

Capto L

Affinity Chromatography

Instructions for Use

Capto™ L is an affinity BioProcess™ 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 particularly 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 process time and amount of medium used.
- High-flow agarose matrix increases productivity, economy and process design flexibility
- Low ligand leakage increases antibody fragment purity and improves media lifetime.

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1 Description

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 makes Capto L suitable for the purification of antibody fragments from lab to process scale.

The characteristics of the medium are summarized in Table below.

Table 1. Characteristics of Capto L

| | |
|---|---|
| Matrix | Rigid, highly cross-linked agarose |
| Ligand | Recombinant Protein L (from <i>E. coli</i>) |
| Coupling chemistry | Epoxy |
| Average particle size (d_{50v})¹ | 85 μm |
| Dynamic binding capacity² | Approx. 25 mg Fab ($M_r \sim 50\,000$)/ml medium |
| Maximum flow velocity | 500 cm/h at bed height 20 cm |
| pH stability³ | |
| Working range | 2 to 10 |
| Cleaning-in-place | 15 mM NaOH |
| Working temperature | 2°C to 40°C |
| Storage | 2°C to 8°C in 20% ethanol |
| Regulatory support | Regulatory support file is available. No material of animal origin is used in the manufacturing process |

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 (cm²).

³ 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 Process development

For initial studies on Capto L, PreDicator™ plates is preferentially used. The PreDicator plates are 96-well plates pre-filled with chromatography media, which can be used for rapid screening of chromatographic conditions in small scale. For further optimization in small-scale columns, we recommend PreDicator RoboColumn™, prepacked HiTrap™/HiScreen™ columns or empty Tricorn™ and HiScale™ columns.

Choose a residence time (see footnote¹) that fulfills your demand on dynamic binding capacity and nominal flow velocity according to [Figure 2, on page 6](#). Ancillary cycle operations including wash, elute and equilibration steps can be run at maximum operational velocities, see [Table 1, on page 3](#). Example of a pressure/flow curve in water is seen in [Figure 1](#) below.

¹ 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 (cm²).

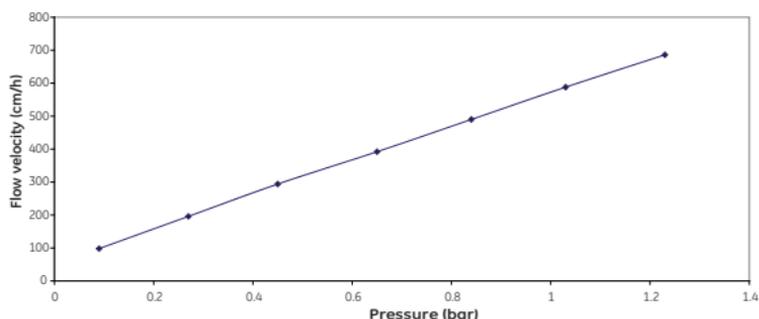


Fig 1. Example of pressure flow curve for Capto L in packed bed. Running conditions: AxiChrom 300 (30 cm i.d.), 20 cm bed height with Packing Factor 1.15 in water at 20°C. Pressure contribution from system, tubings and column is excluded.

Make sure the chosen bed height and flow velocity do not conflict with the large-scale pressure/flow limitations (Figure 2 below).

