by mapping ligand structures based on their functional group similarities. Each point represents a different resin, with its position determined by the properties of its ligand groups rather than a simple characteristic such as charge.

Clusters in the middle of the graph indicate resins with similar ligand structures, suggesting comparable binding behaviors. In contrast, HCPure™ resin is positioned distinctly to the right, highlighting its unique ligand composition and different mode of action.

The distinct ligand chemistry of HCPure™ resin, as shown in Figure 1, offers unique purification advantages compared to other resins with similar structures that may behave alike. This underscores the importance of selecting appropriate adsorbents for specific purification needs. This reinforces the value of a toolbox approach, allowing for resin selection based on specific process needs rather than a one-size-fits-all solution.

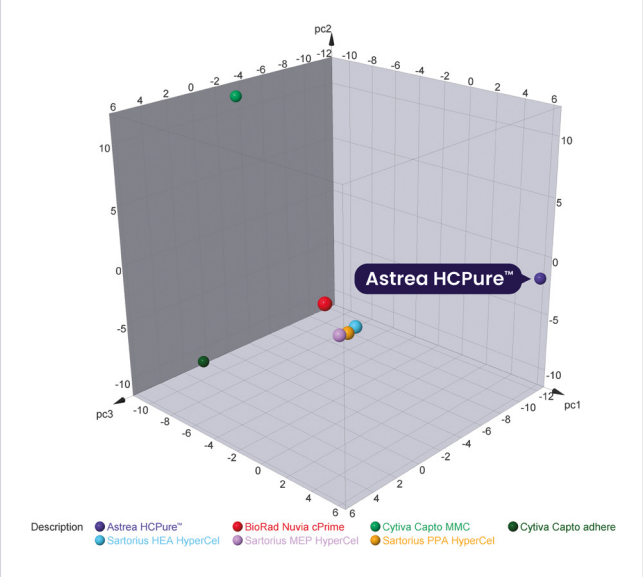

Figure 1: Chemical space diagram of commercial mixed-mode adsorbents.

Exploring the pH wash regime for Protein A capture

Clarified immunoglobulin G (IgG) produced in Chinese hamster ovary (CHO) cells was loaded onto a 1 mL GORE® Protein Capture Device with Protein A equilibrated with Phosphate-Buffered Saline (PBS). The device was washed with PBS, followed by a high-salt wash of PBS and 1.8 M NaCl. The salt was washed off with PBS before being eluted with 100 mM sodium citrate pH 3.4. An identical run was performed with a 100 mM sodium citrate pH 5 wash added prior to elution. IgG concentration was measured by UV, while aggregates, HCP levels, and HC DNA were assessed using SEC HPLC, ELISA, and PicoGreen, respectively. Table 1 summarizes the analytical results, showing that the run with the pH 5 wash had a similar impurity profile but a lower IgG yield.

Reverse-phase HPLC	IgG recovery (mg)	IgG recovery (%)	Aggregate (%)	HCP concentration (ng/mg IgG)	HC DNA concentration (ng/mg IgG)
Control	22	83	7	185	9
With pH 5 wash	12	44	4	184	11

Table 1: Comparison of IgG recovery and various impurity levels between the control capture step and the capture step with a pH wash.

Exploring an arginine wash regime for Protein A primary capture

An additional batch of CHO IgG was purified with two additional runs on a 9 mL GORE® Protein Capture Device. One of the runs eluted the IgG immediately after the PBS post-load wash, while the other was run with a high-salt wash, followed by an arginine wash prior to elution (Table 2). IgG concentration was measured by UV280, and HCP was measured by ELISA. High-salt and arginine washes reduced HCP impurities by approximately threefold while having minimal impact on IgG yield (Figure 2).

