GE Healthcare
Life Sciences

A platform approach for the purification of antibody fragments (Fabs)

This Application note describes a three-step purification process of a Fab originating from an *E. coli* supernatant. First, chromatography media (resins) were screened in 96-well PreDictor™ plates, using wide wash and elution conditions. Next, capacity studies were performed in small columns with purified protein, and elution studies in columns were performed to find the optimal elution pH. Design of Experiments (DoE) was used to further optimize the conditions for each step. This general approach supports Quality by Design (QbD), where the plates give the characterized space and the DoE in columns render both the design and the control space. Using Capto™ L, Capto SP ImpRes, and Capto Q media in the three step process resulted in efficient removal of the main contaminants and high yields (≥ 87%) over the entire process. This platform approach enables increased efficiency and productivity in developing therapeutics based on antibody fragments.

Antibody fragments (e.g., Fab, scFv, domain antibodies, etc.) are set to become the next important class of protein-based biotherapeutics after monoclonal antibodies (MAbs). One of the advantages is that due to their structure and smaller size, antibody fragments possess properties (e.g., easier tissue penetration) that suit a range of diagnostic and therapeutic applications.

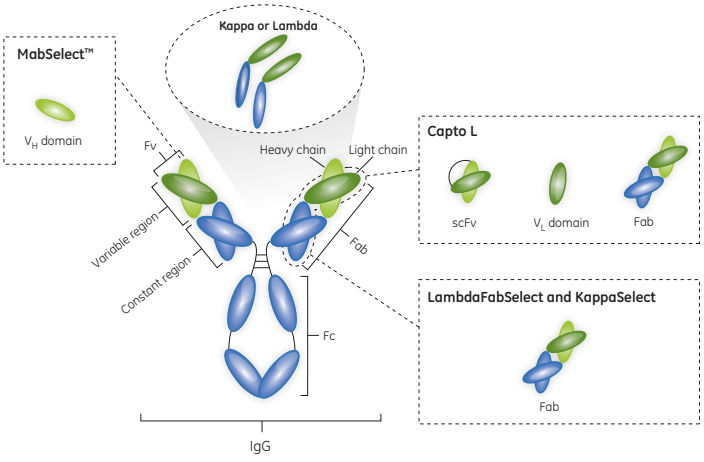

Fig 1. The protein L ligand in Capto L binds to the variable region of an antibody's kappa light chain. LambdaFabSelect binds to Fabs with lambda light chain, KappaSelect to Fabs containing kappa light chain, and MabSelect media can be used for fragments containing the VH3 domain.

The industry standard for purifying MAbs is a platform approach using affinity chromatography with protein A as the capture step. The high purification factor and generic conditions associated with this approach have proven particularly attractive to biopharmaceutical manufacturers. Antibody fragments, however, have previously lacked such a platform solution.

With the introduction of Capto L, the first industrial platform for the purification of antibody fragments is now emerging. With its recombinant protein L ligand, Capto L is a BioProcess™ chromatography medium with a broad range affinity for antibody fragments of different sizes containing kappa light chains (Fig 1).

A three-step Fab purification process using Capto media

Here we describe an example of using Capto media in a purification process of a kappa subclass Fab originating from an *E. coli* supernatant. Table 1 shows the properties of the Fab.

Table 1. Characteristics of the Fab used in the purification process

Fab origin	from IgG1 in *E. coli* supernatant
Theoretical pI	8.5
Molecular weight	48 kDa
Concentration in feed	1 mg/mL
Aggregate content	3.5%

During the set-up of this three-step process, a general workflow was used with the purpose of minimizing the development time by using High Throughput Process Development (HTPD) tools:

1. Choose media and wash or elution conditions by screening in plates
2. Perform capacity studies (column) with purified protein (only binding steps)
3. Make elution study in columns
4. Perform DoE in columns

This general approach also supports QbD, where the plates give the characterized space and the DoE in columns provides both the design and the control space. The DoE for all steps is outlined in Figure 2.

Step 1 – Capture step with Capto L

For the capture step, Capto L was chosen and wide wash and elution conditions were evaluated in a 96-well format using PreDictor plates. Second, capacity studies were performed in small columns with purified protein. Third, an elution pH study was performed to find the optimal elution pH. This might be especially important for some molecules that are sensitive to acidic pH values. DoE (Fig 2) was used to find the best conditions for the step.

