HiScreen™ Capto™ L
HiTrap™ Protein L, 1 ml and 5 ml

Capto L is an affinity chromatography medium (resin) for capture 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 particular suitable for capture of a wide range of different-sized antibody fragments such as Fabs, single-chain variable fragments (scFv) and domain antibodies (dAbs).

Key performance features of Capto L include:

- 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.

HiScreen Capto L (4.7 ml) and HiTrap Protein L (1 ml and 5 ml) are prepacked columns for optimization of methods and parameters, such as sample load and binding conditions, as well as small scale purifications.

HiScreen Capto L and HiTrap Protein L columns provide fast, reproducible and easy separations in convenient formats. The columns are used in an optimal way with liquid chromatography systems such as ÄKTA™.

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Please read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the products in a safe way, please refer to the Safety Data Sheets.

1 Product description

HiScreen column characteristics

HiScreen columns are made of biocompatible polypropylene that does not interact with biomolecules. The columns are delivered with stoppers at the inlet and outlet. The arrow on the column label indicates the column orientation and the recommended flow direction, see Figure 1.

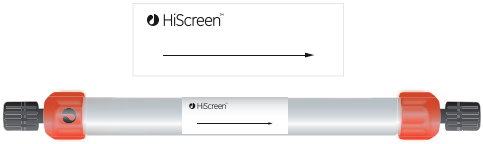

Fig 1. HiScreen column

For easy scaling-up and when a higher bed height is required, two columns can easily be connected in series using a union to give 20 cm bed height (see Section *Scaling up*).

Note: *HiScreen columns cannot be opened or refilled*

Note: *Make sure that the connector is tight to prevent leakage.*

Table 1. Characteristics of HiScreen column

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

Note: *The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography medium, sample/liquid viscosity and the column tubing used.*

HiTrap column characteristics

The columns are made of biocompatible polypropylene that does not interact with biomolecules.

The columns are delivered with a stopper at the inlet and a snap-off end at the outlet. Table 2 lists the characteristics of HiTrap columns.

Fig 2. HiTrap, 1 ml column.

Fig 3. HiTrap, 5 ml column

Note: *HiTrap columns cannot be opened or refilled.*

Note: *Make sure that the connector is tight to prevent leakage.*

Table 2. Characteristics of HiTrap columns

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

Note: *The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography medium, sample/liquid viscosity and the column tubing used.*

Supplied Connector kit with HiTrap column

Connectors supplied	Usage	No. supplied
Union 1/16" male/ luer female	For connection of syringe to HiTrap column	1
Stop plug female, 1/16"	For sealing bottom of HiTrap column	2, 5 or 7

Properties of Capto L

The protein L ligand is produced in *Escherichia coli*. Fermentation and subsequent purification are performed in the absence of animal products. The specificity of binding to the variable region of kappa light chain of antibodies provides excellent purification in one step. The epoxy-based coupling chemistry ensures low ligand leakage. 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.

The product is based on a high flow agarose base matrix, which gives good flow properties.

Table 3. Characteristics of Capto L

Matrix	Rigid, highly cross-linked agarose
Ligand	Recombinant Protein L (from *E. coli*)
Coupling chemistry	Epoxy
Average particle size (d_{50v}) [1]	85 µm
Dynamic binding capacity [2]	Approx. 25 mg Fab (M_r ~50 000)/ml medium
Maximum flow velocity [3]	500 cm/h
pH stability [4]	
Working range	2 to 10
Cleaning-in-place	15 mM NaOH
Working temperature	2°C to 40°C
Chemical stability	All commonly used aqueous buffers, e.g., 15 mM sodium hydroxide, 8 M urea, 6 M guanidine hydrochloride, 1 M acetic acid
Storage	2°C to 8°C in 20% ethanol

1 d_{50v} is the average particle size of the cumulative volume distribution.

2 Determined at 10% breakthrough by frontal analysis at a mobile phase velocity of 150 cm/h in a column with a bed height of 10 cm, i.e. residence time is 4.0 min. Residence time is equal to bed height (cm) divided by nominal flow velocity (cm/h) during sample loading. Nominal flow velocity is equal to volumetric flow rate (ml/h) divided by column cross-sectional area (cm^2).

