Capto L

AFFINITY CHROMATOGRAPHY

Capto™ L is an affinity chromatography medium (resin) for capture of antibodies and antibody fragments (Fig 1). It combines a rigid, high-flow agarose matrix with the immunoglobulin-binding recombinant protein L ligand, which has strong affinity for the variable region of an antibody's kappa light chain (Fig 2). Capto L is therefore suitable for capture of a wide range of antibody fragments such as Fabs, single-chain variable fragments (scFv), and domain antibodies (Dabs).

Key performance features of Capto L include:

- High dynamic binding capacity and selectivity for a broad range of antibody fragments
- Enables a platform solution for the purification for antibody fragments containing kappa light chain
- Rigid base matrix allowing for high flow rates and high productivity as well as low ligand leakage
- BioProcess™ medium with regulatory support and security of supply for commercial manufacturing

Capto L is available in bulk sizes as well as prepacked formats to support screening and optimization of binding and elution conditions. Prepacked formats include PreDictor™ 96 well plates and PreDictor RoboColumn™ units, as well as HiTrap™ and HiScreen™ prepacked columns (Fig 1).

Fig 1. Capto L for capture of antibody fragments.

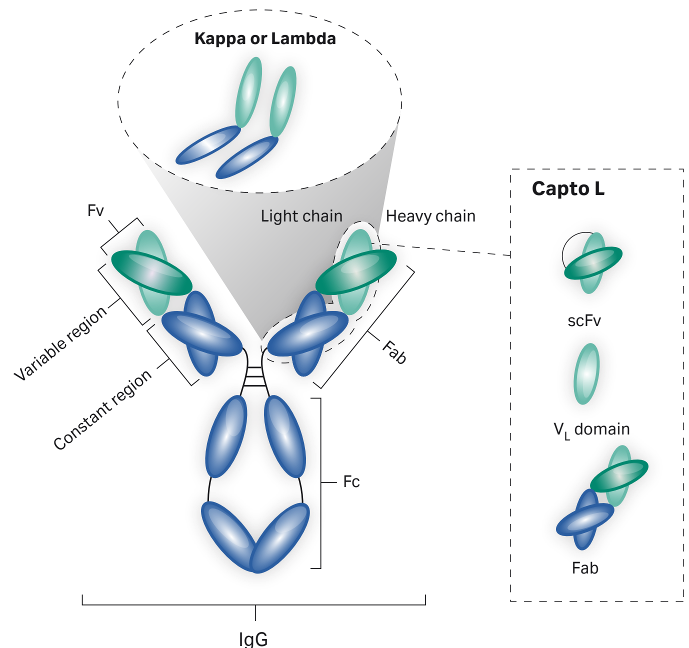


Fig 2. The protein L ligand in Capto L binds to the variable region of an antibody's kappa light chain without interfering with its antigen-binding site.

cytiva

Capto L characteristics

The key characteristics of Capto L are listed in Table 1. It is based on the same high flow agarose base matrix as MabSelect SuRe™ and enables processing of large volumes of feed with high productivity. The ligand, recombinant protein L, has four binding domains and binds to the variable region of the kappa light chain of immunoglobulins and immunoglobulin fragments.

Protein L binds to three of four kappa light chain subtypes in humans (1, 3, and 4) and kappa 1 in mice. Table 2 maps the full protein L binding affinity.

