Three-step monoclonal antibody purification processes using modern chromatography media

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Three-step monoclonal antibody purification processes using modern chromatography media

This application note describes monoclonal antibody (MAb) purification processes using the expanded MAb purification toolbox of GE Healthcare's Life Sciences business, covering modern chromatography media (resins) for standard as well as more challenging purification tasks. The most established approach for purifying MAbs is a three-step process, where the initial capture step using a protein A medium is followed by two polishing steps using cation exchange (CIEX) and anion exchange (AIEX) media. Typical results from such an approach will be presented. However, for more challenging MAb purifications, an expanded MAb purification toolbox is beneficial. A study, in which a multimodal Capto adhere ImpRes AIEX medium was used as an alternative in the final purification step, will be presented. Using this second approach, the set criteria for the polishing steps were achieved, with a recovery of above 90% over each step and an aggregate content below 1% in the final product.

Introduction

As a class of molecules, MAbs exhibit many shared properties, making them well-suited for a platform approach to downstream bioprocessing. A three-step downstream chromatography purification platform commonly includes a protein A-based capture step, an initial polishing step using a CIEX medium in bind-elute (B/E) mode, and a second polishing step using an AIEX medium in flow-through (FT) mode. MabSelect SuRe™ LX is well suited for the capture step, with high dynamic binding capacities (generally above 60 mg/mL) and high alkali stability that enables cleaning under harsh conditions. Capto™ S ImpAct and Capto Q media are suitable options for the polishing steps.

Capto S ImpAct is a strong CIEX medium designed for MAb polishing. The medium has a polymer-grafted ligand that combines a surface extender with functional groups, resulting in high binding capacity. At the same time, this medium displays good resolution between MAb monomer and impurities, resulting in efficient removal of host cell proteins (HCP); leached protein A ligand; and MAb aggregates, fragments, and other isoforms during elution. Capto Q is a traditional high-capacity AIEX medium, typically operated in FT mode. The functional group of this medium, a quaternary amine, is linked to the matrix via a dextran surface extender that increases the capacity for DNA, viruses, HCP, and protein A.

Though sharing many properties, all MAbs will not behave in a similar manner. Certain MAbs will be more challenging to purify, for example, due to a complex impurity profile or aggregate formation at certain conditions. In such cases, an expanded MAb purification toolbox with different options in primarily the polishing steps can be beneficial. For example, multimodal chromatography media can be suitable alternatives for more challenging purification tasks.

From the first study, typical results from highly productive three-step processes using traditional CIEX and AIEX media in the polishing steps will be presented (Fig 1A). This three-step approach will be suitable for the majority of cases and should be the first option to evaluate when developing processes for new MAb targets.

From the second study, results a from purification of a more challenging MAb, prone to aggregation at pH values > 6, will be presented (Fig 1B). Capto adhere ImpRes is an excellent choice for purification of MAbs with a challenging aggregate content. This multimodal AIEX medium also allows a wider window of operation compared with traditional AIEX media such as Capto Q.

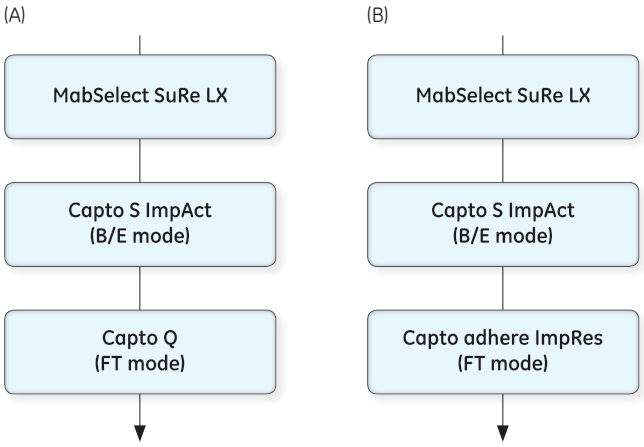

Fig 1. (A) Standard three-step MAb purification process using Capto Q AIEX medium in the last polishing step. (B) Alternative tree-step MAb purification process with Capto adhere ImpRes as multimodal AIEX medium in the last polishing step.