3 Water at room temperature.
For viscous buffers and samples - the flow velocity must be optimized and starting with a low flow rate is recommended in order not to exceed pressure limits.

4 Working range: pH interval where the medium can be operated without significant change in function.
Cleaning-in-place: pH stability where the medium can be subjected to cleaning-in-place without significant change in function.

2 General process development

HiScreen column format is ideal to use for parameter and method optimization and for robustness testing when developing a new purification process. The small column volume, 4.7 ml, and the 10 cm bed height makes it possible to perform scalable experiments at relevant process flow rates. If necessary, two columns can easily be connected in series with a union to give 20 cm bed height (see Section *Scaling up*).

The figure below outlines typical steps during general process development.

Already from start in process development it is necessary to consider process cost, cleaning of the medium and environmental constraints.

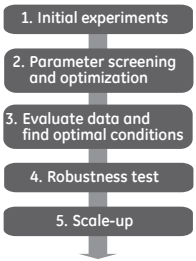

Fig 4. Typical steps during process development.

Design of Experiments (DoE) is an effective tool for investigating the effect of several parameters on protein recovery in order to establish the optimal purification protocol. A common approach in DoE is to define a reference experiment (center point) and perform representative experiments around that point. Some initial experiments are required in order to define the center point and the variable ranges. DoE may be used for parameter screening and optimization as well as robustness testing.

The robustness of a process is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters, and provides an indication of its reliability during normal usage. An objective of a robustness test is the evaluation of factors potentially causing variability in the responses of the method, for example, purity and yield. For this purpose, small variations in method parameters are introduced.

For scale-up, see Section *Scaling up*.

3 Operation

Prepare the sample

Step	Action
1	Adjust the sample to the composition of the binding buffer, using one of the following methods: • Dilute the sample with binding buffer. • Exchange buffer using a HiPrep™ 26/10 Desalting, a HiTrap Desalting or a PD-10 Desalting column (see Table 4).
2	Filter the sample through a 0.45 µm filter or centrifuge immediately before loading it to the column. This prevents clogging and increases the longevity of the column when loading large sample volumes. ***Note:*** *Water and chemicals used for buffer preparation should be of high purity. Filter buffers through a 0.22 µm or a 0.45 µm filter before use.*

Table 4. Prepacked columns for desalting

Column	Code No.	Loading volume	Elution volume	Comments	Application
HiPrep 26/10 Desalting	17-5087-01	2.5 to 15 ml	7.5 to 20 ml	Prepacked with Sephadex™ G-25 Fine. Requires a laboratory pump or a chromatography system to run.	For desalting and buffer exchange of protein extracts (M_r > 5000).
HiTrap Desalting	17-1408-01	0.25 to 1.5 ml	1.0 to 2.0 ml	Prepacked with Sephadex G-25 Superfine. Requires a syringe or pump to run.	
PD-10 Desalting	17-0851-01	1.0 to 2.5 ml[1] 1.75 to 2.5 ml[2]	3.5 ml[1] up to 2.5 ml[2]	Prepacked with Sephadex G-25 Medium. Runs by gravity flow or centrifugation	For desalting, buffer exchange, and cleanup of proteins and other large biomolecules (M_r > 5000).
PD MiniTrap™ G-25	28-9180-07	0.1 to 0.5 ml[1] 0.2 to 0.5 ml[2]	1.0 ml[1] up to 0.5 ml[2]		
PD MidiTrap™ G-25	28-9180-08	0.5 to 1.0 ml[1] 0.75 to 1.0 ml[2]	1.5 ml[1] up to 1.0 ml[2]		

[1] Volumes with gravity elution
[2] Volumes with centrifugation

Recommended flow rates

To allow antibody fragments to bind properly the flow rate during sample application should not be too high.

A good starting point is 1.2 ml/min for HiScreen, 1.0 ml/min for HiTrap 1 ml and 5.0 ml/min for HiTrap 5 ml (150 cm/h).