Table 1. Main characteristics of Capto L

Matrix	Rigid, highly cross-linked agarose
Ligand	Recombinant protein L (*E. coli*), mammalian free
Coupling chemistry	Epoxy activation
Average ligand density	10 mg/mL
Average particle size (d_{50v})*	85 µm
Dynamic binding capacity ($Q_{b10\%}$)[†]	Approx. 25 mg human Fab (M_r 50 000) /mL medium at 4 min residence time
Maximum flow velocity	500 cm/h at bed height 20 cm
pH stability[‡]:	
Working range	2 to 10
Cleaning-in-place	Recommended cleaning-in-place protocol: 15 mM NaOH
Working temperature	2°C to 40°C
Storage	2°C to 8°C in 20% ethanol
Regulatory Support File	Regulatory Support File is available. No material of animal origin is used in the manufacturing process

* Medium particle size distribution of the cumulative volume distribution.
[†] Determined at 10% breakthrough by frontal analysis. Binding capacity depends on the specific antibody fragment and on the molecular weight of the target.
[‡] Working range=pH interval where the medium can be operated without significant change in function, Cleaning-in-place=pH where the medium can be subjected to cleaning-in-place without significant change in function.

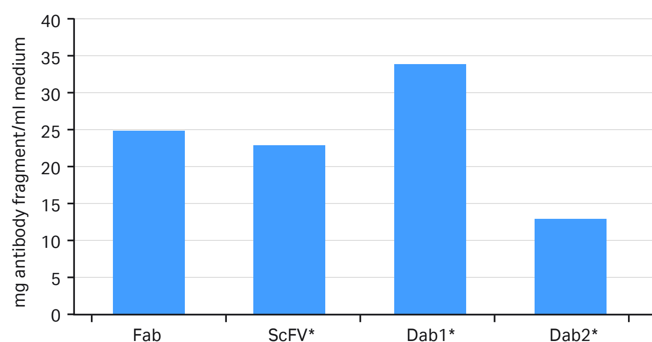
Table 2. Protein L binding affinities*

Species	Antibody class	Affinity[†]
General	Kappa light chain (subtypes 1,3,4)	Strong
	Lambda light chain	No binding
	Heavy chain	No binding
	Fab	Strong
	ScFv	Strong
	Dab	Strong
Human	IgG1	Strong
	IgG2	Strong
	IgG3	Strong
	IgG4	Strong
	IgA	Strong
	IgD	Strong
	IgE	Strong
	IgM	Strong
Mouse	IgG1	Strong
	IgG2a	Strong
	IgG2b	Strong
	IgG3	Strong
	IgM	Strong
Rat	IgG1	Strong
	IgG2a	Strong
	IgG2b	Strong
	IgG2c	Strong
Pig	Total IgG	Strong
Dog	Total IgG	Weak
Cow	IgG1	No Binding
	IgG2	No Binding
Goat	IgG1	No Binding
	IgG2	No Binding
Sheep	IgG1	No Binding
	IgG2	No Binding
Chicken	Total IgG	No Binding

* De Chateau, M. *et al.* On the interaction between protein L and immunoglobulins of various mammalian species. *Scand. J. Immunol.* **37**, 399-405 (1993).
[†] Binding to protein L occurs only if the immunoglobulin has the appropriate kappa light chains. Stated binding affinity refers only to species and subtypes with appropriate kappa light chains. Lambda light chains and some kappa light chains will not bind.

High binding capacity and selectivity for antibody fragments of different sizes and species

Capto L is designed to be used in the capture step of antibody fragments of different sizes and species. Due to the high affinity binding of protein L to the variable region of the kappa light chain, Capto L purifies conventional Fabs as well as the smallest functional entity of antibodies, so-called domain antibodies (Dabs). Figure 3 shows the dynamic binding capacity ($Q_{b10\%}$) of Capto L for a number of different antibody fragments. Note that because dynamic binding capacity is normally measured in mg/mL, the molecular weight of the target molecule is an important factor to consider. Table 3 presents the dynamic binding capacity in relation to the molecular weight and the corresponding molar binding capacity.



* The results for scFv and the two Dabs have been obtained through customer collaborations

Fig 3. Dynamic binding capacity (DBC $Q_{b10\%}$) of Capto L for four human antibody fragments. Fab fragment kindly provided by UCB Celltech.

