



HiScreen™ Capto™ Core 400

HiScreen™ Capto™ Core 700

HiTrap™ Capto™ Core 400, 1 mL

HiTrap™ Capto™ Core 700, 1 mL

Instructions for Use

The Capto™ Core 400 and Capto Core 700 chromatography resins are designed for intermediate purification and polishing of viruses and other large biomolecules in flow-through mode. The products are based on the core bead concept. Each bead has a ligand-activated core and an inactive shell. The shell prevents larger molecules from entering the core, but smaller proteins and impurities can enter the core and bind to the hydrophobic and positively charged octylamine ligands.

The prepacked columns are used for optimization of methods and parameters, such as sample load and binding conditions, as well as for small-scale purifications. The columns provide fast and reproducible separations in convenient formats. The columns are optimally used with liquid chromatography systems such as ÄKTA™.

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Important

Read these instructions carefully before using the products.

Safety

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

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.

1 Product description

HiScreen column characteristics

HiScreen™ columns are made of biocompatible polypropylene that does not interact with biomolecules. The arrow on the column label shows the recommended flow direction.

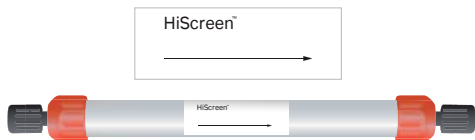


Fig 1. HiScreen column.

Note: Do not open or refill HiScreen columns.

Note: Make sure that the column is sealed properly to prevent leakage when stored.

If scale-up requires a larger bed height, two columns can be connected in series using a union to give a 20 cm bed height (see [Chapter 5 Scale-up, on page 16](#)).

Table 1. Characteristics of HiScreen column

Column volume (CV)	4.7 mL
Column dimensions	0.77 × 10 cm
Column hardware pressure limit	0.8 MPa (8 bar, 116 psi)

Note: The pressure over the packed bed varies depending on parameters such as resin characteristics, sample/liquid viscosity, and column tubing used.

HiTrap column characteristics

HiTrap™ 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. The first table below lists the characteristics of HiTrap columns.



Fig 2. HiTrap, 1 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
Column dimensions	0.7 × 2.5 cm
Column hardware pressure limit	0.5 MPa (5 bar, 72 psi)

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

Supplied Connector kit with HiTrap column

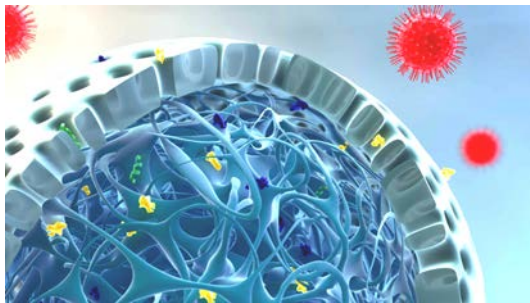
Connectors supplied	Usage	No. supplied
Union 1/16" male/luer female	For connection of syringe to HiTrap column	1
Stop plug female, 1/16"	For sealing bottom of HiTrap column	2, 5, or 7

Resin properties

Capto Core 400 and Capto Core 700 are composed of a ligand-activated core and an inactive shell. The inactive shell excludes large molecules (average cut-offs: $M_r \sim 400\,000$ for Capto Core 400, and $M_r \sim 700\,000$ for Capto Core 700) from entering the core through the pores of the shell, see image below. These larger molecules are collected in the column flow-through, while smaller impurities bind to the internalized ligands.

The core of each bead is functionalized with ligands that are both hydrophobic and positively charged, resulting in a highly efficient multimodal binding of various impurities that are small enough to enter the core. The octylamine ligand ensures strong binding with most impurities over a wide range of pH and salt concentrations.

A schematic representation of the principle for Capto Core 400 and Capto Core 700 is given in the image below.



The products are based on a highly cross-linked agarose base matrix, which gives good flow properties.

The design of the bead, with an inactive shell that prevents binding of the large target molecules, allows for high resolution at high flow rates and short residence times. Further resin characteristics are found in the table below.

