Capture of human single-chain Fv (scFv) fusion protein on Capto L affinity medium

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Application note 29-0144-56 AA Affinity chromatography

Capture of human single-chain Fv (scFv) fusion protein on Capto™ L affinity medium

We describe the capture of a single-chain Fv fusion protein from a challenging feedstock (animal plasma) on the affinity chromatography medium (resin) Capto L. Initial work on small prepacked HiScreen™ Capto L columns demonstrated high selectivity and high capacity. Wash and elution conditions were then optimized and the separation performed in larger columns. This gave high purity of the target protein as well as good yields and substantial log reductions of host cell proteins. The potential to further improve productivity by processing stocks with increased expression levels or by shortening residence time in the column was also noted.

Introduction

Different variants of classic monovalent antibody fragments (e.g. Fab, scFv, Dab, etc.) are now emerging as credible alternatives to monoclonal antibodies. Today, several types of antibody fragments that lack the Fc-portion yet retain the Kappa light chain and the targeting specificity of a full antibody can generally be produced economically in, for example, prokaryotic systems. Due to their structure and smaller size, they also possess other unique properties that suit a range of diagnostic and therapeutic applications, e.g. easier tissue penetration.

The industry standard for purifying monoclonal antibodies 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.

This Application Note describes the single-step capture and purification of a plasma-derived single-chain Fv (scFv) fusion protein on Capto L as performed by a contract manufacturing organization (CMO). Capto L comprises a high-flow agarose base matrix to which recombinant protein L (*E. coli*) has been immobilized. A laboratory-scale purification step developed previously means that some parameters, e.g. loading conditions and residence time, had already been determined prior to technology transfer to the CMO. This earlier work is not described here.

Materials and Methods

Sample

Human scFv fusion protein obtained from animal plasma with a molecular weight of 57 kDa was precipitated by PEG/PBS buffer prior to chromatography on Capto L. The protein's expression level was approximately 20 to 40 mg/L. Precipitates were dissolved in equilibration buffer (buffer A) before loading on the columns.

Affinity chromatography

The equilibration and first wash buffer comprised phosphate buffered saline (PBS) with 0.1 M glycine, pH 8. The second wash buffer was 0.05 M glycine + 0.05 M citrate, pH 4 or 3.5. Elution was performed with 0.05 M glycine + 0.05 M citrate, pH 2. Flow rates were varied to keep a constant residence time of 4.6 min. Columns used included prepacked HiScreen Capto L (column volume [CV] 4.7 mL); Kronlab ECO (aqueous buffer) 10 mm packed with 2.4 mL Capto L, bed height 3.1 cm; XK 16/20 packed with 21.1 mL Capto L, bed height 10.5 cm; and XK 26/20 packed with 34 mL Capto L, bed height 6.4 cm.

SDS-PAGE

12% SDS-PAGE gels from Bio-Rad (Mini-Protean™ TGX Precast Gels) were run for 35 min at 200 V and 110 mA with silver staining as detection method. The marker was SeeBlue™ Plus 2 Prestained (Invitrogen).

ELISA

Albumin, IgG and plasma proteins were detected using ELISA kits from Cygnus Technologies.

Results

The first chromatographic run on HiScreen Capto L with a CV of 4.7 mL is shown in Figure 1. Approximately 11 mg sample were loaded at pH 8 and the predefined residence time (RT) was 4.6 min. Flow-through, wash fractions and the elution pool were collected for analysis by SDS-PAGE.

The silver-stained gels are seen in Figure 2. These SDS-PAGE results indicate an absence of scFv fusion protein in the flow-through (Fig 2, lane 2). The two wash steps, the first at pH 8 and the second at pH 4, remove a large proportion of the contaminants. The elution pool (Fig 2, lane 5) contains highly enriched scFv fusion protein. Nevertheless, some contaminants (albumin, IgGs) still remain after these two wash steps.

To improve the purity of the eluted fraction, the chromatographic step was optimized. Firstly a linear pH gradient from pH 4 to pH 2 was run. This resolved two peaks (Fig 3). The first peak (eluting at approx. pH 3.5) contained contaminants with molecular weights of about 65 kDa, 50 kDa and 25 kDa (Fig 4). The second peak was mainly scFv fusion protein with only small amounts of the 65 kDa contaminant visible.

This 65 kDa contaminant could potentially be serum albumin. Albumin was found to form aggregates with the scFv fusion protein and these could not be completely resolved by the affinity chromatography.