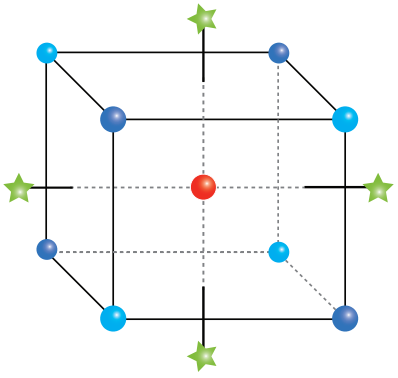

Fig 2. For all three steps, the optimization study used a Central Composite Circumscribed (CCC) design. This high resolution design supports quadratic interactions, while the star distance points eliminate confounding between interactions and quadratic terms.

For the dynamic binding capacity (DBC) studies Fab in an *E. coli* supernatant was first purified with Capto L. All runs were performed on pre-equilibrated columns on an ÄKTAexplorer™ 100 system equipped with a fraction collector.

DBC for Capto L in the capture step was 21 mg/ml at 4 min residence time. The elution pH for this Fab was 3.2 when running a gradient from pH 6 to 2.5.

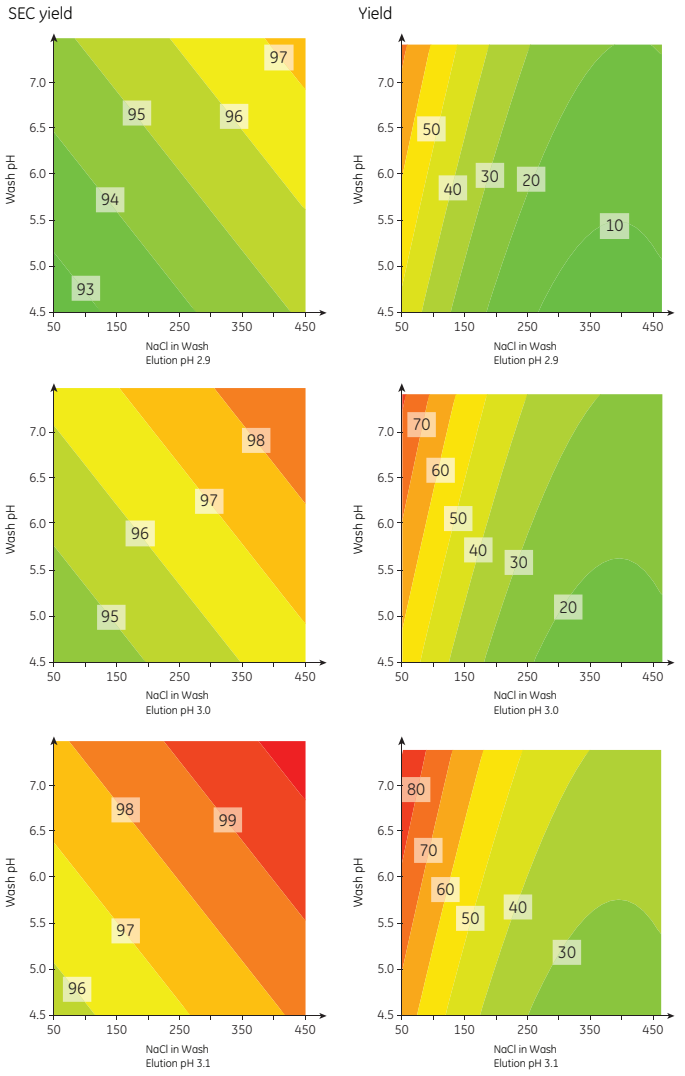

Fig 3. Contour plots at three different elution pH values, showing the responses for yield determined by size exclusion chromatography (SEC) and ECP. Optima for yield and ECP did not coincide. The SEC yield is highest in the upper right corner (high salt content in the wash and high wash pH) while the lowest values for ECP were obtained in the lower right corner (high salt content in the wash and low wash pH).

Following screening experiments, a three-factor DoE was performed. The studied factors were wash pH, NaCl-concentration in the wash and elution pH. Some factors were kept constant. For instance, the load was 15 mg/ml, which corresponds to 70% of the DBC, a representative load for a production scenario. The residence time during the load was 4 min and the wash volume was 7 column volumes. The most important responses in this study were the *E. coli* protein (ECP*) content and the yield. Figure 3 shows the DoE results.

To combine the information achieved for ECP and yield and to find the overall optimum, sweet spot analysis was used (Fig 4). The green surface shows conditions where both criteria were met. A verification run was performed where the wash step contained an acetate buffer with a pH of 5 and 400 mM NaCl. The results were as expected: 96% Fab yield containing only 12 ppm of ECP.