Materials and methods

Start material

The MAbs used in these studies were expressed from Chinese hamster ovary (CHO) cells and purified from clarified cell culture supernatant. Characteristics of the used MAbs are listed in Table 1.

Table 1. MAb characteristics

Antibody	MAb A	MAb B	MAb C
pI	7.3 to 7.5	8.9 to 9.0	8.4
Aggregate concentration post protein A	2%	2%	2% to 3%

Capture on MabSelect SuRe LX using standard conditions

MabSelect SuRe LX medium was packed in a HiScale™ 26 column (column volume [CV] 108 mL, 20 cm bed height), which was connected to an ÄKTA™ chromatography system. The column was equilibrated with 3 CV of 20 mM sodium phosphate, 0.5 M NaCl, pH 7.0. Cell culture supernatant was loaded at 50 mg MAb/mL medium and the column was washed with 5 CV of equilibration buffer. Before elution, the column was subjected to a second wash with 1 CV of 100 mM sodium acetate, pH 6.0. Elution was performed with 50 mM sodium acetate, pH 3.5 and the elution peak was collected. The column was cleaned with 3 CV of 0.1 M NaOH at a contact time of 40 min. Re-equilibration was conducted with 5 CV of 20 mM sodium phosphate, 0.5 M NaCl, pH 7.0. A flow rate of 18 mL/min, corresponding to a column residence time of 6 min, was used during feed load and wash. During elution and cleaning-in-place (CIP) operations, the flow rate was decreased to 8.8 and 8.0 mL/min, respectively. For equilibration and re-equilibration, a flow rate of 20 mL/min was used.

First polishing step using Capto S Impact

The pH and conductivity of the protein A eluate containing MAb A was adjusted to pH 5.3 (6.8 mS/cm) by addition of acetic acid. The buffer of the MAb B eluate from the protein A capture step was exchanged for 50 mM sodium acetate, pH 5.3 (3 mS/cm). MAb C eluate from the protein A capture step was adjusted to pH 5.0 and NaCl was added to a concentration of 50 mM.

For polishing of MAb A and MAb B, Capto S ImpAct was packed in a Tricorn™ 5/100 column (CV 2.0 mL, 10 cm bed height), which was connected to an ÄKTA system. The column was equilibrated with 5 CV of binding buffer. The dynamic binding capacity (DBC) of Capto S ImpAct medium under these conditions was determined for MAb A and B in previous experiments (data not shown). Sample load was performed at 64 g MAb/L medium for MAb A and 85 g MAb/L medium for MAb B, corresponding to 70% of DBC at 10% breakthrough (Q_{B10}). After wash with 5 CV of binding buffer, the MAbs were eluted using a gradient from 0 to 350 mM NaCl in binding buffer over 20 CV. Fractions of 0.5 mL (MAb A) or 1.0 mL (MAb B) were collected during elution. The column was cleaned with 3 CV of 1.0 M NaOH before re-equilibrated with 5 CV of binding buffer. The flow rate was 0.8 mL/min during equilibration and re-equilibration. During sample load, wash, elution, and CIP, the flow rate was 0.39 mL/min, corresponding to a column residence time of 5.4 min.

For polishing of MAb C, a Capto S ImpAct prepacked HiScreen™ column (CV 4.7 mL, 10 cm bed height) was connected to an ÄKTA system. Running conditions were optimized in a separate study (1). The column was equilibrated with 5 CV of 50 mM sodium acetate, 50 mM NaCl, pH 5.0. Sample load was performed at 76 mg MAb/mL medium, corresponding to 70% of Q_{B10}. A flow rate of 0.87 mL/min, corresponding to a residence time of 5.4 min was used. After wash with 5 CV of binding buffer, a linear gradient from 50 to 400 mM NaCl in binding buffer over 20 CV was applied for elution. Fractions of 2.0 mL were collected during elution. CIP was performed with 3 CV of 1.0 M NaOH. Re-equilibration was performed with 5 CV of binding buffer. Samples of 150 µL from the eluted fractions were transferred to a 96-well plate. To stabilize MAb C, 7.5 µL of 1.25 M sodium acetate, pH 4.5 was added to the wells.