Table 3. Dynamic binding capacity for a number of different antibody fragments

Molecule	DBC (mg/mL)	M_r (kDa)	Molar equivalence*
Fab	25	50	0.5 µmol Fab /mL medium
scFv fusion protein	23	57	0.5 µmol scFv/mL medium
Dab1	34	25	1.4 µmol Dab/mL medium
Dab2	13	10	1.3 µmol Dab/mL medium

* Note as a comparison that typical Protein A media capture app. 0.3 µmol IgG/mL medium.

High purity and yield in one step

Antibody fragments are often expressed in microbial systems and the homogenate entering downstream purification is often crude and challenging. A domain antibody was purified from clarified *E. coli* fermentation broth using Capto L.

Figure 4 shows approximately 11.4 mg Dab/mL medium loaded at pH 7.0 at a flow rate of 300 cm/h and a residence time of 4 min. A wash step followed at pH 5.0 to remove weakly bound impurities. Elution of bound material was performed with a step gradient using sodium acetate buffer pH 3.0. Flow through and eluted fractions were collected and analyzed by SDS-PAGE. The elution pool contained highly enriched Dab protein, Figure 5, lane 4. Product recovery was 87% and the *E. coli* host cell protein (HCP) levels were reduced from ~28 million ppm to ~6000 ppm.

This single capture step resulted in a HCP level clearance log reduction of 3.6 and a final purity of Dab protein of 93.2%, see Table 4.

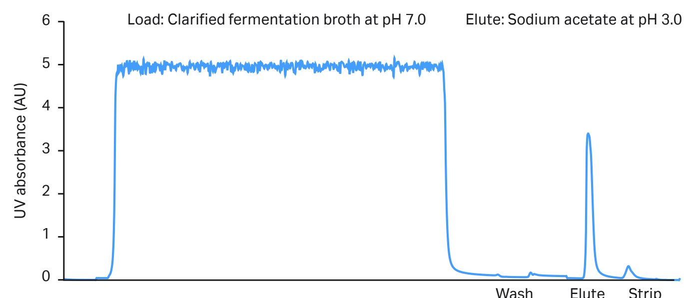


Fig 4. Purification of Dab from *E. coli* with Capto L.

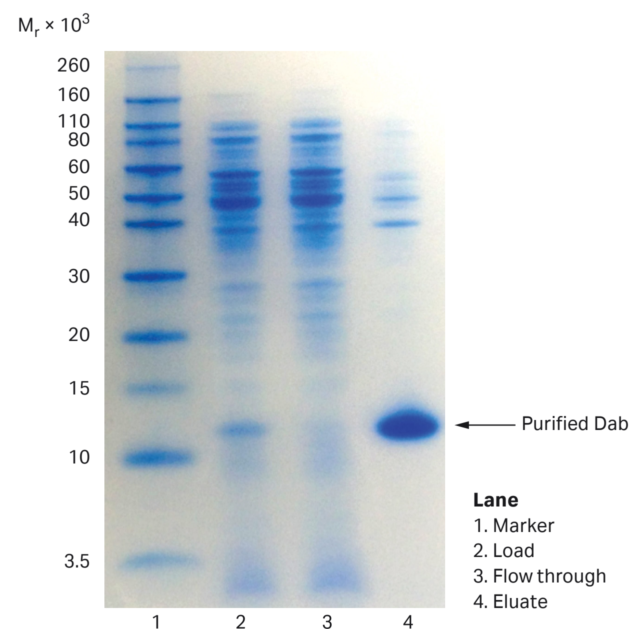


Fig 5. SDS PAGE of load, flowthrough, and eluted fractions from the purification of the Dab molecule. SDS-PAGE (Invitrogen, 4-12% Bis/Tris) run under reducing conditions with Coomassie™ stain.

Table 4. Results from purification of Dab protein from clarified *E. coli* broth using Capto L medium. The results presented were obtained through customer collaboration

Parameter	Result
E. coli HCP Clearance log	3.6 log reduction
Purity (evaluated by SEC)	93.2 % monomer 6.5 % aggregates
Yield	87%

Affinity for mouse kappa light chain

The protein L ligand also has affinity for mouse and rat antibody fragments, which makes it very useful in research applications as well. Figure 6 demonstrates the affinity of Capto L for mouse Fabs containing the kappa light chain.