Step	Buffer
Equilibration	PBS
Load	CHO harvest
PLW	PBS
High-salt wash	PBS + 1.8 M NaCl
EQ wash	PBS
Arginine wash	**PBS + 0.1 M arginine pH 7.5**
Elution	100 mM sodium citrate pH 3.4
Strip	0.1 M citric acid
CIP	0.1 M NaOH

Table 2: Conditions for Protein A primary capture, including an arginine wash step.

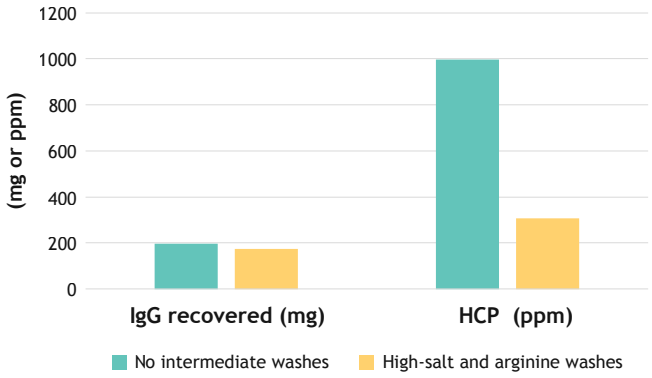

Figure 2: Comparison of HCP content post-Protein A with high-salt and arginine washes.

Flow rate recovery of GORE® Protein Capture Device during cleaning

The same 9 mL device was used for more than 20 cycles of CHO IgG feedstock. The flow rate was automatically reduced to maintain the pressure below the limit (0.4 MPa), indicating an increase of pressure in subsequent runs (Figure 3). The flow rate was recovered after the extensive cleaning method (1), as indicated by the teal bars below (Figure 3).

Polishing host cell impurities with HCPure™ adsorbent

HCPure™ is a tunable resin effective for post-affinity polishing of full-length IgG, IgG fragments, and bispecific antibodies from CHO, HEK293, *E. coli*, and Pichia cell lines. Optimal running conditions for HCPure™ resin rely on the host cell and the target protein, therefore a screen of pH (4–9) and conductivities (6–18 mS/cm) should be performed for each target. Astrea Bioseparations offers 0.2 mL RoboColumns® and 1 mL pre-packed columns with HCPure™ which are invaluable tools for screening.

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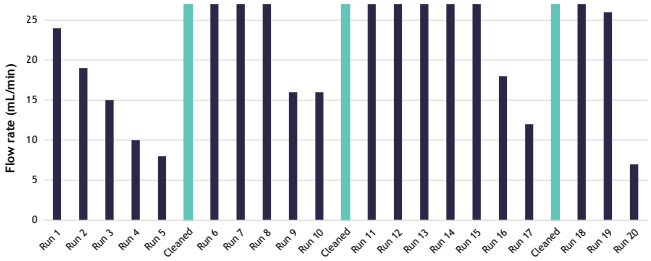
Extended cleaning method recovers flow rate on GORE® Protein A device loading



Buffer	CV
200 mM arginine pH 2.3	5
50 mM Tris, 100 mM NaCl, 1% SDS, 10 mM DTT pH 10.4	10
(10-minute hold)	
200 mM arginine pH 2.3	10
0.2 M NaOH	3.6
PBS	10

(1) GORE® cleaning regime

Figure 3: Effect on extended cleaning on the GORE® Protein A device flow rate.

Broad range screen for buffer conditions using HCPure™ adsorbent

A broad-range pH and conductivity screen was performed at the extremes of the recommended range in the HCPure™ user guide (pH 4-9, 8-18 mS/cm), using RoboColumns® for a factorial design of experiment (DOE) approach.

For this study, the post-Protein A neutralized elution fraction was buffer-exchanged into the following buffer conditions. An additional test condition of the neutralized elution without buffer exchange was also included. Three replicates of the midpoint were included for precision analysis.