During column equilibration and wash steps higher flow rates can be used, up to 3.9 ml/min for HiScreen, 3.2 ml/min for HiTrap 1 ml and 16 ml/min for HiTrap 5 ml (500 cm/h), see Table below.

Table 5. Recommended flow rates for different operations.

Type of operation	Flow rate HiScreen (ml/min)	Flow rate HiTrap, 1 ml (ml/min)	Flow rate HiTrap, 5 ml (ml/min)	Flow velocity (cm/h)
Equilibration [1]	up to 3.9	up to 3.2	up to 16.0	up to 500
Wash[1]	up to 3.9	up to 3.2	up to 16.0	up to 500
Load of sample	0.8 to 2.3	0.6 to 1.9	3.3 to 10.0	100 to 300
Cleaning-in-place [2]	0.16 to 0.31	0.13 to 0.26	0.7 to 1.4	20 to 40

[1] Stated flow rates are for buffers with the same viscosity as water at 20°C. For solutions with higher viscosities, e.g., 20% ethanol, lower flow rates should be used.

[2] See also Section *Cleaning-In-Place (CIP)*.

Purification

Flow rate: See Table above for recommended flow rates for the different operations. Collect fractions throughout the separation.

Column tubing: Choose the optimal tubing kit for the column and the application you intend to run. (i.d.: 0.25, 0.50 or 0.75 [mm]). A tubing with wider inner diameter gives broader peaks whereas a tubing with a smaller inner diameter gives higher back pressure.

Step	Action
1	Before connecting the column to a system: • Remove the two stoppers from the HiScreen column. • Remove the stopper in the top and the snap-off in the bottom of the HiTrap column.
2	Connect the column to the system with a drop-to-drop connection to avoid introducing air into the column. ***Note:*** *To prevent leakage, make sure that the connectors are tight. Use fingertight 1/16" connector (28-4010-81).*
3	Wash with 1 column volume (CV) distilled water. This step removes the ethanol and avoids the precipitation of buffer salts upon exposure to ethanol. 20% ethanol has higher viscosity than water. Therefore the recommended flow rate for this step is: • 1.9 ml/min for HiScreen (250 cm/h) • 1.6 ml/min for HiTrap 1 ml (250 cm/h) • 8.4 ml/min for HiTrap 5 ml (250 cm/h) ***Note:*** *This step can be omitted if precipitation is not likely to be a problem.*
4	Equilibrate the column with at least 5 CV binding buffer or until the UV baseline, pH and conductivity are stable. Make sure to reduce flow rates (see step 3) if no equilibration with water has been done first. Examples of suitable buffers: • Binding buffer: 20 mM sodium phosphate, 150 mM NaCl, pH 7.2 • Elution buffer: 0.1 M sodium citrate, pH 2.0-3.5.
5	Load the sample at flow rates described in Table 4.
6	Wash with 5 CV binding buffer or until the UV trace of the flowthrough returns to near baseline.

Step	Action
7	Elute with either a one-step gradient, approx. 5 CV 100% elution buffer or with a 10 to 20 CV linear gradient from 0% to 100% elution buffer.
8	Collect fractions into tubes containing approx. 60 µl 1.0 M Tris-HCl, pH 8.0, per ml fraction to preserve antibody activity (as elution buffer has very low pH). ***Note:*** *Do not exceed the maximum recommended flow rate (see Table 5) or the column hardware pressure limit (see Tables 1 and 2).*
9	Wash the column with 3 CV binding buffer.
10	If necessary, perform Cleaning-in-Place (CIP) with 5 CV 15 mM NaOH.
11	Re-equilibrate the column with at least 5 CV binding buffer or until the pH is neutral.
12	If the column is going to be stored, wash with 2 CV distilled water followed by 2 CV 20% ethanol. Store at 2°C to 8°C.