Second polishing step

The buffer of the MAb eluate from the first polishing step was exchanged for 25 mM sodium phosphate, pH 7.5 for the Capto Q step and 25 mM phosphate, 150 mM NaCl, pH 6.3 for the Capto adhere ImpRes step.

Capto Q in FT mode

Capto Q was packed in a Tricorn 5/50 column (CV 1.1 mL, 5.6 cm bed height), which was connected to an ÄKTA system. The column was equilibrated with 7 CV of 25 mM sodium phosphate, pH 7.5. Sample was loaded at 150 mg MAb/mL

medium. Wash was performed with 5 CV of loading buffer followed by a strip with 5 CV of 50 mM sodium phosphate, 1.0 M NaCl, pH 7.5. CIP was performed with 6 CV of 1.0 M NaOH. The column was re-equilibrated with 7 CV of 25 mM sodium phosphate, pH 7.5. Load and wash was performed using a flow rate of 0.5 mL/min, corresponding to a column residence time of 2 min. For equilibration, strip, and re-equilibration, a flow rate of 1 mL/min was used. CIP was performed at a flow rate of 0.33 mL/min, corresponding to a contact time of 20 min.

Capto adhere ImpRes in FT mode

Capto adhere ImpRes was packed in a Tricorn 5/50 column (CV 0.53 mL, 2.7 cm bed height), which was connected to an ÄKTA system. The general recommendations for optimization of MAb polishing using Capto adhere ImpRes are the same as for Capto adhere (2). Furthermore, the optimal conditions for Capto adhere in FT mode will also be applicable for Capto adhere ImpRes in FT mode. Here, the conditions determined using Capto adhere in a separate study applying design of experiments (DoE) were used for this final polishing step for MAb C. The column was equilibrated with 10 CV of 25 mM phosphate, 150 mM NaCl, pH 6.3. Sample was loaded at 150 mg MAb/mL medium. Wash was performed with 10 CV of equilibration buffer. The flowthrough and part of the wash was collected based on determination of protein content by UV absorbance at 280 nm (> 100 mAU). Strip was performed with 7 CV of 100 mM acetic acid and CIP with 5 CV of 1.0 M NaOH. Re-equilibration was performed with 20 CV of 25 mM phosphate, 150 mM NaCl, pH 6.3. Sample load was performed at a flow rate of 0.098 mL/min, corresponding to a column residence time of 5.4 min. Wash was performed at a flow rate of 0.25 mL/min and equilibration, strip, and re-equilibration was performed at a flow rate of 0.5 mL/min. CIP was also performed at a flow rate of 0.5 mL/min, corresponding to a contact time of 5 min.

Determination of aggregate concentration and aggregate clearance

Fractions from the chromatography runs were collected and analyzed by size exclusion chromatography (SEC) on a Superdex™ 200 Increase 10/300 GL column. The peaks were integrated and the dimer and aggregate concentrations (in percent) were determined. For the gradient elution runs, cumulated recovery of monomers was plotted against cumulated aggregates.

Determination of HCP and protein A content

HCP content in the elution fractions and product pools were analyzed using commercially available anti-CHO HCP antibodies (Cygnus Technologies Inc.) and Gyrolab™ workstation (Gyros AB). Protein A content was determined using a commercially available ELISA kit (Repligen Corp.).

Results and discussion

Capture step using MabSelect SuRe LX

MabSelect SuRe LX was selected for the capture step because of its high binding capacity for MAbs and its alkali stability that enables CIP with 0.1 to 0.5 M NaOH. Direct MAb capture from cell culture supernatants on MabSelect SuRe LX was performed using standard conditions (Fig 2). After MAb capture, the column was washed with a salt-containing buffer. MabSelect SuRe LX is based on a high-flow agarose base matrix with hydrophilic properties that minimize unspecific adsorption of HCP to the matrix. Nevertheless, salt in the post load wash helps remove any residual HCP that might be interacting nonspecifically with the protein A medium.