A polyclonal mouse IgG Fab fragment was loaded onto a HiTrap Protein L, prepacked with Capto L. The Fab fragments containing the lambda light chain did not bind to the protein L ligand indicated by the peak in the flowthrough. The Fabs containing the kappa light chain bound to Capto L medium and was eluted when the pH was decreased. These results demonstrate the affinity of Capto L medium for mouse kappa light chain.

Column:	HiTrap Protein L
Sample:	2 mg Fab/mL medium, polyclonal mouse IgG Fab fragment (Jackson ImmunoResearch laboratories)
Binding buffer:	PBS, pH 7.4
Wash buffer:	0.025 M sodium citrate + 0.025 M sodium phosphate, pH 7.4
Elution buffer:	0.025 M sodium citrate + 0.025 M sodium phosphate, pH 2.3
Flow rate loading:	0.25 mL/min (residence time 4 min)

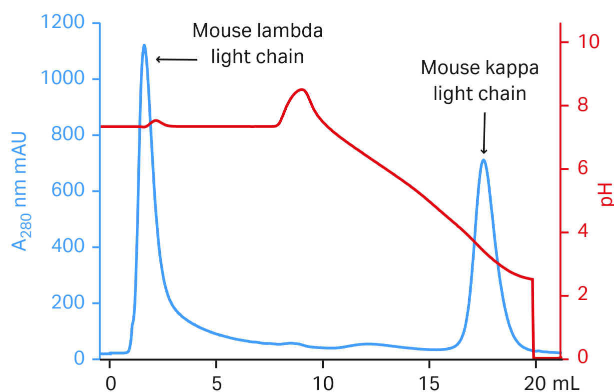


Fig 6. Affinity of Capto L for mouse polyclonal IgG Fab containing kappa light chain. Fraction containing kappa light chain elutes in the pH gradient.

Broad specificity enables a platform approach for antibody fragment purification

Monoclonal antibodies are typically purified using a platform approach where capture by protein A affinity chromatography has become the industry standard. However, a corresponding solution for antibody fragments has been lacking. One reason is the high diversity of the antibody fragments; and another is that chromatography media currently available on the market do not meet the demands for industrial scale purification. The introduction of Capto L provides the foundation for a purification platform approach for this class of biomolecules.

Affinity capture allows very robust and generic processes compared to ion exchange and hydrophobic interaction chromatography. Purification with Capto L requires minimal optimization and gives high purity in one separation step. This results in significant savings in costs, time, and effort during process development. Gains can also be made in manufacturing as the high selectivity of affinity capture will help reduce the number of subsequent polishing steps as compared to other, less selective purification media.

In cases where Capto L lacks affinity for the antibody fragment of interest there are a number of complementary affinity chromatography media available from Cytiva to enable a comprehensive capture toolkit. KappaSelect binds kappa Fabs (constant region) and LambdaFabSelect binds lambda Fabs (constant region). In addition MabSelect™ can be used to capture antibody fragments due to its affinity for the heavy chain subtype V_{H3}. Figure 7 is a general guide for media selection.