Table 3. Characteristics of Capto Core 400 and Capto Core 700

	Capto Core 400	Capto Core 700
Matrix	Highly cross-linked agarose	
Median particle size, d_{50v}¹	90 μm	85 μm
Ligand	Octylamine	
Dynamic binding capacity, Q_{B10}²	~22 mg ovalbumin/mL resin	~13 mg ovalbumin/mL resin
Exclusion limit, M_r	~400 000	~700 000
Recommended operating flow velocity, maximum³	700 cm/h	500 cm/h
pH stability		
Operational⁴	3 to 13	
CIP⁵	3 to 14	
Chemical stability	Stable in commonly used aqueous buffers, 1 M NaOH ⁶ , 6 M guanidine hydrochloride, 30% isopropanol, 70% ethanol	
Avoid	Oxidizing agents, anionic detergents	
Storage	4°C to 30°C in 20% ethanol	

¹ Median particle size of the cumulative volume distribution.

² Dynamic binding capacity at 10% breakthrough with a residence time of 3 minutes (1.6 mL/min = 200 cm/h) in HiScreen columns. Buffer: 20 mM Tris-HCl, 0.15 M NaCl, pH 7.5.

³ At 20 cm bed height, at room temperature using buffers and samples with the same viscosity as water. < 2 bar (0.2 MPa). Recommended operating flow velocity, maximum must be optimized. Start with a lower flow rate to prevent exceeding pressure limits.

⁴ pH range where resin can be operated without significant change in function.

⁵ pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

⁶ 1 M NaOH should only be used for cleaning purposes.

2 Optimization

General

The aim of designing and optimizing a separation process is to identify conditions that promote binding of the highest amount of impurities, in the shortest possible time, with highest possible product recovery and purity.

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

The HiScreen column format is suitable for method optimization when developing a new purification process. The small column volume (CV) of 4.7 mL, and the 10 cm bed height allow for scalable experiments at relevant process flow rates. Two columns can be connected in series with a union to give a 20 cm bed height (see [Chapter 5 Scale-up, on page 16](#)).

The HiTrap column format is suitable for initial screening of binding and elution conditions.

Buffer and pH

The octylamine ligand is multimodal (both hydrophobic and positively charged), giving a broad window of operation. The function of the ligand has been verified in buffers containing up to 1 M NaCl, see [Fig. 3, on page 8](#).

Capto Core 400 and Capto Core 700 are compatible with most commonly used buffers for size exclusion chromatography and ion exchange chromatography, such as phosphate and Tris. The use of citrate buffer might result in decreased binding capacity.

Most host cell proteins are negatively charged above pH 7. Since the ligands are positively charged, it is advised to use a pH of 7 to 9 to provide good binding of the host cell proteins to the ligands.

DNA is negatively charged over a wider pH range and the efficiency of DNA removal is thus less dependent on pH. To remove DNA with Capto Core 400 and Capto Core 700, it is strongly recommended to treat the sample with Benzonase™ endonuclease first, see next section.

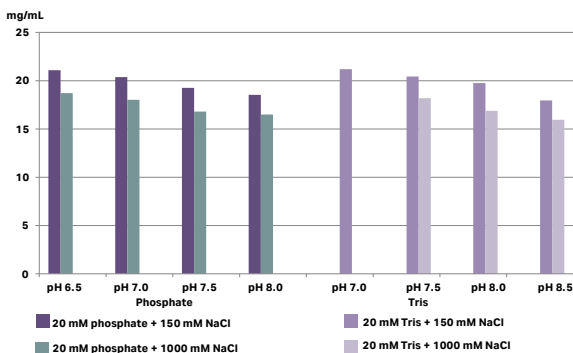


Fig 3. Static binding capacity (SBC) of Capto Core 700 for ovalbumin in sodium phosphate and Tris buffers with different NaCl concentrations and pH values.

Reduction of DNA and RNA

In some cases, high levels of DNA and RNA can affect the performance of the Capto Core resins. It is therefore recommended to reduce DNA and RNA levels before the purification step, for example, by using an anion exchange step, or by treatment with Benzonase endonuclease.

Benzonase endonuclease degrades all nucleic acids down to oligonucleotides of approximately 3 to 5 base pairs in length. These small fragments and Benzonase endonuclease itself can enter the core of the resins where they are bound to the octylamine ligands.