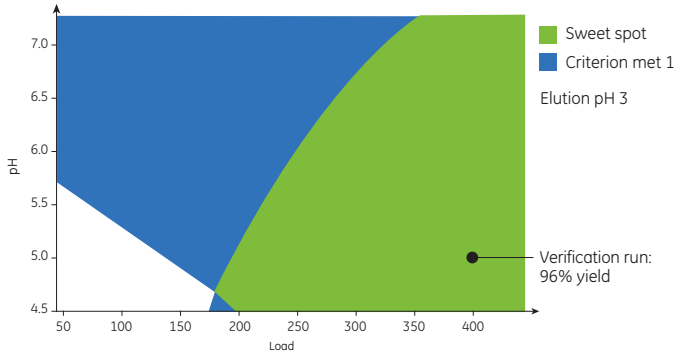

Fig 4. Sweet spot analysis for yield and ECP content using Capto L in the capture step. Criteria were an ECP content of 2-30 ppm and a yield of 95%-100%. The green surface shows conditions where both criteria were met. A verification run was performed within the sweet spot and results were: 96% Fab yield containing only 12 ppm of ECP.

Step 2 – Purification with Capto SP ImpRes

For the second purification step, the high resolution cation exchanger Capto SP ImpRes was chosen due to its ability to efficiently separate aggregates from monomers. DBC at 10% breakthrough was 80 mg/mL and 71 mg/mL at pH 4.0 and 5.5, respectively. The lowest DBC value was used to calculate the maximum load for the DoE study, where 70% of QB_{10} (i.e., approx. 50 mg/mL) was used as the center point.

A DoE was used, where pH, gradient length (with NaCl) and load were studied. Some factors (e.g., residence time and wash length and final NaCl concentration) were kept constant.

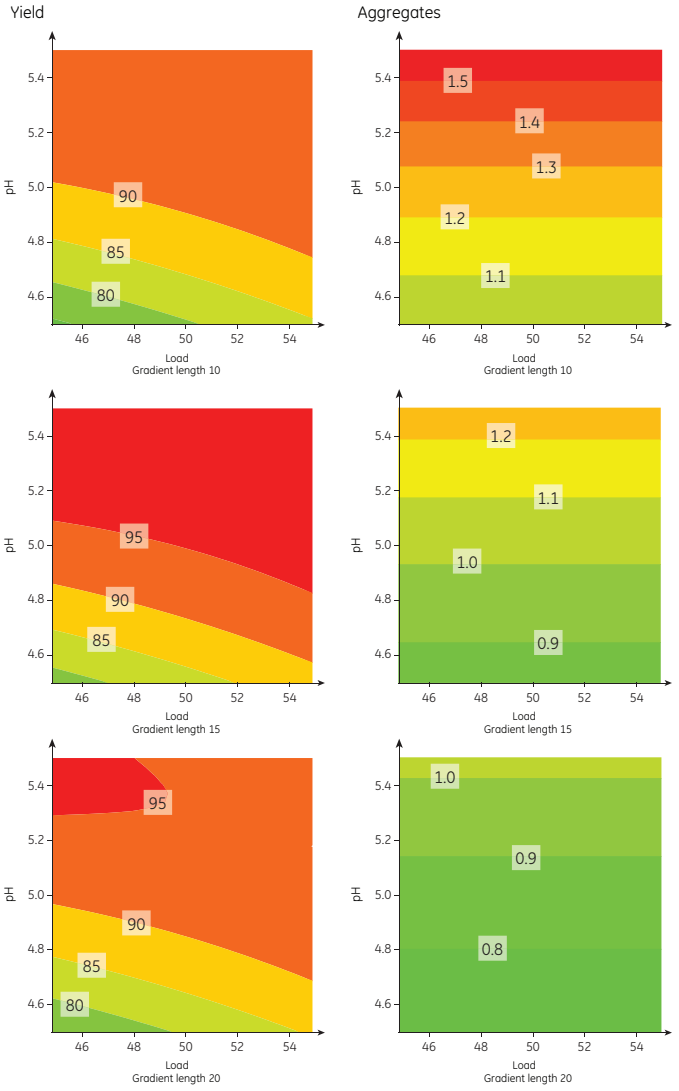

Fig 5. Contour plots of yield (left) and aggregates (right) at three different gradient lengths. To achieve a high yield the general trend is to work at a pH in the upper range. At pH 4.5 the cleaning-in-place (CIP) peak was quite large, explaining the lower yield under these conditions. The Capto L elution pool contained 3.5% aggregates. The DoE with Capto SP ImpRes showed that the aggregate reduction was better at the lower pH values and was independent of load. The aggregate values were lower when using a 20 column volume gradient, as seen on the lower right figure.