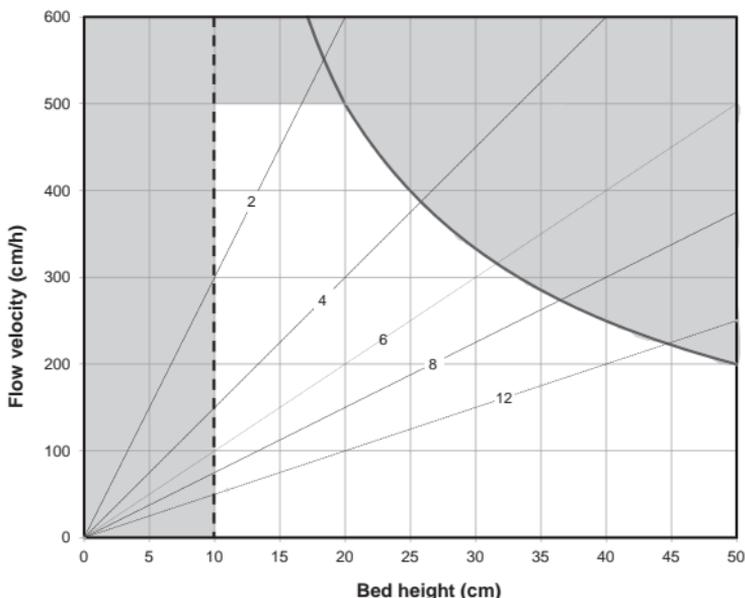


Fig 2. Operating window for Capto L (white area). Choose bed height and operating flow velocity in terms of residence time, pressure restrictions and large-scale column packing challenges.

Figure 2 above shows the possible combinations of bed height and operational nominal flow velocity for Capto L. The figure also displays the residence time in the interval 2 to 12 minutes for any bed height and flow velocity. Included are also pressure drop limitations and packing limitations at large scale. The solid curved line shows the calculated large-scale column pressure restriction which is 2 bar according to specification (500 cm/h at 2 bar and 20 cm bed height). The dashed vertical line indicates that operating at below 10 cm bed height is not favorable. The reason for this is that large diameter columns have a very different aspect ratio, and that packing short wide beds is a greater challenge.

Figure 2 above can be used as a guide when determining suitable bed height and operating flow velocity in terms of residence time and thus capacity and pressure drop.

3 Recommended screening conditions

Examples of suitable buffers:

- Buffer A: 20 mM sodium phosphate, 150 mM NaCl, pH 7.2
- Buffer B: 0.1 M sodium citrate, pH 2.0 – 3.5

Experimental conditions:

| Step | Action |
|------|--|
| 1 | Equilibrate the column with 5 column volumes (CV) of buffer A. |
| 2 | Apply a small sample of antibody fragment at residence time > 3 mins. |
| 3 | Wash the column with 5 CV of buffer A. |
| 4 | Elute the column with a 10 CV linear gradient from 0% to 100% buffer B. |
| 5 | Collect fractions into titrating diluent (e.g. 1.0 M Tris-HCl, pH 8.0 so that the diluent volume equals 5% of the programmed fraction volume). |
| 6 | Regenerate the column with 5–10 CV of 100% buffer B. |
| 7 | Wash the column with 3 CV of buffer A. |
| 8 | Perform CIP with 5 CV of 15 mM NaOH. |

Step Action

- 9** Re-equilibrate the column with buffer A.
-

To minimize the use of buffer, however, we recommend optimizing the washing procedure with respect to residence time, volumes, pH and conductivity.

When optimizing elution conditions, determine the highest pH that allows efficient desorption of antibody fragment from the column. This will prevent denaturing sensitive proteins due to exposure to low pH. Step-wise elution (Figure 3 below) 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.

Figure 3 below shows an example of purification of a Fab from papain digested Mab, spiked in *E. coli* supernatant on Capto L. The load was 15 mg Fab/ml medium, and the yield was 85%. A Tricorn 5/50 column with a CV of 1 ml and a bed height of 5 cm was used. Elution pool was analyzed for ligand leakage.