- 50 mM Sodium citrate, pH 4, 8 mS/cm
- 50 mM Sodium citrate, pH 4, 18 mS/cm
- 50 mM Tris, pH 9, 8 ms/cm
- 50 mM Tris, pH 9, 18 ms/cm
- Midpoint: 50 mM Sodium phosphate, pH 6.5, 13 mS/cm
- Neutralized elution: ~80 mM Citrate, ~160 mM Tris, pH 6, 12 mS/cm

For this feedstock, IgG recovery and HCP clearance performed consistently strong across all conditions tested (Figure 4), with good repeatability observed between the three replicates of the midpoint. The neutralized elution without buffer exchange performed equally well, offering the added advantage of elimitnating the need to condition the feedstock between the Protein A and HCPure™ steps.

In another example, a post-Protein A elution fraction of Vκ-overexpressed in *E. coli* was screened for HCP removal on HCPure™ RoboColumns®, using a factorial DOE with the following pH and conductivity conditions in 40 mM sodium phosphate:

- pH 6, 6 mS/cm
- pH 6, 18 mS/cm
- pH 7, 12 mS/cm
- pH 8, 6 mS/cm
- pH 8, 6 mS/cm

In this example, the DOE identified a significant improvement in HCP reduction at pH 6 compared to pH 8, from 1.6 log to 2 log (Figure 5). The Vκ yield ranged from 75-96; however, no significant correlation was observed between the Vκ yield and pH or conductivity parameters. Based on the predictions from Design-Expert® 13 software, optimal conditions of pH 6 and 18 mS/cm were expected to achieve a 2.19 log reduction in HCPs and an 89% yield of the Vκ fragment.

These two examples demonstrate the importance of optimizing HCPure™ resin conditions based on the specific requirements of each target protein.

Screening pH and conductivity conditions for IgG recovery and HCP reduction on HCPure™

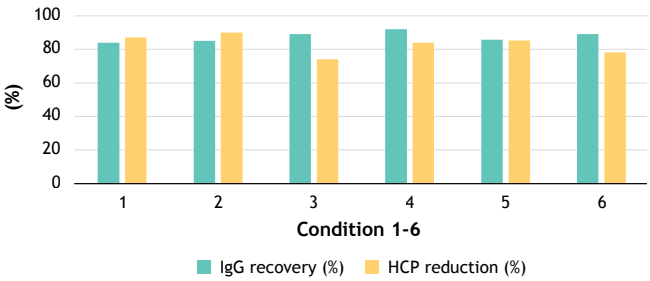


Figure 4: Broad range screen for HCP removal conditions from a CHO-produced IgG using HCPure™ resin.

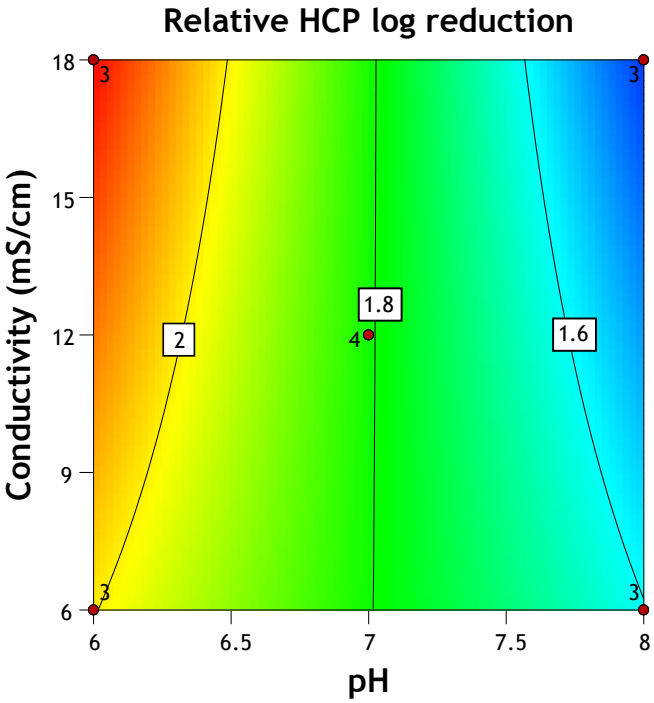


Figure 5: Heat map illustrating the impact of various pH and conductivity conditions on HCP removal efficiency for a Vκ fragment produced in *E. coli*. The analysis suggests optimal conditions for both HCP reduction and Vκ yield represented by the red regions. Sub-optimal conditions are highlighted by the blue regions.. These examples demonstrate the importance of optimizing HCPure™ conditions to the specific requirements of each target protein.