Before elution, an extra wash with 100 mM sodium acetate, pH 6.0 was included to remove any remaining phosphate before elution with 20 mM sodium acetate, pH 3.5. This elution condition resulted in a narrow elution peak with a pool volume of 1.1 CV at a MAb concentration of 45.6 g/L. Despite the high binding capacity, the volume of the elution pool was not larger compared with the elution volume for a protein A medium with lower binding capacity. Consequently, capture using MabSelect SuRe LX will not require larger footprint or new investments in terms of larger hold tanks. The yield over the capture step was more than 95%. For MAb C, the aggregate concentration in the pool was 2.1%, the HCP concentration was 298 ppm, and the protein A concentration was 3.6 ppm.

Sample:	MAb from CHO cell supernatant
Medium:	MabSelect SuRe LX
Column:	HiScale 26
Load:	50 mg MAb/mL medium
Residence time:	6 min
Equilibration buffer:	20 mM sodium phosphate, 0.5 M NaCl, pH 7.0
Wash:	5 CV of equilibration buffer 1 CV 100 mM sodium acetate, pH 6.0
Elution buffer:	50 mM sodium acetate, pH 3.5
System:	ÄKTA system

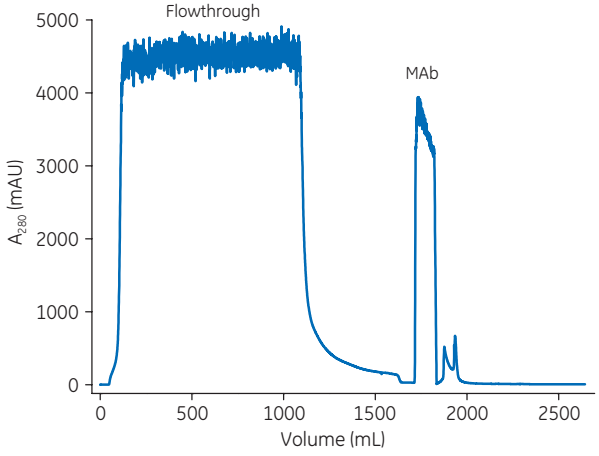

Fig 2. Direct MAb capture from CHO cell culture supernatant on MabSelect SuRe LX using standard run conditions. The first UV peak represents the loading phase, where MAb binds to the column and impurities such as HCP and other process related impurities flows through. The second peak corresponds to elution of purified MAb.

Initial MAb polishing step using Capto S ImpAct

The protein A capture step was followed by a polishing step using Capto S ImpAct CIEX medium. For MAb A, the aggregate concentration was reduced from 2% to 0.6% and the HCP concentration was reduced from 1800 ppm to 42 ppm at a MAb monomer yield of 90% after this step. The concentration of leached protein A in the elution pool was below 1 ppm. Similar results were obtained for MAb B, for which the start HCP concentration was 454 ppm. For MAb C, the aggregate concentration was reduced from 3% to 1.2% at a MAb monomer yield of 91%. The MAb concentration in the pool was determined to 11.3 g/L. The HCP and protein A concentrations were reduced from 298 to 151 ppm and from 3.6 to < 1 ppm, respectively. The good selectivity of Capto S ImpAct between MAb monomer, aggregates, and HCP can be seen from the chromatograms in Figures 3 to 5.

Sample:	MAb A in 50 mM sodium acetate, pH 5.3 (6.8 mS/cm)
Medium:	Capto S ImpAct (B/E mode)
Column:	Tricorn 5/100
Load:	64 g MAb/L medium (70% of Q_{B10})
Residence time:	5.4 min
Binding buffer:	50 mM sodium acetate, pH 5.3 (6.8 mS/cm)
Wash:	5 CV of binding buffer
Elution buffer:	50 mM sodium acetate, pH 5.3, 0 to 350 mM NaCl in 20 CV
System:	ÄKTA system

