Selection guide

Capto L
HiTrap Protein L
HiScreen Capto L

cytiva

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

The specificity of binding to the variable region of kappa light chain of antibodies provides excellent purification in one step.

The high capacity, low ligand leakage and high flow properties make Capto L suitable for the purification of antibody fragments from lab to process scale.

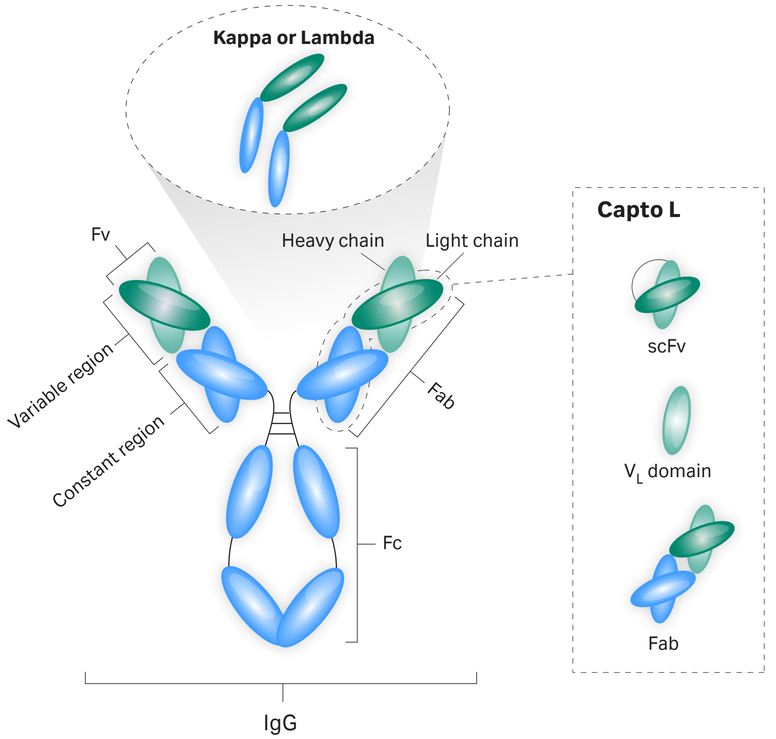
Available products

Prepacked HiTrap™ Protein L 1 ml and 5 ml, HiScreen™ Capto L as well as Capto L 5 ml, 25 ml and 200 ml lab packs.

Key features Capto L chromatography medium and prepacked formats

- High specificity for kappa light chain allows efficient capture of a broad selection of antibodies and antibody fragments
- High dynamic binding capacity reduces purification time and amount of medium used
- Low ligand leakage increases antibody fragment purity
- Prepacked HiTrap and HiScreen columns available for convenient and reproducible small scale purifications, screening and method development

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 (see illustration).



Applications

Binding of mouse Fab

The protein L ligand has affinity for mouse and rat antibody fragments. Figure 1 demonstrates the affinity of Capto L for mouse Fabs containing the kappa light chain. A polyclonal mouse IgG Fab fragment was loaded onto 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 flow through. 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 1 ml
Sample:	2 mg Fab. Polyclonal mouse IgG Fab fragment (Jackson ImmunoResearch laboratories)
Binding buffer:	PBS, pH 7.4
Wash buffer:	25 mM sodium citrate, 25 mM sodium phosphate, pH 7.4
Elution buffer:	25 mM sodium citrate, 25 mM sodium phosphate, pH 2.3
Flow rates:	Equilibration, wash and elution: 1 ml/min Sample load: 0.25 ml/ml
Gradient:	pH 7.4-2.3 in 10 column volumes
System:	ÄKTA™ avant 25

Fig 1. Affinity of Capto L for mouse polyclonal IgG Fab containing kappa light chain. Fraction containing kappa light chain elutes in the pH gradient.

Purification of a human domain antibody (Dab) expressed in *E. coli*

Antibody fragments are often expressed in microbial systems and the sample to be purified is often crude and challenging. Here a domain antibody was purified from clarified *E. coli* homogenate using Capto L.

Approximately 11.4 mg Dab/ml chromatography medium was loaded at pH 7.0 at a flow rate of 300 cm/h. 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 (Fig 2). Flow through and eluted fractions were collected and analyzed by SDS-PAGE. The elution pool contained highly enriched Dab protein, (Fig. 3, lane 4). Product recovery was 87% and final purity (determined by gel filtration) of the Dab protein was 93.2%. The results were obtained through customer collaboration.

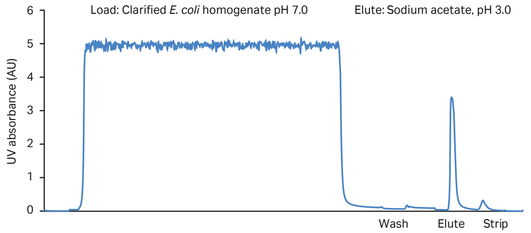


Fig 2. Purification of Dab from *E. coli* with Capto L.

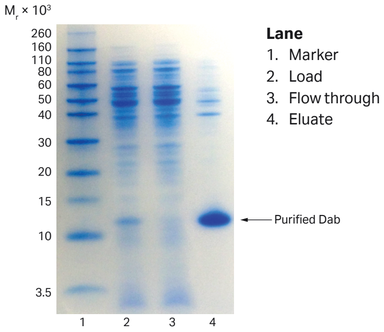


Fig 3. SDS PAGE of load, flowthrough, and eluted fractions from the purification of the Dab molecule. SDS-PAGE (Invitrogen, 4-12% Bis/Tris) run under reducing conditions with Coomassie™ stain.

