Polishing host cell proteins with HCPure™ mixed-mode adsorbent

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Abstract

The use of Protein A adsorbents to capture monoclonal antibodies is a routine process step. However, to reach the purity required, intermediate and polishing steps are often employed in multistep purification processes. Here we demonstrate clearance of host cell impurities with a novel adsorbent comprising a proprietary mixed-mode ligand on beaded agarose, HCPure™.

Primary capture of monoclonal antibodies and fragments was achieved with the GORE® Protein Capture Device with Protein A, a composite PTFE membrane with a native Protein A ligand. The use of intermediate washes with high salt, low pH, or arginine were investigated for optimizing host cell protein removal on the GORE® Protein Capture Device. A stringent wash regime was demonstrated to recover pressure increases to allow more than 20 cycles on the same device.

Post-affinity mAbs and fragments were then polished using HCPure™, to remove host cell impurities such as host cell proteins (HCP) and DNA (HC DNA), aggregates, Protein A leachate, viruses, and endotoxin. HCPure™ adsorbent was highly effective for post-Protein A polishing of an IgG produced in CHO and HEK293 cells.

1 Exploring the pH wash regime for Protein A capture

Clarified CHO IgG was loaded on to a 1 mL GORE® Protein Capture Device with Protein A equilibrated with PBS. The device was washed with PBS followed by a high salt wash of PBS + 1.8 M NaCl. The salt was washed off with PBS before being eluted with 100 mM sodium citrate pH 3.4. As a comparison to this, an identical run was performed with a 100 mM sodium citrate pH 5 wash added prior to the elution.

The run with the pH 5 wash had a similar impurity profile but a lower IgG yield.

	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

IgG concentration was measured by analytical Protein A HPLC, aggregates by SEC HPLC, host cell protein (HCP) by ELISA and HC DNA by PicoGreen.

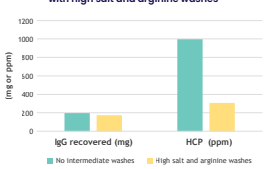
2 Exploring an arginine wash regime for Protein A primary capture

An additional batch of CHO IgG was purified with two further 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 the elution.

Three times less host cell protein impurities were eluted with the high salt and arginine washes, while still demonstrating minimal impact on IgG yield.

Step	Buffer
Equilibration	PBS
Load	CHO harvest
Post-load wash	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

Comparing HCP content post Protein A with high salt and arginine washes



IgG concentration was measured by UV280 and host cell protein (HCP) by ELISA

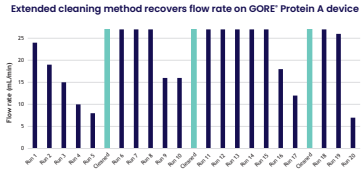
3 Flow rate recovery of GORE® Protein Capture Device during cleaning

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

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

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



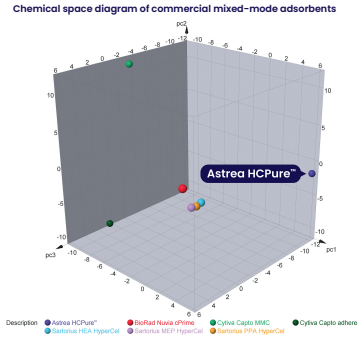
4 Polishing host cell impurities with HCPure™ adsorbent

HCPure™ is a mixed-mode adsorbent which has a unique chemical space compared to other commercially available adsorbents. HCPure™ primarily utilizes hydrogen bonding and hydrophobic interaction chromatography to bind host cell impurities whilst leaving the target protein in the non-bound.

HCPure™ has been shown effective for post-affinity polishing of full-length IgG, IgG fragments, and Bispecific IgG from CHO, HEK293, E. coli, and Pichia cell lines.

The optimal running conditions for HCPure™ relies on the host cell and the target protein. A screen of pH (4 - 9) and conductivities (6 - 18 mS/cm) should be performed for each target.

Chemical space diagram of commercial mixed-mode adsorbents



5 Polishing of CHO IgG using HCPure™ adsorbent

This Protein A purified CHO IgG had relatively low impurities, but the host cell protein (HCP) concentration is above the FDA recommended 100 PPM (2). Astrea Bioseparations' HCPure™ adsorbent was used as a polishing step following the Protein A device.

50 mg of IgG from a neutralized Protein A elution fraction was loaded directly on to a 1 mL HCPure™ pre-packed column in flow through mode. The column was equilibrated and washed with 80 mM sodium citrate, 170 mM Tris pH 6, 12 mS/cm to match the buffer conditions of the neutralized Protein A elution. The IgG was recovered in the flow through.

The IgG recovery was 96% and the HCP was reduced from 380 PPM to 50 PPM, within the FDA recommendation.

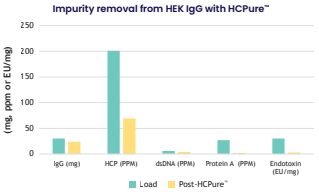
	IgG recovery (mg)	Aggregate (%)	HCP concentration (ppb)	HC DNA concentration (ppb)	Protein A leachate (ppb)
Post-Protein A	80	1	465	652	4
Post-HCPure™	96	1	50	559	2

IgG concentration was measured by UV, aggregates by SEC HPLC, host cell protein (HCP) and Protein A leachate by ELISA and HC DNA by PicoGreen.

6 Polishing of HEK293 IgG using HCPure™ adsorbent

An additional mAb produced in HEK293 cells was purified with 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 dialysed into the equilibration buffer then 30 mg loaded on to a 1 mL HCPure™ pre-packed column and the IgG collected in the non-bound. The IgG recovery was 80% and had reduction of HCP, dsDNA, Protein A leachate and endotoxin impurities.

Impurity removal from HEK IgG with HCPure™



IgG concentration was measured by UV, host cell protein (HCP) and Protein A leachate by ELISA, HC DNA by PicoGreen and endotoxin by LAL assay

Summary

HCPure™ adsorbent is a mixed-mode chromatography resin which can be used for polishing of post-Protein A purified products. Reduction of host cell proteins, host cell DNA, Protein A leachate and endotoxin from IgG produced in CHO and HEK293 cells lines is shown. By utilizing a unique chemical space compared to other commercially available adsorbents, HCPure™ offers a valuable addition to a purification toolbox.

(1) GORE® application note: Cleaning protocols to reduce the effects of pressure rise over the lifetime of a GORE® Protein Capture Device
(2) doi: 10.1080/19420862.2021.1955811
HCPure™ Adsorbent is available at astreabioseparations.com

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