Based on the above results, the pH of the second wash step was decreased from pH 4 to pH 3.5. A new purification with these modified wash conditions was run on a packed XK 16/20 column with a CV of 21 mL (Fig 5). This modification removed the 65 kDa contaminant from the elution pool. Furthermore, the yield of the scFv fusion protein was estimated to be 93% (Fig 6).

Column:	HiScreen Capto L column (volume 4.7 mL)
Sample:	Precipitated plasma containing scFv fusion protein
Loading:	2.3 mg scFv fusion protein/mL medium
Buffer A:	PBS + 0.1 M glycine, pH 8
Buffer W2:	0.05 M glycine + 0.05 M citrate, pH 4
Buffer B:	0.05 M glycine + 0.05 M citrate, pH 2
Flow rate:	1 mL/min (residence time 4.6 min)
System:	ÄKTAexplorer™ 100
Detection:	Absorbance at 280 nm

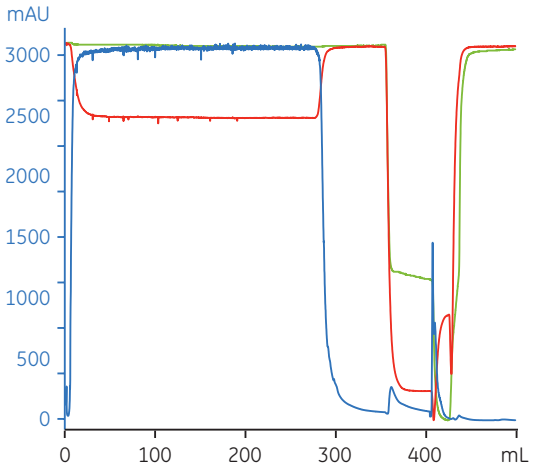
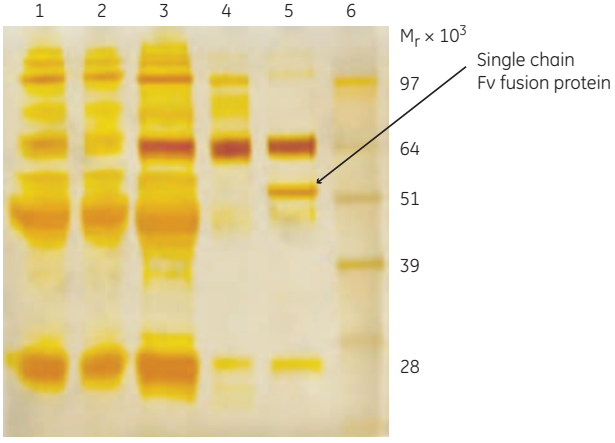


Fig 1. Capture of scFv fusion protein on HiScreen Capto L.



Lane
1. Sample (1:10 diluted)
2. Flow-through (1:10 diluted)
3. Wash 1
4. Wash 2
5. Eluate (1:4 diluted)
6. Marker

Fig 2. SDS-PAGE (silver staining, reducing conditions) of samples collected from the first chromatographic run on HiScreen Capto L shown in Figure 1.

Column:	Kronlab ECO 10 mm packed with 2.4 mL Capto L, bed height 3.1 cm
Sample:	Precipitated plasma containing scFv fusion protein
Loading:	0.16 mg scFv fusion protein/mL medium
Buffer A:	PBS + 0.1 M glycine, pH 8
Buffer W2:	0.05 M glycine + 0.05 M citrate, pH 4
Buffer B:	0.05 M glycine + 0.05 M citrate, pH 2
Gradient:	10 CV gradient elution, pH 4.0 to 2.0
Flow rate:	0.53 mL/min (residence time 4.6 min)
System:	ÄKTApurifier™ 100
Detection:	Absorbance at 280 nm

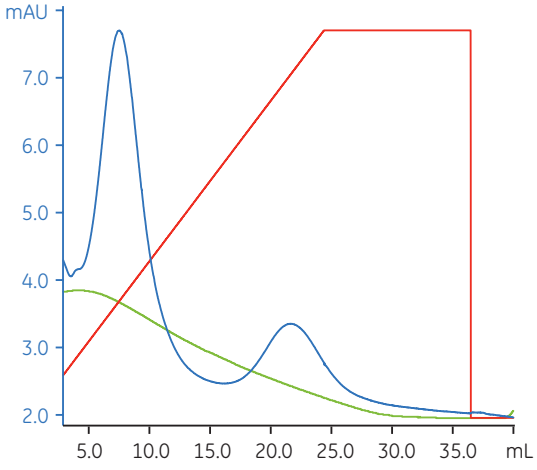


Fig 3. Elution on Capto L with a linear pH gradient from pH 4 to pH 2 resolved two peaks.