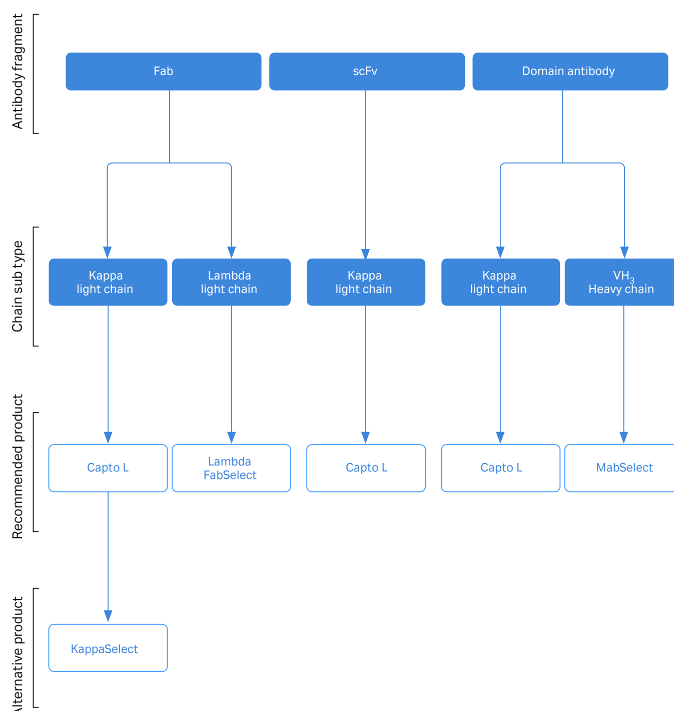


Fig 7. Guide to affinity media for antibody fragments.

Antibody fragments that fall outside of this toolkit include scFv (single-chain Fv) fragments with the lambda light chain, and heavy-chain domain antibodies with subtypes V_{H1}, V_{H2} and V_{H4}. For these fragments, we recommend ion exchange chromatography or multimodal media, e.g. Capto MMC.

Cleaning-in-place and sanitization

The recommended CIP method for Capto L is 15 mM NaOH for 15 min after each cycle. The CIP protocol was verified in column experiments using Fab-containing *E. coli* homogenate. CIP was performed in every cycle using a contact time of 15 min. The results show that Capto L retained more than 90% of its initial binding capacity for 80 cycles with the recommended 15 mM NaOH CIP solution, see Figure 8 below.

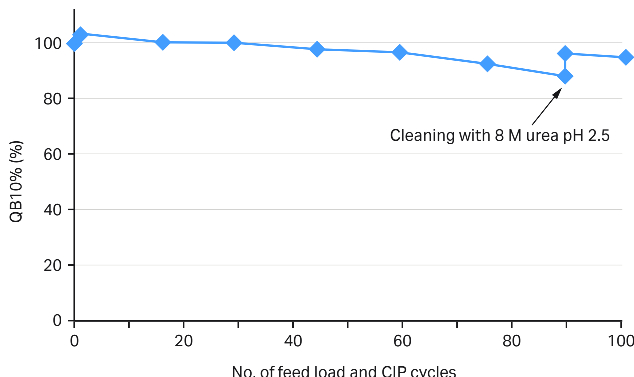


Fig 8. DBC capacities of Capto L during 100 chromatographic cycles of *E. coli* homogenate containing Fab fragment using 15 mM NaOH as CIP agent.

In cases with reduced performance, the cleaning procedure can be complemented with 8 M urea, 0.05 M citric acid pH 2.5 to remove strongly bound impurities. This cleaning solution can be used at regular intervals or when considered necessary, depending on the nature of the feed. At cycle 89 in Figure 8 above, the Capto L column was cleaned with this solution with positive effects on binding capacity.

Stronger NaOH can be used, but might reduce the lifetime of the medium.

The stability of Capto L towards caustic conditions was investigated and compared to MabSelect, a protein A-based affinity medium used to purify monoclonal antibodies. Capto L and MabSelect were both exposed to repeated CIP cycles of 100 mM NaOH, contact time 15 min. Figure 9 shows that approximately 80% of Capto L binding capacity remains after 20 CIP cycles at 100 mM NaOH, whereas MabSelect shows slightly higher resistance to 100 mM NaOH and can be used for more than 30 cycles with this NaOH concentration.