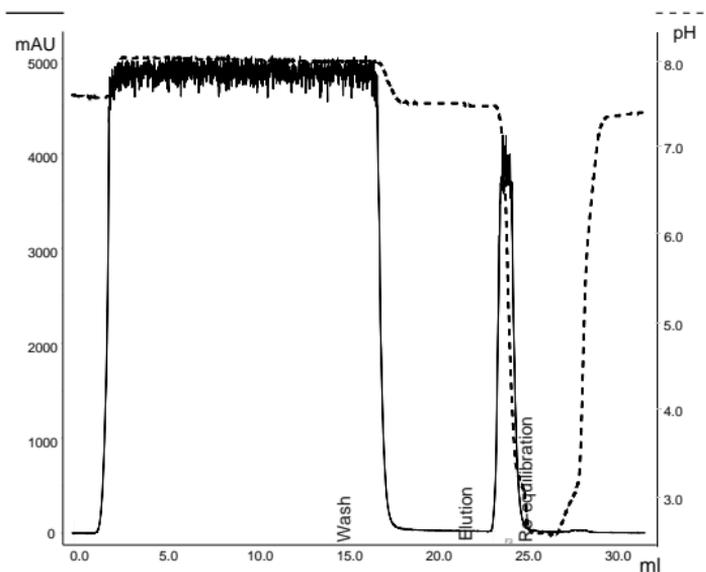


Fig 3. Purification of a Fab from papain digested Mab on Canto L.

The dynamic binding capacity for the target antibody fragment should be determined by breakthrough capacity experiment using real process feedstock. 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.

4 Removal of leached ligand from final product

The ligand leakage from Capto L is generally low. For example, the eluate from the purification run shown in [Figure 3, on page 9](#) contained <10 ppm (ng ligand/mg Fab) of leached ligand. 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 28907892](#). Methods used for removal of leached ligand from MabSelect SuRe is applicable also for removal of leached ligand from Capto L.

5 Packing columns

Recommended columns

| Column | Inner diameter (mm) | Bed volume ¹ | Maximum Bed height (cm) |
|------------------|---------------------|-------------------------|-------------------------|
| Lab scale | | | |
| Tricorn 5/100 | 5 | 2 ml | 10 |
| Tricorn 10/100 | 10 | 8 ml | 10 |
| HiScale 16/20 | 16 | 20 to 40 ml | 20 |
| HiScale 16/40 | 16 | 20 to 70 ml | 35 |
| HiScale 26/20 | 26 | 53 to 106 ml | 20 |
| HiScale 26/40 | 26 | 53 to 186 ml | 35 |

| Column | Inner diameter (mm) | Bed volume ¹ | Maximum Bed height (cm) |
|-------------------------------------|---------------------|-------------------------|-------------------------|
| Production scale | | | |
| AxiChrom ² | 50 to 200 | 0.2 to 12.5 l | 40 |
| AxiChrom ² | 300 to 1 000 | 7 to 314 l | 40 |
| BPG ³ | 100 to 300 | 1 to 28 l | 40 |
| Chromaflow™ standard ^{4 5} | 400 to 800 | 12 to 151 l | 30 |

¹ Bed volume range calculated from 10 cm bed height to maximum bed height.

² Intelligent packing method according to MabSelect SuRe LX can be used.

³ The pressure rating of BPG 450 is too low to use with Capto L media.

⁴ Packing instructions for MabSelect in Chromaflow columns are described in Application Note 11000752 and can be used for Capto L.

⁵ Larger pack stations might be required at larger diameters.

All large-scale columns can be supplied as variable bed height columns.

For practical instructions in good packing techniques, see *CD-ROM 18116533*. For more details about packing HiScale columns, see *instruction 28967470*. For information on packing of process scale columns, please contact your local Cytiva representative.

Packing Tricorn columns

Introduction

The following instructions are for packing Tricorn 5/100 and Tricorn 10/100 columns with a 10 cm bed height.

For more details about packing Tricorn columns, see *instruction 28409488*.

Packing preparations

Materials

- Capto L
- Plastic spoon or spatula
- Glass filter G3
- Vacuum suction equipment
- Filter flask
- Measuring cylinder
- Thin capillary
- 20% ethanol with 0.4 M NaCl
- Tricorn 5/100 column, Tricorn Glass Tube 5/100 (to be used as packing tube), and Tricorn Packing Connector 5-5, or
- Tricorn 10/100 column, Tricorn Packing Equipment 10/100, which includes the 10-mm packing connector, 100-mm glass tube (to be used as packing tube).
- Bottom unit with filter holder, cap, and stop plug.