Column:	XK 16/20 packed with 21.1 mL Capto L, bed height 10.5 cm
Sample:	Plasma containing scFv fusion protein
Loading:	1.8 mg scFv fusion protein/mL medium
Buffer A:	PBS + 0.1 M glycine, pH 8
Buffer W2:	0.05 M glycine + 0.05 M citrate, pH 3.5
Buffer B:	0.05 M glycine + 0.05 M citrate, pH 2
Flow rate:	4.6 mL/min (residence time 4.6 min)
System:	ÄKTApurifier 10
Detection:	Absorbance at 280 nm

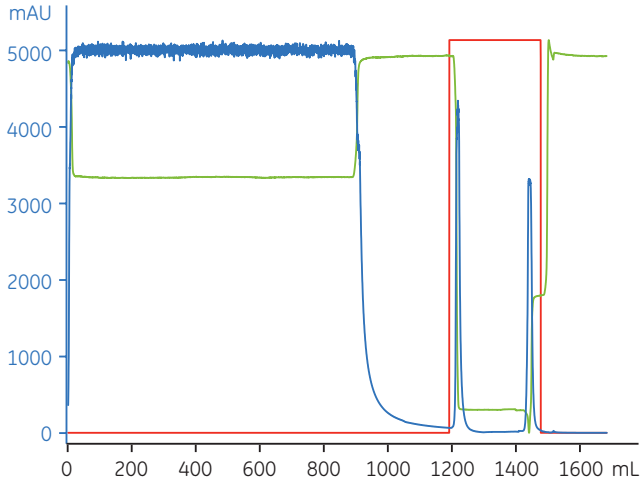
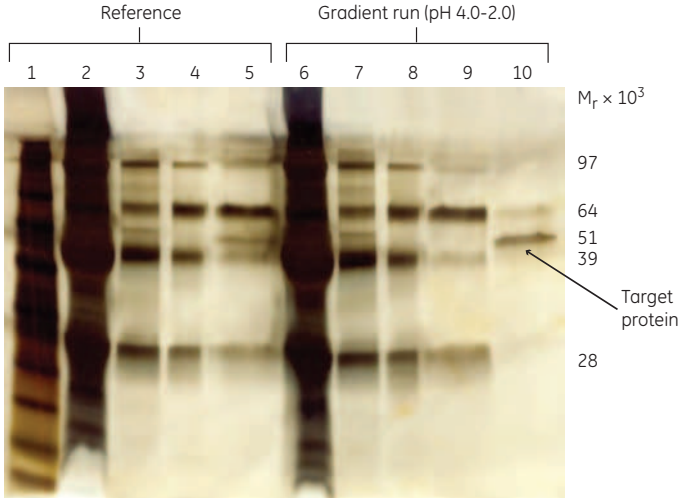


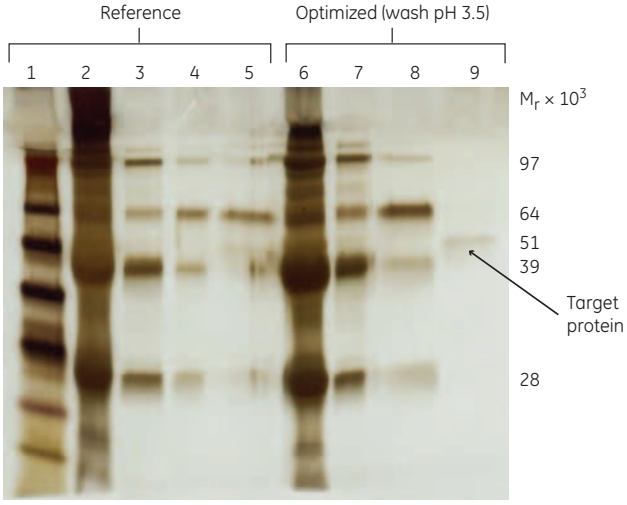
Fig 5. Optimized chromatographic run on Capto L with the second wash buffer modified from pH 4.0 to pH 3.5.