Addition of salt (e.g., NaCl or Na_2SO_4) to the caustic CIP solution can increase the ligand stability but decreases the cleaning efficiency. Another alternative is using a two-step sequence with reducing agent (e.g. 100 mM 1-Thioglycerol) followed by 15 mM NaOH. Experiments have shown positive effects on the cleaning ability of the CIP solution with reducing agent as a pre-step*.

Studies† demonstrate that Capto L retains consistent capacity after 24 h exposure to sanitization solutions such as 0.1 M acetic acid in 20% ethanol.

* Gronberg, A. *et al.* A tool for increasing the lifetime of chromatography resins. mAbs, **3**, 192-202 (2011).
† Capto L regulatory support file, 11-0029-90, Edition AA (2012).

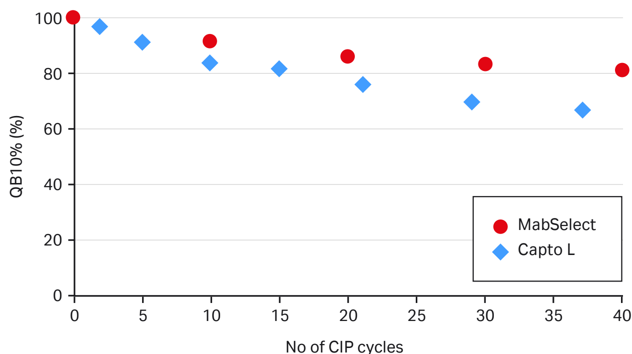


Fig 9. Stability of Capto L and MabSelect in 100 mM NaOH. Capto L retains approximately 80% of binding capacity of Fab after 20 CIP cycles.

Rigid, highly cross-linked matrix allows high flow rates

Capto L is based on the same rigid, highly cross-linked agarose matrix used for the MabSelect platform of affinity media. This rigid base matrix allows for pressure/flow properties that result in high productivity and improved process economy. The ability to run at higher flow rates and higher bed heights also increases flexibility in process design. Figure 10 shows an example of the pressure flow curve for Capto L.

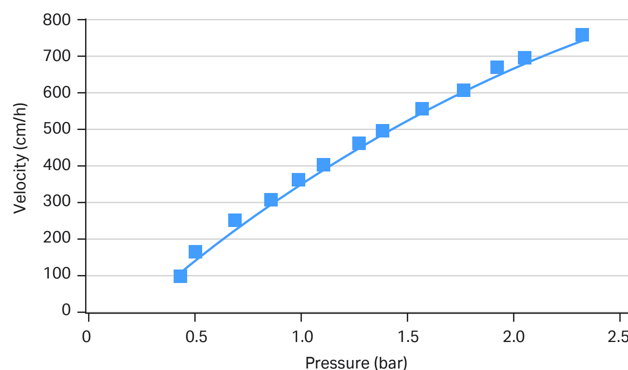


Fig 10. Example of pressure flow curve for Capto L in packed bed. Running conditions: AxiChrom™ 300 (30 cm i.d.), 20 cm bed height with packing factor 1.15 in water at 20°C. Pressure contributions from system, tubing, and column are excluded.

Low ligand leakage

The protein L ligand is immobilized to the agarose base matrix via a multi-point coupling that gives Capto L high chemical stability and low ligand leakage. The protein L ligand leakage from Capto L has been analyzed using a commercial non-competitive ELISA, protein L ligand leakage kit‡. The ELISA was developed to analyze recombinant protein L in the presence of Fab. Typical values found in the Fab-containing eluates from purification of *E. coli* homogenate feed showed ligand leakage below the quantitation limit of 5.7 ng/mL. This corresponds to a protein L level of <1.4 ppm of purified Fab.

‡ Sold and manufactured by Medicago AB, Uppsala Sweden. (www.medicago.se)

Optimization of elution conditions

After washing, the target antibody fragment is eluted by decreasing the pH. Elution at a pH between 2 and 3.5 results in high yields and low pool volumes. Antibody fragment dimers have been observed to bind more tightly to the Capto L resin and elute later than corresponding monomers.