Equipment

ÄKTA™ systems, or a stand-alone pump such as Pump P-900, depending on the flow rate required, can be used for packing.

Equilibrate all materials to room temperature.

Washing the chromatography medium

Mount the glass filter funnel onto the filtering flask. Suspend the medium by shaking and pour into the funnel and wash according to the following instructions:

Step Action

- 1 Wash 5 times with 5 ml 20% ethanol with 0.4 M NaCl/ml medium.
 - 2 Gently stir with a spatula between additions.
 - 3 Move the washed medium from the funnel into a beaker and add 20% ethanol with 0.4 M NaCl to obtain a 50% slurry concentration.
-

Preparing the packing slurry

Check the slurry concentration after settling overnight in a measuring cylinder or use the method for slurry concentration measurement described in *application note 28925932*.

Tricorn columns can be packed with an excess of medium to be removed after packing.

Packing procedure

Main features

Table 2. Main features of the packing method.

| | Tricorn 5/100 | Tricorn 10/100 |
|-------------------------|-----------------------------|----------------|
| Slurry packing solution | 20% ethanol with 0.4 M NaCl | |
| Slurry concentration | 50% | 50% |
| Phase 1 | | |
| Packing velocity | 1830 cm/h | 1830 cm/h |
| Packing flow | 5 ml/min | 20 ml/min |
| Packing time | 10 min | 10 min |
| Phase 2 | | |
| Packing velocity | 1830 cm/h | 1830 cm/h |
| Packing flow | 5 ml/min | 20 ml/min |

| | Tricorn 5/100 | Tricorn 10/100 |
|--------------|---------------|----------------|
| Packing time | 5 min | 5 min |

Procedure

Preparing packing

| Step | Action |
|------|--------|
|------|--------|

- | | |
|---|--|
| 1 | Assemble the column according to the column <i>instruction 28409488</i> . For additional information, please visit Technical support at cytiva.com . |
| 2 | Put a stop plug in the bottom of the column tube and pour the suspended media slurry (50%) into the top of the packing tube, filling both column tube and packing tube. Avoid formation of air bubbles in the medium by pouring it along a thin capillary. |
| 3 | Attach an extra bottom unit or an adapter unit to the top of the packing tube. Place a beaker beneath the column tube and connect a pump to the top of the packing unit. Remove the stop plug from the bottom of the column tube. |

Phase 1

| Step | Action |
|------|--------|
|------|--------|

- | | |
|---|--|
| 1 | Pack the media at 5 ml/min (Tricorn 5/100) or 20 ml/min (Tricorn 10/100) for 10 minutes. |
|---|--|

Step Action

- 2** When the medium is packed, switch off the pump, attach the stop plug into bottom of the column tube, disconnect the pump and remove the packing tube and packing connector. If necessary, remove excess medium by re-suspending the top of the packed bed and remove with a Pasteur pipette or spatula.
 - 3** Fill up the column with the same fluid as used for packing the column.
 - 4** Place a pre-wet filter on top of the fluid in the column and gently push it into the column tube with the filter tool.
 - 5** Prepare the adapter unit by screwing the guiding ring inside the adapter unit out to its outer rim and then turn it back 1.5 turns.
 - 6** Wet the O-ring on the adapter unit by dipping it into water or buffer.
 - 7** Screw the adapter unit onto the column tube, ensuring the inner part of the guiding ring fits into the slot on the column tube threads. Ensure that there are no trapped air bubbles.
 - 8** Screw the adapter down to until the filter reaches the surface of the bed.
 - 9** Connect the pump to the adapter unit. Remove the stop plug in the bottom of the column.
-