Lane

1. Marker
2. Flow-through
3. Wash PBS
4. Wash pH 4.0
5. Elution pH 2.0
6. Flowthrough
7. Wash PBS
8. Wash pH 4.0
9. Gradient peak 1
10. Gradient peak 2

Fig 4. SDS-PAGE (silver staining, reducing conditions) of samples from the runs shown in Figures 1 and 3.



Lane

1. Marker
2. Flow-through
3. Wash PBS
4. Wash pH 4.0
5. Elution pH 2.0
6. Flowthrough
7. Wash PBS
8. Wash pH 3.5
9. Elution pH 2.0

Fig 6. SDS-PAGE (silver staining, reducing conditions) of samples from the runs shown in Figures 1 and 5.

Dynamic binding capacity

Dynamic binding capacity (DBC) at 10% breakthrough was determined at 4.6 min residence time. The sample was purified scFv fusion protein at a concentration of 651 mg/L. Results shown in Figure 7 indicate a DBC of 23 mg/mL at 10% breakthrough.

Column: Kronlab ECO^{PLUS}, 5 mm i.d.
Sample: Pre-purified scFv fusion protein, c = 651 mg/L
Fraction volume: 1 mL
System: ÄKTA™ avant 25

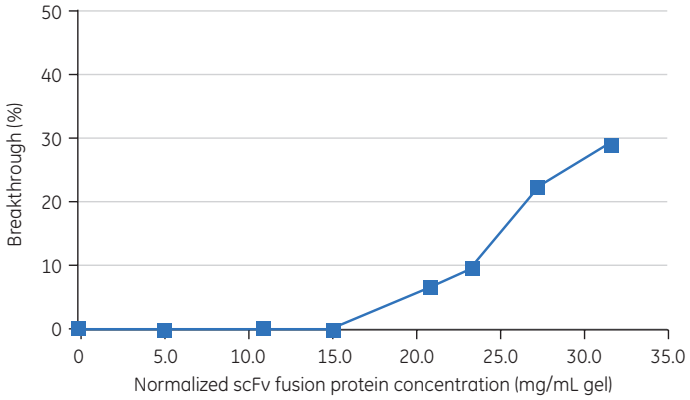


Fig 7. Breakthrough curve for scFv fusion protein at 4.6 min residence time. Breakthrough is shown as a percentage of the starting concentration of the purified scFv fusion protein.

Following optimization of the wash and elution steps and determination of DBC, two consecutive runs were performed on a larger column (CV 34 mL) with increased loading density (6.7 mg scFv fusion protein/mL medium).

Figure 8 shows the results. Table 1 compares contaminant reduction (measured by ELISA) between both runs and the original plasma sample, as well as yield. For all contaminants measured, the log reduction achieved is both large and reproducible. The yield from both runs was good.

In addition, SDS-PAGE results shown in Figure 9 confirm the high purity of the target protein. Fractions analyzed are from the first of the two scaled-up runs.

Table 1. Reduction of process-related impurities during the two consecutive purifications shown in Figure 8

Host cell protein levels	Plasma	Run 1	Run 2	Reduction
Albumin (ppm)	3.7×10^7	2.8×10^4	2.8×10^4	3 log
IgG (ppm)	1.8×10^8	7.6×10^3	7.9×10^3	4.5 log
Plasma proteins (ppm)	1.3×10^9	1.6×10^4	1.8×10^4	5 log
Yield (%)	—	105	89	—
Protein L leakage (ppm)	—	≤ 2	≤ 2	—

Column: XK 26/20 packed with 34 mL Capto L, bed height 6.4 cm
Sample: Plasma containing scFv fusion protein
Loading: 6.7 mg scFv fusion protein/mL medium
Buffer A: PBS + 0.1 M glycine, pH 8
Buffer W2: 0.05 M glycine + 0.05 M citrate, pH 3.5
Buffer B: 0.05 M glycine + 0.05 M citrate, pH 2
Flow rate: 7.4 mL/min (residence time 4.6 min)
System: ÄKTAexplorer 100
Detection: Absorbance at 280 nm