Prepacked columns

HiTrap Protein L

HiTrap Protein L 1 ml and 5 ml columns are prepacked with Capto L. HiTrap Protein L columns can be operated with a syringe, peristaltic pump, or chromatography system such as ÄKTA. The columns are made of biocompatible polypropylene that does not interact with biomolecules. Columns are delivered with a stopper on the inlet and a snap-off end on the outlet. Note that HiTrap Protein L columns cannot be opened or refilled.

HiScreen Capto L

HiScreen Capto L columns are prepacked with Capto L chromatography medium. They are part of the process development platform available from Cytiva and are well suited for method optimization, parameter screening, and robustness testing. Process flow velocities can be applied, since the 10 cm bed height gives enough residence time and the results can then serve as basis for linear process scale-up. If necessary, two columns can easily be connected in series to give a bed height of 20 cm. The small bed volume, 4.7 ml, requires low sample and buffer volumes. HiScreen columns can be used several times with highly reproducible results.

HiScreen columns cannot be opened and repacked.

Characteristics of HiTrap columns

Column volume:	1 ml and 5 ml
Column dimensions:	0.7 × 2.5 cm and 1.6 × 2.5 cm
Column hardware pressure limit:	5 bar (0.5 MPa)

Characteristics of HiScreen columns

Column volume:	4.7 ml
Column dimensions:	0.77 × 10 cm
Column hardware pressure limit:	8 bar (0.8 MPa)

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 Da) /ml medium at 4 min residence time
Maximum flow velocity	500 cm/h at bed height 20 cm
pH stability‡:	
Working range	2-10
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

* 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.

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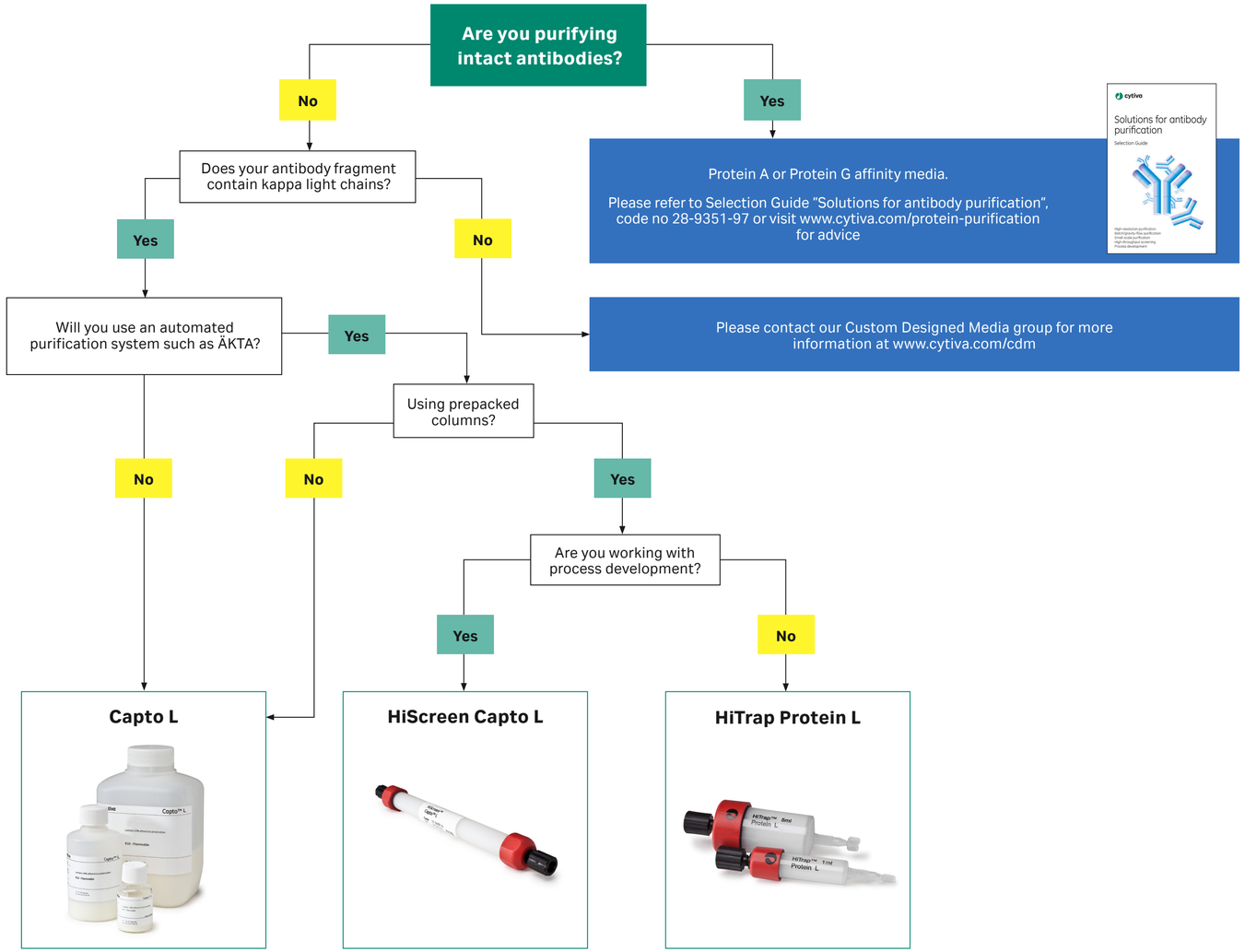

Ordering information

Product	Quantity	Code no.
Capto L	5 ml	17-5478-06
Capto L	25 ml	17-5478-01
Capto L	200 ml*	17-5478-02
HiScreen Capto L	1 × 4.7 ml	17-5478-14
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

* Larger pack sizes are available

cytiva.com/protein-purification

For local office contact information, visit cytiva.com/contact

CY14736-23Nov20-SG

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