Recommendations

When optimizing elution conditions, determine the highest pH that allows efficient elution of antibody fragment from the column. This will prevent denaturing sensitive proteins due to exposure to low pH. Step-wise elution is often preferred in large-scale applications since it allows the target molecule to be eluted in a more concentrated form, thus decreasing buffer consumption and shortening cycle times.

The dynamic binding capacity for the target antibody should be determined by frontal analysis. The dynamic binding capacity is a function of the sample residence time and should therefore be defined over a range of different sample residence times.

Removal of leached ligand from final product

The ligand leakage from Protein L is generally low. However, in many applications it is a requirement to eliminate leached ligand from the final product. There are a number of chromatographic solutions, such as cation and anion exchange chromatography, or multimodal anion exchange chromatography, which can be used to remove leached ligand.

For more details about removal of leached ligand, see the Application Note, *Two step purification of monoclonal IgG_1 from CHO cell culture supernatant* (28-9078-92). Methods used for removal of leached ligand from MabSelect SuRe™ is applicable also for removal of leached ligand from Protein L.

4 Cleaning-In-Place (CIP)

Cleaning-In-Place (CIP) is the removal of very tightly bound, precipitated or denatured substances from the chromatography medium. If such contaminants are allowed to accumulate, they may affect the chromatographic properties of the column, reduce the binding capacity and, potentially, come off in subsequent runs. If the fouling is severe, it may block the column, increase back pressure and reduce flow rate.

Regular CIP prevents the build up of contaminants, and helps to maintain the binding capacity, flow properties and general performance of Capto L . We recommend performing a blank run, including CIP, before the first purification.

For recommended flow rates, see Table 5 in Section *Operation*.

CIP protocol

Step	Action
1	Wash the column with 3 CV binding buffer.
2	Wash with at least 2 CV 15 mM NaOH. Contact time 10 to 15 minutes.
3	Wash immediately with at least 5 CV binding buffer.

CIP is usually performed immediately after the elution. Before applying 15 mM NaOH, we recommend washing the column with binding buffer in order to avoid the direct contact between low-pH elution buffer and high-pH NaOH solution on the column.

Recommended cleaning-in-place method for Capto L is 15 mM NaOH for 10 to 15 minutes after each cycle. However, in cases when reduced performance is observed, the medium needs to be cleaned with an additional procedure to remove contaminants. Experiments have shown that cleaning with 8 M urea, 50 mM citric acid, pH 2.5 for 30 minutes can be used in such cases. This could be performed after each 10 to 20 cycle, or when necessary, depending on the nature of the sample.

5 Scaling up

After optimizing the method at laboratory scale, the process is ready for scaling up. For quick small scale-up of purification, either two HiTrap columns can conveniently be connected (one in the top of the other) or two HiScreen columns can easily be connected in series with a union (18-1120-93) to give 20 cm bed height. Note that the back pressure will increase. This can easily be addressed by lowering the flow rate.

Factors, such as clearance of critical impurities, may change when column bed height is modified and should be validated using the final bed height.

Scaling up is otherwise typically performed by keeping bed height and linear flow velocity (cm/h) constant while increasing bed diameter and volumetric flow rate (ml/min or l/h).

Bulk media is available for further scaling up, see *Ordering information*.

6 Adjusting pressure limits in chromatography system software

Pressure generated by the flow through a column affects the packed bed and the column hardware, see Figure below. Increased pressure is generated when running/using one or a combination of the following conditions:

- High flow rates
- Buffers or sample with high viscosity
- Low temperature
- A flow restrictor

Note: *Exceeding the flow limit (see Table 3) may damage the column.*

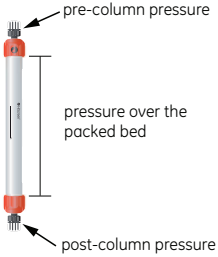

Fig 5. Pre-column and post-column measurements.

ÄKTA avant and ÄKTA pure

The system will automatically monitor the pressures (pre-column pressure and pressure over the packed bed, Δp). The pre-column pressure limit is the column hardware pressure limit (see Tables 1 and 2).

The maximum pressure the packed bed can withstand depends on medium characteristics and sample/liquid viscosity. The measured value also depends on the tubing used to connect the column to the instrument.

ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC and other systems with pressure sensor in the pump

To obtain optimal functionality, the pressure limit in the software may be adjusted according to the following procedure:

Step	Action
1	Replace the column with a piece of tubing. Run the pump at the maximum intended flow rate. Note the pressure as *total system pressure*, P1.
2	Disconnect the tubing and run the pump at the same flow rate used in step 1. Note that there will be a drip from the column valve. Note this pressure as P2.
3	Calculate the new pressure limit as a sum of P2 and the column hardware pressure limit (see Tables 1 and 2). Replace the pressure limit in the software with the calculated value. The actual pressure over the packed bed (Δp) will during run be equal to actual measured pressure - *total system pressure* (P1).

Note: *Repeat the procedure each time the parameters are changed.*

7 Storage

Wash with 2 column volumes (CV) distilled water followed by 2 CV 20% ethanol.

Store at 2°C to 8°C. Do not freeze.

Make sure that the column is tightly sealed to avoid drying out.

8 Troubleshooting

Problem	Possible cause/*corrective action*
High back pressure during the run	The column is clogged. *Clean the column, see Section "Cleaning-In-Place (CIP)".* High viscosity of solutions. *Reduce the flow rate.*
Unstable pressure curve during sample loading	Air bubbles trapped in the sample pump. *If possible degas the sample using a vacuum degasser.*
High back-pressure during CIP	Proteins precipitated on column. *Reduce the flow rate.*
Gradual increase in CIP peaks	Sub-optimal CIP. *Optimize the CIP protocol and/or perform CIP more frequently.*
Decreased column performance despite of optimized CIP	*Change to a new column. The longevity of the column depends mainly on the sample and sample preparation.*

9 Ordering information

Product	Quantity	Code No
HiScreen Capto L	1 × 4.7 ml	17-5478-14
HiTrap Protein L	5 × 1 ml	17-5478-51
	1 × 5 ml	17-5478-15
	5 × 5 ml	17-5478-55

Releated product	Quantity	Code No
Capto L	5 ml	17-5478-06
	25 ml	17-5478-01
	200 ml [1]	17-5478-02

[1] Process-scale quantities are available. Please visit www.gelifesciences.com/bioprocess or contact your local representative.

Accessories HiScreen	Quantity	Code No
HiTrap/HiPrep, 1/16" male connector for ÄKTA *(For connection of columns with 1/16" fittings to ÄKTA)*	8	28-4010-81
Union 1/16" male/1/16" male with 0.5 mm i.d. *(For connecting two columns with 1/16" fittings in series)*	2	18-1120-93
Fingertight stop plug, 1/16" [1] *(For sealing a HiScreen column)*	5	11-0003-55

[1] One fingertight stop plug is connected to the inlet and the outlet of each HiScreen column at delivery.

Accessories HiTrap	Quantity	Code No
1/16" male/luer female *(For connection of syringe to top of HiTrap column)*	2	18-1112-51
Tubing connector flangeless/M6 female *(For connection of tubing to bottom of HiTrap column)*	2	18-1003-68
Tubing connector flangeless/M6 male *(For connection of tubing to top of HiTrap column)*	2	18-1017-98
Union 1/16" female/M6 male *(For connection to original FPLC System through bottom of HiTrap column)*	6	18-1112-57
Union M6 female /1/16" male *(For connection to original FPLC System through top of HiTrap column)*	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep, 1/16" male connector for ÄKTA	8	28-4010-81
Stop plug female, 1/16" (For sealing bottom of HiTrap column)	5	11-0004-64
Fingertight stop plug, 1/16"	5	11-0003-55

Related literature	Code No
Data File: Capto L	29-0100-08
Prepacked chromatography columns for ÄKTA systems, Selection Guide	28-9317-78

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For local office contact information, visit
www.gelifesciences.com/contact

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

www.gelifesciences.com/protein-purification

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GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare UK Limited
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327, USA

GE Healthcare Japan Corporation
Sanken Bldg. 3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073, Japan

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