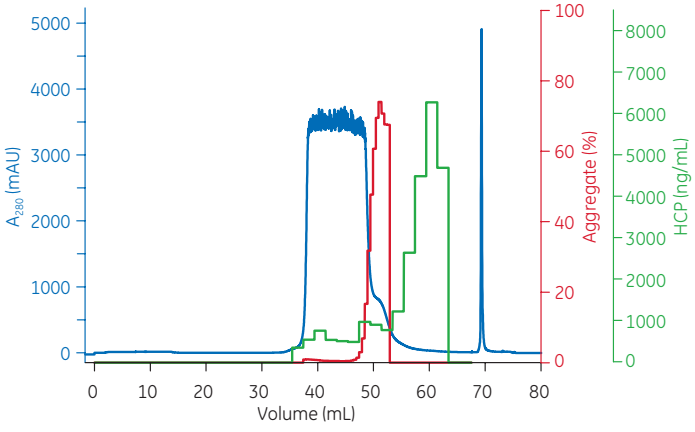

Fig 3. Initial polishing of MAb A. Aggregates elute in the tail of the elution peak (red histogram), whereas most of the HCP (green histogram) elutes after the elution peak (blue UV trace).

Sample:	MAb B in 50 mM sodium acetate, pH 5.3 (3 mS/cm)
Medium:	Capto S ImpAct (B/E mode)
Column:	Tricorn 5/100
Load:	85 g MAb/L medium (70% of Q_{B10})
Residence time:	5.4 min
Binding buffer:	50 mM sodium acetate, pH 5.3 (3 mS/cm)
Wash:	5 CV of binding buffer
Elution buffer:	50 mM sodium acetate, pH 5.3, 0 to 350 mM NaCl in 20 CV
System:	ÄKTA system

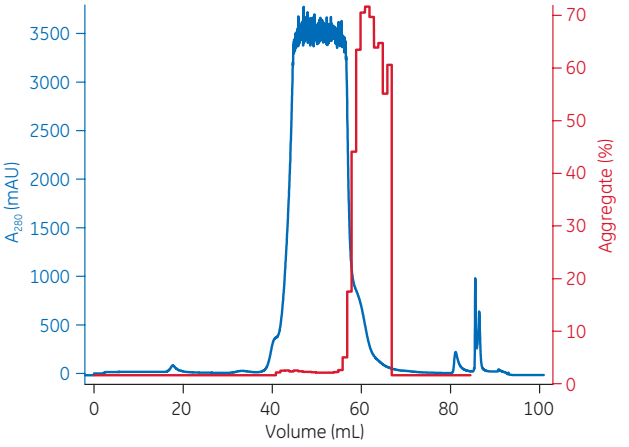

Fig 4. Initial polishing of MAb B. Aggregates (red histogram) elute in the tail of the elution peak (blue UV trace).

Sample:	MAb C in 50 mM sodium acetate, 50 mM NaCl, pH 5.0
Medium:	Capto S ImpAct (B/E mode)
Column:	HiScreen column
Load:	76 mg MAb/mL medium (70% of Q_{B10})
Residence time:	5.4 min
Binding buffer:	50 mM sodium acetate, 50 mM NaCl, pH 5.0
Wash:	5 CV of binding buffer
Elution buffer:	50 mM sodium acetate, 50 to 400 mM NaCl in 20 CV
System:	ÄKTA system

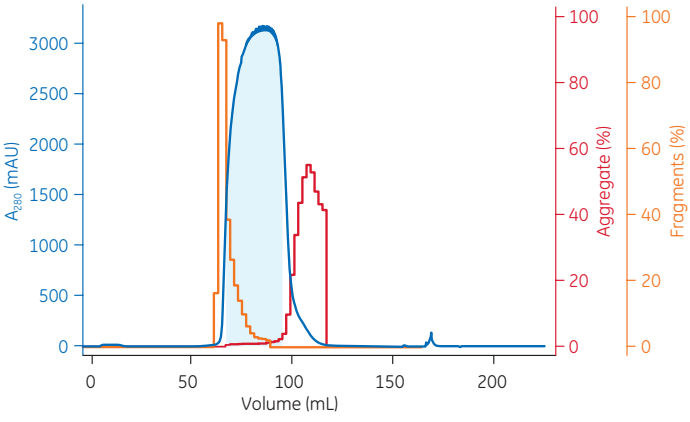

Fig 5. Initial polishing of MAb C. The fragments (orange histogram) elutes at the front of the elution peak (blue UV trace), whereas the aggregates (red histogram) elutes in the tail of the elution peak. The light blue area under the curve corresponds to pooled product fractions.