Polishing of HEK293 IgG using HCPure™ adsorbent

A mAb produced in HEK293 cells was purified using a GORE® Protein Capture Device, followed by polishing with HCPure™ adsorbent. The column was equilibrated with 40 mM sodium phosphate, 160 mM NaCl, pH 6, 18 mS/cm. The Protein A elution fraction was dialyzed into the equilibration buffer before loading 30 mg on to a 1 mL HCPure™ pre-packed column. Unbound IgG was collected, and its concentration was measured by UV. HCP and Protein A leachate were measured by ELISA, HC DNA using PicoGreen, and endotoxin levels using a limulus amebocyte lysate (LAL) assay.

The IgG recovery was 80% with a significant reduction of HCP, dsDNA, Protein A leachate, and endotoxin impurities (Figure 6).

Conclusion

These studies demonstrate the effectiveness of HCPure™ mixed-mode adsorbent as a polishing step across multiple expression systems, for the removal of HCP, Protein A leachate, and other impurities following Protein A capture. High-salt and arginine washes during Protein A chromatography significantly reduced HCP levels while maintaining IgG yield. Further purification with HCPure™ resin efficiently reduced residual impurities, including HCP, host cell DNA, Protein A leachate, and endotoxin, bringing them within regulatory guidelines.

By utilizing a unique chemical space compared to other commercially available adsorbents, HCPure™ offers a valuable addition to a purification toolbox. HCPure™ resin's unique mixed-mode ligand chemistry provides a versatile and effective solution for polishing full-length IgG, IgG fragments, and bispecific antibodies across multiple expression systems, including CHO and HEK293. Its ability to selectively remove impurities while preserving target protein yield reinforces its value as a robust tool for achieving high-purity biologics. By screening each target protein and optimizing wash and polishing conditions, researchers and manufacturers can enhance process efficiency and meet stringent purity requirements for downstream applications.

References

(1) GORE® application note: Cleaning protocols to reduce the effects of pressure rise over the lifetime of a GORE® Protein Capture Device

(2) Molden R, et al. (2021). Host cell protein profiling of commercial therapeutic protein drugs as a benchmark for monoclonal antibody-based therapeutic protein development. mAbs, 13(1). https://doi.org/10.1080/19420862.2021.1955811

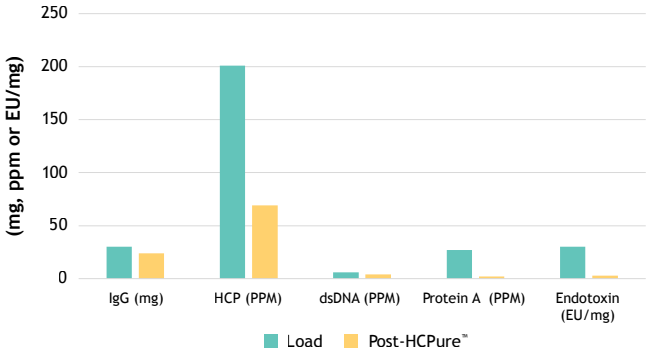


Figure 6: Impurity removal from HEK IgG with a HCPure™ adsorbent polishing step.

Astrea Bioseparations is a world class provider of chromatography adsorbent and resin services. With over 30 years of chromatography manufacturing expertise, we deliver a unique and trusted service in close partnership with our clients. For more information, please don't hesitate to reach out at sales@astrea-bio.com or visit astreabioseparations.com.