Phase 2

| Step | Action |
|------|--------|
|------|--------|

- | | |
|---|---|
| 1 | Pack the media at 5 ml/min (Tricorn 5/100) or 20 ml/min (Tricorn 10/100) for 5 minutes. During this step a small void can be formed. |
| 2 | Switch off the pump, attach the stop plug into bottom of the column tube and disconnect the pump slowly. If a void has been formed, turn the adapter down again until the filter reaches the bed surface. |
| 3 | Press the adapter lock down into the locked position. |
| 4 | Screw a stop plug into the adapter unit. The column is now ready to be used. |
-

Testing the packed column

Note: *Due to unfavorable volume ratio between chromatography system and Tricorn column, asymmetry factor and theoretical plate numbers may be inaccurate. Hence, the method described in Section "Evaluation of column packing" is not fully applicable for Capto L in Tricorn columns.*

Packing HiScale columns

Introduction

The following instructions are for packing HiScale 16/20, 16/40 and HiScale 26/20, 26/40 with 10, 20 and 35 cm bed heights.

For more details about packing HiScale columns, see *instruction 28967470*.

Materials needed

- Capto L
- HiScale column
- HiScale packing tube (depending on bed height)
- Plastic spoon or spatula
- Glass filter G3
- Vacuum suction equipment
- Filter flask
- Measuring cylinder
- 20% ethanol with 0.4 M NaCl

Equipment

ÄKTA systems, or a stand-alone pump such as Pump P-900, depending on the flow rate required, can be used for packing.

Equilibrate all materials to room temperature.

Definitions

The bed height of a gravity settled bed differs from the bed height of a bed settled at a given flow (consolidated).

Therefore, the compression factor (CF) has to be separated from the packing factor (PF).

| | |
|----------------------|--|
| L_{settled} | Bed height measured after settling by gravity. |
| L_{cons} | Consolidated bed height Bed height measured after settling the medium at a given flow velocity. |
| L_{packed} | Packed bed height |
| CF | Compression factor $CF = L_{\text{settled}}/L_{\text{packed}}$ |
| PF | Packing factor $PF = L_{\text{cons}}/L_{\text{packed}}$ |

| | |
|---------------------|--|
| A_C | Crosssectional area of the column |
| V_C | Column volume $V_C = L_{\text{packed}} \times A_C$ |
| C_{slurry} | Concentration of the slurry |

Preparation of the slurry

To measure the slurry concentration, let the medium settle in 20% ethanol at least overnight in a measuring cylinder or use the method for slurry concentration measurement described in *application note 28925932*. This method can also be used for HiScale columns.

Washing the medium

Mount a glass filter funnel onto a filtering flask. Suspend the medium by shaking and pour into the funnel and wash according to the following instructions:

- 5 times with 5 ml 20% ethanol with 0.4 M NaCl/ml medium
- Gently stir with a spatula between additions
- Move the washed medium from the funnel into a beaker and add 20% ethanol with 0.4 M NaCl to obtain a 50% slurry concentration.

Packing parameters

Table 3. Main features of the packing method for HiScale 16/20 and HiScale 16/40

| Product | HiScale 16/20 | HiScale 16/40 | |
|----------------------------|-----------------------------|---------------|------|
| Bed height (cm) | 10 | 20 | 35 |
| Slurry/ packing solution | 20% ethanol with 0.4 M NaCl | | |
| Slurry concentration (%) | 50 | 50 | 50 |
| Packing factor (PF) | 1.10 | 1.10 | 1.06 |
| Packing velocity (cm/h) | 300 | 300 | 300 |
| Packing flow rate (ml/min) | 10 | 10 | 10 |
| Flow condition (cm/h) | 750 | 450 | 260 |
| Flow condition (ml/min) | 25 | 15 | 8.6 |