Final MAb polishing step using Capto Q medium

Final polishing in a standard three-step MAb purification process is often performed using Capto Q medium conducted under FT conditions (Fig 6). After this step, the aggregate concentrations are typically below 1%, HCP concentrations below 5 ppm, and leached ligand concentration below 1 ppm at high monomer yields of 99%.

The results summarized in Table 2 shows a typical outcome of this standard three-step process.

Sample:	MAb in 25 mM sodium phosphate, pH 7.5
Medium:	Capto Q (FT mode)
Column:	Tricorn 5/50
Equilibration buffer:	25 mM sodium phosphate, pH 7.5
Load:	150 mg MAb/mL medium at a flow rate of 0.5 mL/min
Residence time:	2 min
System:	ÄKTA pure system

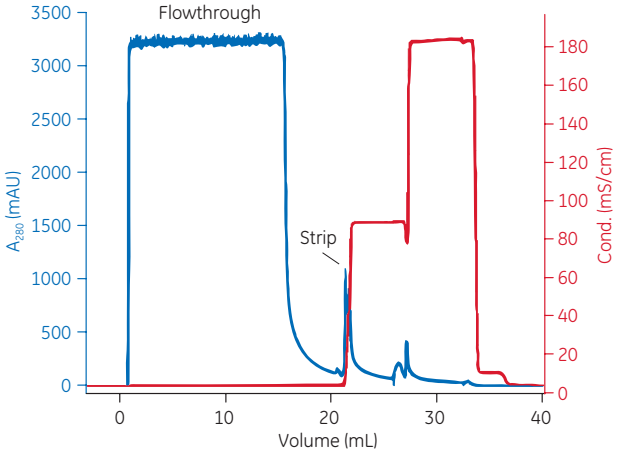

Fig 6. Representative chromatogram of MAb polishing using Capto Q AIEX medium. The first UV peak at approximately 0 to 16 mL represents the loading phase (product pool), where the MAb flows through the column, whereas impurities such as HCP, protein A, DNA and viruses bind to the medium. The peak at 22 mL contains mainly impurities that are stripped of the column with 1.0 M NaCl.

Table 2. Results reflecting a typical outcome of a traditional tree-step process

Process step	MAb yield (%)	Aggregates (%)	HCP (ppm)	Leached ligand (ppm)
MabSelect SuRe LX	95	2 to 3	300 to 2000	4 to 15
Capto S ImpAct	90	< 1	< 100	< 1
Capto Q	99	< 1	< 5	< 1
Total process yield	~ 86			

Final polishing using the expanded MAb toolbox

For the second polishing step of the more challenging MAb C, Capto Q using standard conditions with loading at a pH of 7.5 was initially evaluated. However, due to a low monomer stability of MAb C at a neutral pH, aggregates tended to generate over the Capto Q step (data not shown). Hence, Capto adhere ImpRes was evaluated as an alternative to Capto Q for the final polishing of MAb C. In addition to efficient removal of HCP, leached protein A, and MAb aggregates, Capto adhere ImpRes has a broader window of operation and can be operated at a lower pH than Capto Q.

A chromatogram for the Capto adhere ImpRes step is shown in Figure 7. The flowthrough and part of the wash fraction was collected and analyzed for MAb and impurity concentrations. The MAb concentration of the collected pool was determined to 5.7 g/L, corresponding to a yield of 94%. The aggregate concentration was 0.9%, HCP concentration was 11 ppm, and protein A concentration was < 1 ppm.

A lower load of 108 mg MAb/mL medium was also evaluated for the Capto adhere ImpRes step and resulted in a similar yield and purity as the higher load of 150 mg MAb/mL medium (data not shown). The observation indicates the robustness in load density for this chromatography step.

After elution, the Capto adhere ImpRes column was stripped with 100 mM acetic acid. The MAb concentration in the strip was low, while containing high levels of MAb aggregates, HCP, and protein A.