* ECP content was analyzed using antibodies from Cygnus Technologies on a Gyrolab™ workstation.

Aggregate content was determined by size exclusion chromatography using an ÄKTAexplorer 10 system and Tricorn™ 5/150 columns. The injection volume was 10 µL and the mobile phase (PBS) was run at a flow rate of 0.4 mL/min. UV detection was performed at 215 and 280 nm.

The optima for yield and low aggregates did not coincide (Fig 5). The highest yield was in the upper region of the plot (pH ≥ 5.0) while the lowest aggregate content was in the lower region of the plot (pH ≤ 5.0). To combine the information from these plots, a sweet spot analysis was performed and showed a wide experimental space where both the criteria (yield > 90% and aggregate content ≤ 1%) were met (Fig 6).

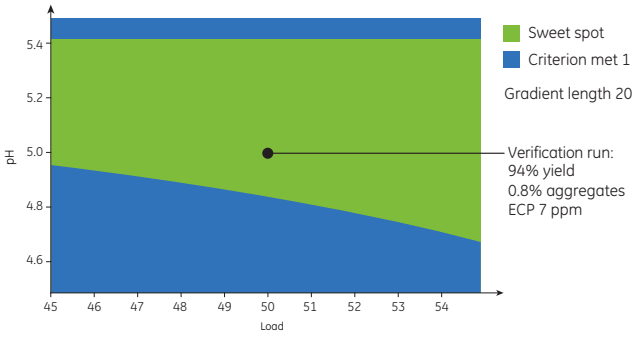

Fig 6. The sweet spot plot, for a gradient length of 20 column volumes, showed a wide experimental space where both the criteria of high yield (> 90%) and maximum aggregate content of 1% were met. A verification run was performed within the sweet spot to further validate the model. When using a load of 50 mg/mL and a pH of 5, the monomer yield was 94%, with 0.8% aggregates and an ECP level of 7 ppm.

Step 3 – Polishing with Capto Q

For the third step Capto Q, an anion exchanger, was used in the process to further reduce contaminant levels. Capto Q is an excellent choice for use in flowthrough mode for proteins with a high isoelectric point, such as this Fab. Since the isoelectric point is approximately 8.5, working at a pH of 8 or less will make the Fab pass in the flowthrough fraction.

Figure 7 shows the contour plots for yield and ECP reduction. High loads generated higher yield, while pH and residence time had negligible impact. ECP reduction was increased with increasing pH, but residence time and load had no impact. For aggregate reduction (data not shown), no significant effects were detected.

Using Capto Q in the third step provided high Fab yield in a broad range of parameters. ECP can be reduced 1.2 to 1.45 times in the pH interval 7.0 to 7.9.

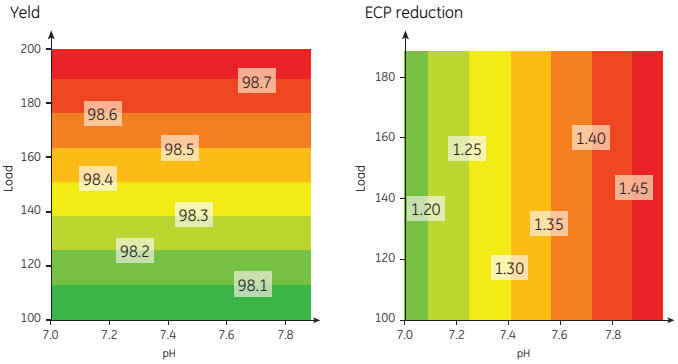

Fig 7. Contour plots for yield (left) and ECP reduction (right) using Capto Q. The only factor significantly affecting yield was the load - the higher the load the higher the yield. The DoE showed that the ECP-reduction factor was independent of load and residence time. The only significant factor was the pH: the higher the pH the better the ECP reduction.

Process verification

Having optimized the three steps and having a better knowledge of the effects of the most significant factors for each step, a process verification was performed. Results of the verification run for the three-step process are shown in Figures 8 and 9, and summarized in Table 2. The total process yield was 87% with 0.8% aggregate cont. ECP and endotoxin reductions both showed excellent results and protein L leakage was below the limit of quantification.