Table 4. Main features of the packing method for HiScale 26/20 and HiScale 26/40

| Product | HiScale 26/20 | HiScale 26/40 | |
|----------------------------|-----------------------------|---------------|------|
| Bed height (cm) | 10 | 20 | 35 |
| Slurry/ packing solution | 20% ethanol with 0.4 M NaCl | | |
| Slurry concentration (%) | 50 | 50 | 50 |
| Packing factor (PF) | 1.10 | 1.10 | 1.10 |
| Packing velocity (cm/h) | 300 | 300 | 300 |
| Packing flow rate (ml/min) | 27 | 27 | 27 |
| Flow condition (cm/h) | 750 | 450 | 250 |
| Flow condition (ml/min) | 66 | 40 | 23 |

Packing procedure

| Step | Action |
|-------------|--|
| 1 | Assemble the column according to the column <i>instruction 28967470</i> . |
| 2 | Mount the column tube in a stand. |
| 3 | Connect the bottom adapter unit to the pump or a syringe and prime the bottom net with a slow flow of packing solution. This is easiest done if the nets are dry, but if air is trapped under the net it can be removed by a light suction with a syringe. |
| 4 | Mount the bottom adapter unit in the bottom of the column tube and tighten the O-ring. |
| 5 | Fill the column with approximately 1 cm packing liquid using the pump/syringe. Disconnect the pump/syringe and put a stop plug on the outlet. |
| 6 | Mount the packing tube on top of the column tube. |
| 7 | Connect the top adapter to the pump and prime it with a slow downward flow. The net needs to be facing the roof as this is done. If air is trapped under the net it can be removed by a light suction with a syringe |
| 8 | Fill the column with slurry suspended in packing solution. If needed, top up the slurry with extra packing solution so the top adapter dips into the slurry to avoid air under the net. |

Step Action

- 9** Mount the top adapter unit on top of the packing tube. Tighten the O-ring firmly and remove the bottom stop plug
- 10** Start a downward flow with packing velocity according to Tables in [Packing parameters on page 19](#).
- 11** Let the flow run until the bed has consolidated.
- 12** Use the scale on the column to measure the bed height. There might be a build up of media at the column wall after the bed is consolidated and to easier see where the top of the bed is, a light source can be used.
- 13** Calculate the final bed height by dividing the consolidated bed height with the desired packing factor.
 $L_{\text{packed}} = L_{\text{cons}} / \text{PF}$. See Tables in [Packing parameters on page 19](#).
- 14** Turn off the flow and put a stop plug in the bottom.
- 15** Dismount the top adapter from the packing tube.
- 16** Over a beaker or a sink, detach the packing tube from the column.
- 17** Remount the top adapter in the column tube. Make sure no air is trapped under the net and lower the adapter down to 1 to 2 cm above the bed, making sure the surface is not disturbed.

Step Action

- 18** Tighten the O-ring on the adapter. Remove the bottom stop plug and carefully start turning the end cap down. While spilling out liquid through the bottom, proceed turning until the calculated final bed height is reached.
 - 19** Make sure that the pressure peaks that occur during turning the end knob down do not exceed the pressure specifications of the media.
 - 20** Start a downward flow to flow condition the bed. The flow rate is shown in Tables in [Packing parameters on page 19](#).
 - 21** Let the flow run for about 10 column volumes. The column is ready to be tested.
-

Testing the packed column

See [Chapter 6 Evaluation of column packing](#), .

6 Evaluation of column packing

Intervals

Test the column efficiency to check the quality of packing. Testing should be done immediately after packing and at regular intervals during the working life of the column and also when separation performance is seen to deteriorate.

Column efficiency testing

The best method of expressing the efficiency of a packed column is in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (A_s). These values are easily determined by applying a sample such as 1% acetone solution to the column. Sodium chloride can also be used as a test substance. Use a concentration of 0.8 M NaCl in water with 0.4 M NaCl in water as eluent.

For more information about column efficiency testing, consult the *application note 28937207*.

