Three-Step Monoclonal Antibody Purification Processes Using Modern Chromatography Media

This application note describes a monoclonal antibody (MAb) purification process using the expanded MAb purification toolbox of GE Healthcare's Life Sciences business, covering modern chromatography media (resins) for standard and 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 (Figure 1A). However, for more challenging MAb purifications, an expanded MAb purification toolbox is beneficial. Here, a multimodal AIEX medium was used as an alternative in final polishing of a MAb with low monomer stability (Figure 1B). Using the described approach, the set criteria for the polishing steps were achieved, with a recovery of >90% over each step and an aggregate content <1% in the final product.

MAb Capture Step

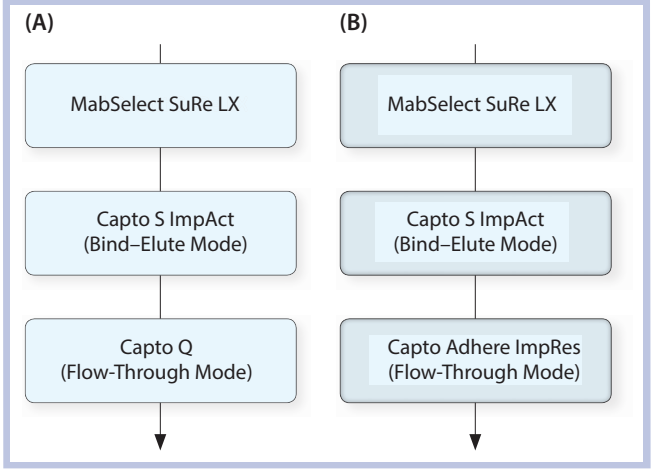
MabSelect SuRe™ LX was selected for the capture step because of its high binding capacity for MAbs and its alkali stability that enables cleaning in place with 0.1–0.5 M NaOH. Direct MAb capture from cell culture supernatant on MabSelect SuRe LX was performed using standard conditions.

A postload wash with 20 mM sodium phosphate, 0.5 M NaCl (pH 7.0) improved host cell protein (HCP) reduction over this step. An extra wash with 100 mM sodium acetate, pH 6.0 was included to remove any remaining sodium chloride and 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 approximately 40 g/L. Despite the high binding capacity, the volume of the elution pool was not larger than 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.

Initial MAb Polishing Step

The protein A capture step was followed by a polishing step using Capto™ S ImpAct CIEX medium. Over this step, 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 among MAb monomer, aggregates, and HCP can be seen from the chromatogram in Figure 2.

Figure 1: (A) Standard three-step MAb purification process using Capto Q AIEX medium in the last polishing step; (B) alternative three-step MAb purification process with Capto adhere ImpRes as multimodal AIEX medium in the last polishing step



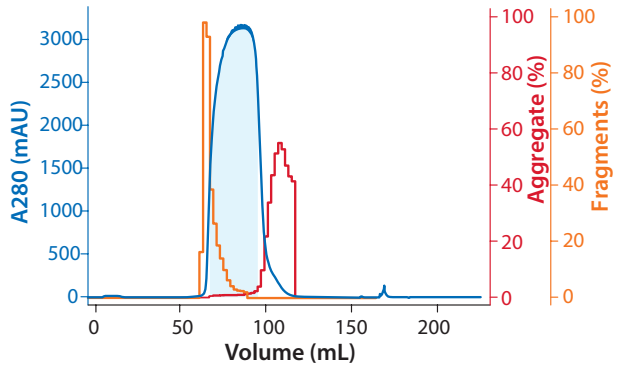
Final Polishing Using the Expanded MAb Toolbox

For the second polishing step, Capto Q using standard conditions with loading at a pH of 7.5 was initially evaluated for this MAb. However, due to a low monomer stability of the MAb 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 step. 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 pH that is lower

Table 1: Results from the three-step MAb purification process

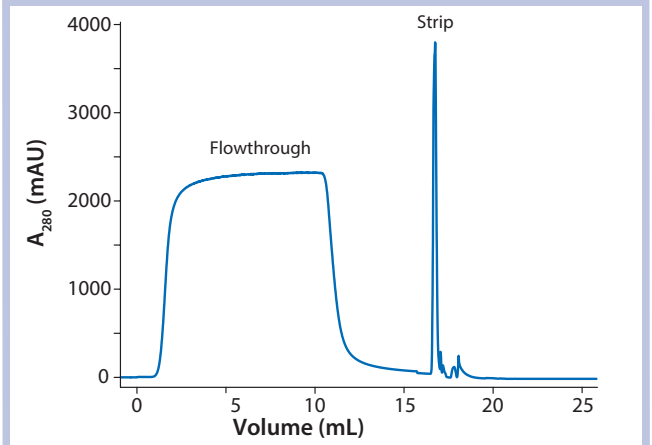
Process Step	MAb Yield (%)	MAb Concentration (mg/mL)	Aggregates (%)	HCP (ppm)	Leached Ligand (ppm)
MabSelect Sure LX	99	37	2.9	298	3.6
Capto S ImpAct + buffer change	91	8.3	1.4	154	<1
Capto adhere ImpRes	94	5.7	0.9	11	<1
Total process yield	85				

Figure 2: Initial MAb polishing; 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.



Sample:	MAb 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% 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, 50-400 mM NaCl in 20 CV
System:	ÄKTA™ system

Figure 3: Final MAb polishing 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.



Sample:	MAb 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

than required for Capto Q. Figure 3 shows a chromatogram for the Capto adhere ImpRes step. The overall results from the described three-step MAb purification process (Table 1) are comparable with typical results from a traditional three-step process.

Conclusions

A toolbox consisting of modern chromatography media is useful in the development of effective MAb purification platforms. This application note describes the use of such media in a three-step purification process for a challenging MAb, prone to aggregation at pH > 6.

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, 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 this step. Capto adhere ImpRes offers the possibility of operation at a lower pH than for 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 consists of chromatography media for both standard and challenging purification applications. A more detailed description of this three-step MAb purification process is given in application note 29132569 AA (1).

References

1 *Application Note: Three-Step Monoclonal Antibody Purification Processes Using Modern Chromatography Media.* GE Healthcare, 29132569, Edition AA (2015).

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