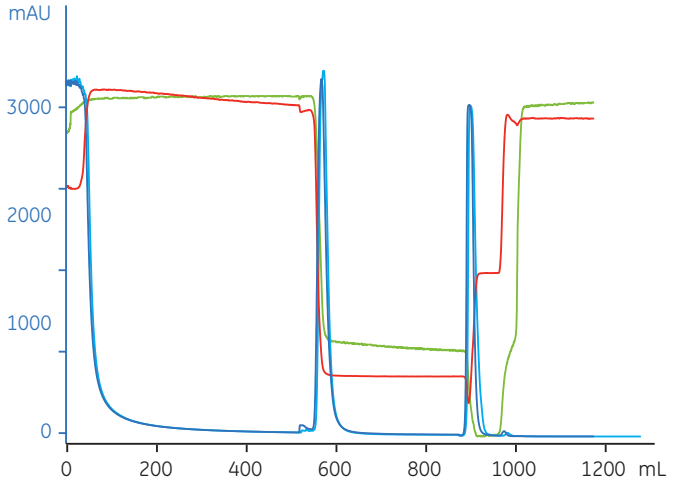
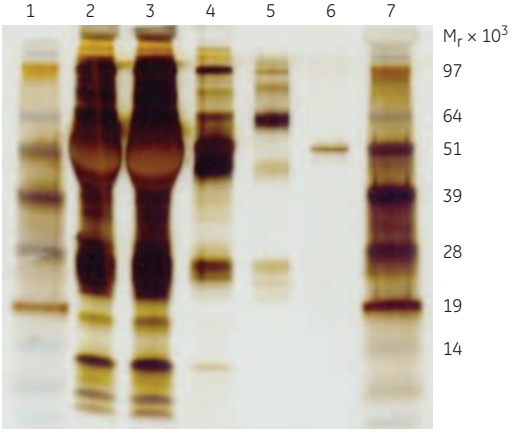


Fig 8. Two consecutive purifications with optimized wash and elution steps on a larger XK 26/20 column packed with 34 mL Capto L and run at increased loading density.



Lane
1. Marker
2. Sample
3. Flowthrough
4. Wash PBS
5. Wash pH
6. Elution pH 2.0
7. Marker

Fig 9. SDS-PAGE (silver staining, reducing conditions) of samples from the first optimized run shown (Fig 5) in XK 26/20 column packed with 34 mL Capto L (Table 1). The molecular weights of the marker are: orange 97 kDa, 64 kDa, 51 kDa, 39 kDa, 28 kDa, red 19 kDa, 14 kDa.

Conclusion

A highly efficient chromatography step for capturing a scFv fusion protein from plasma has been developed on Capto L, an affinity medium that features a recombinant protein L ligand. Using affinity chromatography for capture offers high selectivity, and concentrates the target molecule with high purity in one step. In addition to saving time, this also meant that the scFv fusion protein was effectively separated from contaminants such as host cell proteins (HCP).

The study demonstrates high selectivity and high capacity when processing a challenging starting sample, i.e. animal plasma. However, practical considerations regarding purification cycle-time duration meant that the CMO chose not to fully utilize the capacity determined for Capto L (loading density 6.7 mg/mL medium) in their process. The potential therefore exists to further improve productivity in this and similar applications by processing samples with higher expression levels or by shortening residence time in the column.

Acknowledgement

We thank BIBITEC GmbH (www.bibitec.de) for fruitful discussions and excellent application work.

Ordering information

Product*	Quantity	Code no.
Capto L	25 mL	17-5478-01
Capto L	200 mL	17-5478-02
Capto L	1 L	17-5478-03
Capto L	5 L	17-5478-04
Capto L	10 L	17-5478-05
PreDictor™ Capto L, 6 µl	4 × 96-well plates	17-5478-30
PreDictor Capto L, 20 µl	4 × 96-well plates	17-5478-31
PreDictor, Capto L, 50 µl	4 × 96-well plates	17-5478-32
PreDictor RoboColumn™, 200 µl	One row of 8 columns	28-0034-20
PreDictor RoboColumn, 600 µl	One row of 8 columns	28-0034-21
HiScreen Capto L	1 × 4.7 mL	17-5478-14
HiTrap™ Capto L	5 × 1 mL	17-5478-51
HiTrap Capto L	1 × 5 mL	17-5478-15
HiTrap Capto L	5 × 5 mL	17-5478-55

* Capto L is available on request in prepacked, prequalified, and presanitized ReadyToProcess™ columns. Please ask your local GE Healthcare sales representative for details.

Related products

Column		
HiScale™ 16/20	1	28-9644-41
HiScale 16/40	1	28-9644-24
HiScale 26/20	1	28-9645-14
HiScale 26/40	1	28-9645-13
HiScale 50/20	1	28-9644-45
HiScale 50/40	1	28-9644-44

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