Note: *The calculated plate number will vary according to the test conditions and it should only be used as a reference value. It is important that test conditions and equipment are kept constant so that results are comparable. Changes of solute, solvent, eluent, sample volume, flow velocity, liquid pathway, temperature, etc. will influence the results.*

Sample volume and flow velocity

For optimal results, the sample volume should be at maximum 2.5% of the column volume and the flow velocity 30 cm/h. If an acceptance limit is defined in relation to column performance, the column plate number can be used as one of the acceptance criteria for column use.

Method for measuring HETP and A_s

Calculate HETP and A_s from the UV curve (or conductivity curve) as follows:

$$\text{HETP} = \frac{L}{N}$$

L = bed height (cm)

N = number of theoretical plates

$$N = 5.54 \times \left(\frac{V_R}{W_h} \right)^2$$

V_R = volume eluted from the start of sample application to the peak maximum

W_h = peak width measured as the width of the recorded peak at half of the peak height

V_R and W_h are in the same units

The concept of reduced plate height is often used for comparing column performance.

The reduced plate height, h , is calculated as follows:

$$h = \frac{\text{HETP}}{d_{50v}}$$

d_{50v} = mean diameter of the beads (cm)

As a guideline, a value of < 3 is very good.

The peak should be symmetrical, and the asymmetry factor as close to 1 as possible (a typical acceptable range could be $0.8 < A_s < 1.8$).

A change in the shape of the peak is usually the first indication of bed deterioration due to excessive use.

Peak asymmetry factor calculation:

$$A_s = \frac{b}{a}$$

a = ascending part of the peak width at 10% of peak height

b = descending part of the peak width at 10% of peak height

The Figure below shows a UV trace for acetone in a typical test chromatogram from which the HETP and A_s values are calculated.

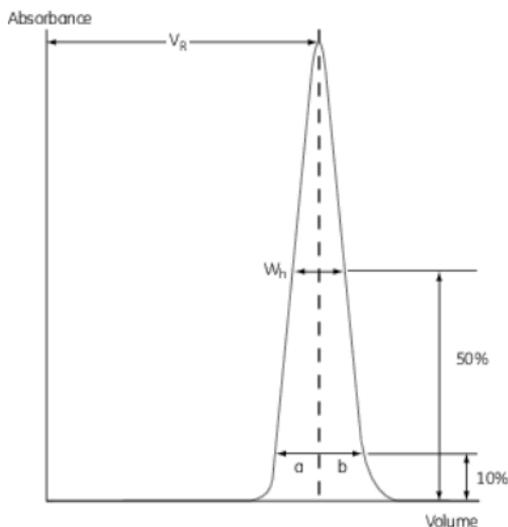


Fig 4. A typical test chromatogram showing the parameters used for HETP and A_s calculations.

7 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.

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.

8 Sanitization

Sanitization reduces microbial contamination of the chromatographic bed to a minimum.

Sanitization protocol

To sanitize Capto L we recommend treatment with solution containing 0.1 M acetic acid in 20% ethanol.

9 Storage

Store unused media in its container at a temperature of 2°C to 8°C. Ensure that the screw top is fully tightened.

Equilibrate packed columns in buffer containing 20% ethanol to prevent microbial growth.

After storage, equilibrate with starting buffer and perform a blank run, including CIP, before use.

10 Scaling up

After optimizing the antibody fragment purification at laboratory scale, the process can be scaled up to pilot and process scales.

- Keep the residence time constant in order to maintain the dynamic binding capacity.
- Select bed volume according to required binding capacity. Verify the purification step with the new bed height, if it is changed.
- Select column diameter according to your volume throughput requirements. Then determine the bed height to give the desired residence time. Bed heights of 10 to 25 cm are generally considered appropriate. Note that the backpressure increases proportionally with increasing bed height at constant nominal flow velocity.
- Keep sample concentration and elution conditions constant.

See also [Figure 2, on page 6](#) for appropriate windows of operation for Capto L.