Elution conditions for a specific antibody fragment can be determined by performing elution in a linear pH gradient. Figure 11 shows how optimal elution pH for an antibody fragment captured on Capto L was determined using a pH gradient from pH 6.5 to 2.3. In this example a pH of 3.2 or lower elutes the antibody fragment.

Sample:	7.4 mg (1 mL Fab)
Column:	Capto L prepacked in Tricorn™ 5/50 (1 mL)
Flow rate:	1 mL/min
Binding and wash buffer:	50 mM sodium citrate, pH 6.5
Elution buffer:	50 mM sodium citrate, pH 2.3
Gradient:	Linear 0-100% elution buffer in 20 column volumes (CV)

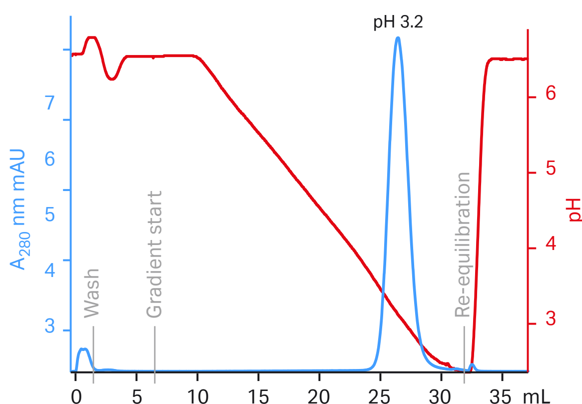


Fig 11. Determination of optimal elution conditions of an antibody fragment captured on Capto L.

Small-scale prepacked formats give fast screening, fast method development, and convenient small-scale purifications

Using prepacked small-scale formats to screen for the most suitable chromatography media and/or process conditions in the early stages of process development saves both time and sample. The primary aim of method development is to establish and optimize the conditions that will bind the highest amount of target protein in the shortest time, to the best economy and with the highest product recovery.

Like other Capto and MabSelect media, Capto L is available in prefilled 96-well PreDictor plates and PreDictor Robocolumn, which support high-throughput process development (HTPD) by allowing parallel screening of chromatographic conditions such as pH and additives.

Defined conditions for loading, washing, elution, CIP etc. can then be verified and optimized with small prepacked columns such as HiTrap Protein L 1 mL and 5 mL and HiScreen Capto L, 4.7 mL. These are easily used together with a chromatography system such as ÄKTA™ avant when developing an efficient and robust purification method or performing small scale purifications.

Basic characteristics of HiTrap and HiScreen prepacked columns are summarized in Tables 5 and 6, respectively.

Table 5. Main characteristics of HiTrap columns prepacked with Capto L

Column volume	1 mL and 5 mL
Column dimensions	0.7 × 2.5 cm (HiTrap 1 mL) 1.6 × 2.5 cm (HiTrap 5 mL)
Maximum flow rates	4 mL/min (HiTrap 1 mL) 20 mL/min (HiTrap 5 mL)
Recommended flow rates	1 mL/min (HiTrap 1 mL) 5 mL/min (HiTrap 5 mL)
Column hardware pressure limit	5 bar (0.5 MPa, 70 psi)

Table 6. Main characteristics of HiScreen columns prepacked with Capto L

Column volume	4.7 mL
Column dimensions	0.77 × 10 cm
Maximum flow velocities	500 cm/h
Recommended flow velocities	120 cm/h to 150 cm/h
Column hardware pressure limit	8 bar (0.8 MPa, 116 psi)

Further development and optimization using HiScale™ columns then permits straightforward scale-up to full-scale manufacturing on AxiChrom columns.

BioProcess medium with full support

Capto L is a BioProcess medium which means that it is developed and supported for the large-scale manufacture of biopharmaceuticals. This support includes validated manufacturing methods, secure long-term media supply, safe and easy handling, and Regulatory Support Files (RSF) to assist process validation and submissions to regulatory authorities. In addition, Fast Trak Training & Education provide high-level, hands-on training for all key aspects of bioprocess development and manufacturing.