The overall results from the three-step MAb C purification process are summarized in Table 3.

Sample:	MAb C in 25 mM sodium phosphate, 150 mM NaCl, pH 6.3
Medium:	Capto adhere ImpRes (FT mode)
Column:	Tricorn 5/50
Equilibration buffer:	25 mM phosphate, 150 mM NaCl, pH 6.3
Load:	150 mg MAb/mL medium at a flow rate of 0.5 mL/min
Residence time:	5.4 min
System:	ÄKTA system

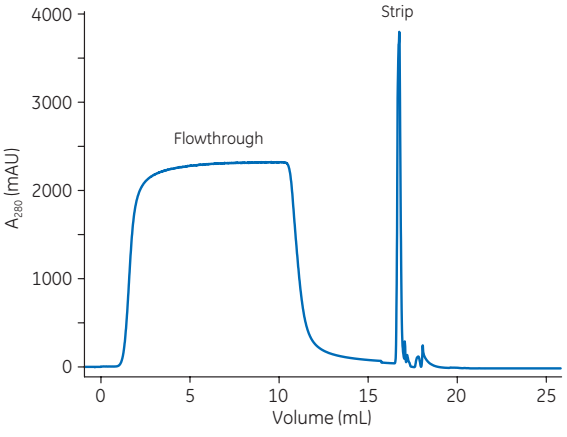

Fig 7. Final polishing of MAb C using Capto adhere ImpRes. The first UV peak represents the loading phase (product pool), where the MAb flows through the column, whereas impurities such as MAb aggregates, HCP, protein A, DNA, and viruses bind to the medium. The second peak contains mainly impurities that are stripped of the column with 100 mM acetic acid.

Table 3. Results from the three-step MAb C purification process

Process step	MAb yield (%)	MAb concentration (mg/mL)	Aggregates (%)	HCP (ppm)	Leached ligand (ppm)
MabSelect Sure LX	99	37	2.9	298	3.6
Capto S ImpAct + buffer change	91	8.3	1.4	154	< 1
Capto adhere ImpRes	94	5.7	0.9	11	< 1
Total process yield	85				

Conclusions

A toolbox comprising modern chromatography media is useful in the development of effective purification platforms for MAbs. This application note describes the use of such media in standard three-step MAb purification processes. The capture step was performed using high-capacity MabSelect SuRe LX protein A medium. For the initial polishing step, Capto S ImpAct was selected. This CIEX medium enables separation of aggregates from the monomer fractions with high resolution. In the final polishing step of the standard three-step process, Capto Q AIEX medium was used. This traditional AIEX polishing medium exhibits high capacity for DNA, viruses, HCP, and protein A due to its functional group being linked to the matrix via a dextran surface extender.

For a more challenging MAb, prone to aggregation at pH values above 6, Capto Q using standard conditions with loading at pH 7.5 was shown to be unsuitable. Instead, Capto adhere ImpRes multimodal AIEX medium was selected for the final polishing of this MAb. Capto adhere ImpRes offers the possibility of operation at a lower pH than Capto Q. The strong anion exchange multimodal ligand of Capto adhere ImpRes displays high selectivity compared with traditional AIEX media. With this polishing medium, aggregates, HCP, and leached protein A, were efficiently separated from the target MAb, resulting in a MAb recovery of 94% and an aggregate content below 1%. The total MAb recovery for this three-step process was 85%.

For efficient MAb processes, GE Healthcare's expanded MAb toolbox comprises chromatography media for both standard and more challenging purification applications.

Reference

1. Application note: Optimization of dynamic binding capacity and aggregate clearance in a monoclonal antibody polishing step. GE Healthcare, 29-1450-68, Edition AA (2015).
2. Procedure: MAb polishing step development using Capto adhere in flow-through mode, GE Healthcare, 29-0192-56, Edition AA (2012).

Ordering information

Product	Size	Product code
MabSelect SuRe LX	25 mL	17547401
Capto S ImpAct	25 mL	17371701
Capto Q	25 mL	17531610
Capto adhere ImpRes	25 mL	17371501
Superdex 200 Increase 10/300 GL	1 × 24 mL	28990944

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