11 Troubleshooting

The list below describes faults observed from the monitor curves.

| Fault | Possible cause/corrective action |
|---|--|
| High backpressure during the run | <ul style="list-style-type: none">• Change the in-line filter• The column is clogged. Perform CIP• The adapter net/filter is clogged. Clean or replace the net/filter. |
| Unstable pressure curve during sample application | <ul style="list-style-type: none">• Remove air bubbles that might have been trapped in the sample pump• Degas the sample using a vacuum degasser or an air trap. |
| Gradual broadening of the eluate peak | <ul style="list-style-type: none">• Might be due to insufficient elution and CIP caused by contaminants accumulating in the column. Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently. |
| Gradual decrease in yield | <ul style="list-style-type: none">• Too high sample load. Decrease the sample load.• Precipitation during elution. Optimize the elution conditions.• Might be due to insufficient elution and CIP. Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently. |
| Gradual increase in CIP peaks | <ul style="list-style-type: none">• Might be due to insufficient elution or CIP. Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently. |
| High ligand leakage during the first purification cycle | <ul style="list-style-type: none">• Perform a blank run, including CIP, before the first purification cycle on a new column. |

12 Ordering information

| Product | Quantity | Product Code. |
|----------------|-----------------|----------------------|
| Capto L | 5 ml | 17547806 |
| | 25 ml | 17547801 |

| Product | Quantity | Product Code. |
|---------|----------|---------------|
| | 200 ml | 17547802 |
| | 1 l | 17547803 |
| | 5 l | 17547804 |
| | 10 l | 17547805 |

| Related product | Quantity | Product Code. |
|---------------------------------------|-----------------------------|---------------|
| HiScreen Capto L | 1 × 4.7 m | 17547814 |
| HiTrap Protein L | 5 × 1 ml | 17547851 |
| HiTrap Protein L | 1 × 5 ml | 17547815 |
| HiTrap Protein L | 5 × 5 ml | 17547855 |
| PreDicator RoboColumn Capto L, 200 µL | One row of eight columns | 29003420 |
| PreDicator RoboColumn Capto L, 600 µL | One row of eight columns | 29003421 |
| PreDicator Capto L, 6 µL | 4 × 96-well plates | 17547830 |
| PreDicator Capto L, 20 µL | 4 × 96-well plates | 17547831 |
| PreDicator Capto L, 50 µL | 4 × 96-well plates | 17547832 |
| Tricorn 5/100 column | 1 | 28406410 |
| Tricorn 10/100 column | 1 | 28406415 |
| Tricorn 5/100 glass tube | 1 | 18115306 |
| Packing Connector 5-5 | 1 | 18115321 |
| Tricorn packing equipment 10/100 | 1 | 18115325 |
| HiScale 16/20 | 1 | 28964441 |
| HiScale 16/40 | 1 | 28964424 |
| HiScale 26/20 | 1 | 28964514 |
| HiScale 26/40 | 1 | 28964513 |

| Related product | Quantity | Product Code. |
|-----------------------------|----------|---------------|
| Packing tube, HiScale 16/20 | 1 | 28986816 |
| Packing tube, HiScale 16/40 | 1 | 28986815 |
| Packing tube, HiScale 26/20 | 1 | 28980383 |
| Packing tube, HiScale 26/40 | 1 | 28964505 |

| Related literature | | Product Code. |
|--------------------|---|---------------|
| Data files | AxiChrom Columns | 28929041 |
| | BPG columns | 18111523 |
| | Chromaflo columns | 18113892 |
| | Capto L | 29010008 |
| Application notes | MabSelect – Column packing | 11000752 |
| | Capture of human single-chain Fv (scFv) fusion protein on Capto L affinity medium | 29014456 |
| | High-throughput process development for design of cleaning-in-place protocol | 28984564 |
| Movie | Column Packing - The Movie | 18116533 |
| Instructions | HiScale columns (16, 26, 50) and accessories | 28967470 |

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