Recommended columns

Capto L can be used with most modern chromatography equipment from laboratory to production scale. Table 7 lists suitable empty columns from Cytiva. Axial compression has been shown to be the preferred packing method for these high-flow agarose resins. To ensure best performance at process scale, Capto L should be packed at bed heights of 10 to 30 cm.

Table 7. Recommended Cytiva column families for packing Capto L

Column family	Inner diameter (mm)
Laboratory scale	
Tricorn	5 and 10
HiScale	16 and 26
Pilot and production scale	
AxiChrom	50 to 1000
BPG™	100 to 300*
Chromaflow™ †	400 to 800‡

* The pressure rating of BPG 450 is too low to allow use with Capto L.
† Packing instructions for MabSelect media in Chromaflow columns are described in Application note 11-0007-52. Since Capto L is based on similar base matrix this might also provide useful guidance for Chromaflow packing.
‡ Larger pack stations may be required for larger diameters.

Storage

Store unused Capto L at 2°C to 8°C in 20% ethanol to prevent microbial growth. Do not freeze.

Store prepacked HiTrap Protein L and HiScreen Capto L columns at 2°C to 8°C in 20% ethanol to prevent microbial growth. Do not freeze.

After storage, equilibrate with suitable binding buffer and perform a blank run, including CIP, before use. An alternative storage solution to prevent microbial growth is 2% benzyl alcohol.

Acknowledgement

The Fab fragment used and presented in this datafile was kindly provided by UCB Celltech, UK.

Ordering information

Product*	Quantity	Code number
Capto L	5 mL	17-5478-06
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	29-0034-20
PreDictor Robocolumn, 600 µl	One row of 8 columns	29-0034-21
HiScreen Capto L	1 × 4.7 mL	17-5478-14
HiTrap Protein L	1 × 1 mL	29-0486-65
HiTrap Protein L	5 × 1 mL	17-5478-51
HiTrap Protein L	1 × 5 mL	17-5478-15
HiTrap Protein L	5 × 5 mL	17-5478-55

* Capto L is available on request in prepacked and prequalified ReadyToProcess™ columns. Please ask your local Cytiva representative for details.

Related products	Quantity	Code number
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

Protein L ligand leakage kit – External supplier	Product	Code number
Medicago AB (www.medicago.se)	Protein L ligand leakage ELISA kit	10-0027

Accessories	Quantity	Code number
1/16” male/Luer female†	2	18-1112-51
Tubing connector flangeless/M6 female	2	18-1003-68
Tubing connector flangeless/M6 male	2	18-1017-98
Union 1/16” female/M6 male	6	18-1112-57
Union M6 female/1/16” male	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep™, 1/16” male connector for ÄKTAdesign	8	28-4010-81
Stop plug female, 1/16”‡	5	11-0004-64
Fingertight stop plug, 1/16”§	5	11-0003-55

† One connector included in each HiTrap package.
‡ Two, five, or seven stop plugs female included in HiTrap packages depending on products.
§ One fingertight stop plug is connected to the top of each HiTrap column at delivery.

Related literature	Code number
Data files	
HiScreen columns	28-9305-81
PreDictor Plates	28-9258-39
PreDictor Robocolumn	28-9886-34
MabSelect	18-1149-94
KappaSelect and LambdaFabSelect	28-9448-22
Application notes	
Capture of scFv fusion protein using Capto L	29-0144-56
Selection Guides	
Prepacked chromatography columns for ÄKTA systems	28-9317-78
Capture toolkit for antibody fragments	29-0164-33
Handbooks	
Antibody purification, Principles and methods	18-1037-46
High-throughput process development with PreDictor plates, Principles and methods	28-9403-58

cytiva.com/bioprocess
cytiva.com/protein-purification

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