	Capto L	Capto SP ImpRes	Capto Q (flowthrough step)
Column:	HiScale™ 16/200	HiScreen™ Capto SP ImpRes (4.7 ml)	HiTrap™ Capto Q (1 ml)
Sample:	Crude feed containing Fab	Elution pool from Capto L step, pH adjusted to 5 and diluted 1/2	Monomer pool from Capto SP ImpRes step, buffer exchanged
Sample load	318 mL feed (15 mg/mL resin)	45 mg/mL resin	100 mg/mL resin
Equilibration and wash buffer:	50 mM sodium acetate, 400 mM NaCl, pH 5	25 mM sodium acetate, pH 5	25 mM sodium phosphate, pH 7.5
Elution buffer:	50 mM acetic acid	25 mM sodium acetate + 500 mM NaCl pH 5	
Residence time:	4 min	4 min	2 min
Gradient length:	–	20 CV	–
Detection:	UV 280 nm	UV 280 nm	UV 280 nm
System:	ÄKTAexplorer 100	ÄKTAexplorer 100	ÄKTAexplorer 100

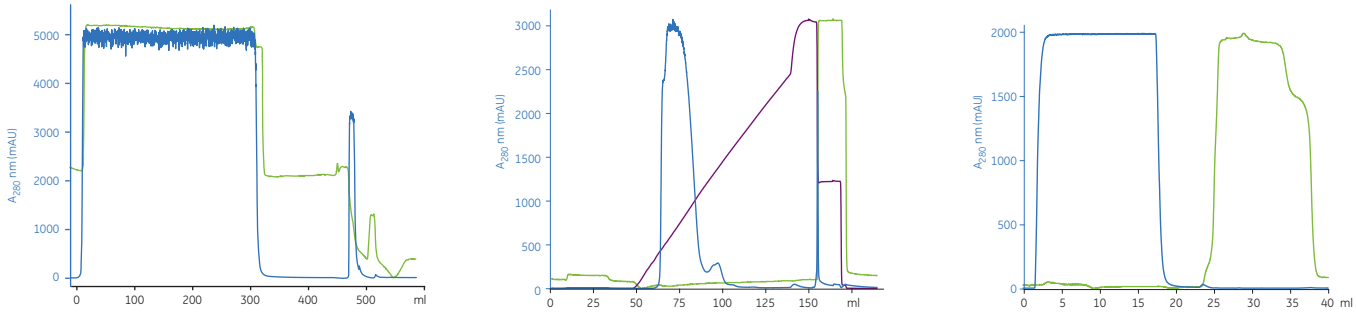

Fig 8. Chromatogram from the verification runs in the three-step process. Traces are UV (blue), conductivity (purple), and pH (green).

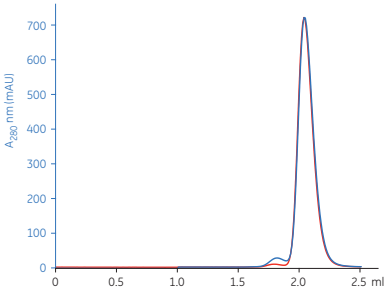

Fig 9. Normalized chromatogram from the SEC analysis of the Capto L elution pool (blue trace) and final product (red trace).

Table 5. Summary of the results from the verification run

Sample	Yield (%)	Aggregates (%)	ECP (ppm)	Endotoxin (EU/mg)	Protein L (ng/mL)*
Feed	100	NA	440 000	1 720 000	
Capto L	97.3	3.30	13	11	< 5.7
Capto SP ImpRes	93.1	0.76	8	0.05	< 5.7
Capto Q	95.8	0.80	6	0.06	< 5.7
Total yield	86.8				

* Protein L leakage was analyzed using an ELISA kit from Medicago AB (Sweden)

Summary

A three-step purification process for a Fab was successfully developed and verified. The step yield ranged between 93% and 97%, giving a total process yield of approximately 87%. Aggregates were reduced from 3.3% to approximately 0.8%. The ECP in the start sample was more than 400 000 ppm whereas the final sample contained only 6 ppm. The endotoxin content in the feed was 1.7 million endotoxin units/mg of protein and the final sample was below 0.1 units/mg. Protein L leakage was below the limit of quantification for all samples.

Market trends forecast an increased effort in developing therapeutics based on antibody fragments. GE Healthcare Life Sciences has developed Capto L, Capto ImpRes, and other Capto media to enable a platform approach for the purification of antibody fragments. Our results for a three-step Fab process using Capto media showed effective removal of the main contaminants, low ligand leakage, and high yields over the entire process.

References

1 Data file: Capto L, GE Healthcare, 29-0100-08, Edition AB (2012).

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