Sample load

Capto Core 400 and Capto Core 700 work as scavengers, meaning that the target passes in the flow-through fraction while the impurities bind to the interior of the beads.

To determine the maximum sample volume that can be loaded onto the column while maintaining the desired purity of target, it is recommended to collect fractions during sample application and analyze the fractions for recovery and purity of target. Typically, 5 to 20 column volumes can be loaded but this is highly dependent on sample composition.

Make sure to start with a high load during method optimization and determine the correct load by analysis of the fractions. After washing the column with binding buffer, the bound impurities will be removed during the cleaning-in-place (CIP) of the resin, see [Chapter 4 Cleaning-In-Place \(CIP\), on page 15](#).

3 Operation

Prepare the sample

Step	Action
------	--------

- | | |
|---|---|
| 1 | Adjust the sample to the composition of the binding buffer, using one of the following methods: <ul style="list-style-type: none">• Dilute the sample with binding buffer.• Exchange the buffer using a prepacked column for desalting, see the table below. |
| 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 must be of high purity. Filter buffers through a 0.22 μm or a 0.45 μm filter before use.

Prepacked columns for desalting and buffer exchange

Table 4. Prepacked columns for desalting and buffer exchange

Column	Loading volume	Elution volume
HiPrep™ 26/10 Desalting ¹	2.5 to 15 mL	7.5 to 20 mL
HiTrap Desalting ²	0.25 to 1.5 mL	1.0 to 2.0 mL
PD-10 Desalting ³	1.0 to 2.5 mL ⁴ 1.75 to 2.5 mL ⁵	3.5 mL Up to 2.5 mL
PD MiniTrap™ G-25	0.1 to 2.5 mL ⁴ 0.2 to 0.5 mL ⁵	1.0 mL Up to 0.5 mL
PD MidiTrap™ G-25	0.5 to 1 mL ⁴ 0.75 to 1 mL ⁵	1.5 mL Up to 1 mL

¹ Prepacked with Sephadex™ G-25 Fine and requires a pump or a chromatography system to run.

² Prepacked with Sephadex G-25 Superfine and requires a syringe or pump to run.

³ Prepacked with Sephadex G-25 and can be run by the gravity flow or centrifugation.

⁴ Volumes with gravity elution.

⁵ Volumes with centrifugation.

Recommended flow rates

To allow proteins and fragmented DNA to bind properly, the flow rate during sample application must not be too high. A good starting point is 1.2 mL/min for HiScreen and 1.0 mL/min for HiTrap (150 cm/h). During column equilibration and wash steps, higher flow rates can be used, see tables below.

Flow rates for Capto Core 400

Table 5. Recommended flow rates for different operations

Type of operation	Flow rate (mL/min)		Flow velocity (cm/h)	
	HiScreen	HiTrap	HiScreen	HiTrap
Equilibration ¹	upto 5.4	upto 4.0	upto 700	upto 600
Wash ¹	upto 5.4	upto 4.0	upto 700	upto 600
Load of sample	0.8 to 2.3	0.6 to 1.9	100 to 300	100 to 300
Cleaning-in-place ²	0.16 to 0.31	0.13 to 0.26	20 to 40	20 to 40

¹ Stated flow rates are for buffers with the same viscosity as water at 20°C. For solutions with higher viscosities, for example, 20% ethanol, use lower flow rates.

² See also [Chapter 4 Cleaning-In-Place \(CIP\), on page 15](#).

Flow rates for Capto Core 700

Table 6. Recommended flow rates for different operations

Type of operation	Flow rate (mL/min)		Flow velocity (cm/h)	
	HiScreen	HiTrap	HiScreen	HiTrap
Equilibration ¹	upto 3.9	upto 3.2	upto 500	upto 500
Wash ¹	upto 3.9	upto 3.2	upto 500	upto 500
Load of sample	0.8 to 2.3	0.6 to 1.9	100 to 300	100 to 300
Cleaning-in-place ²	0.16 to 0.31	0.13 to 0.26	20 to 40	20 to 40

¹ Stated flow rates are for buffers with the same viscosity as water at 20°C. For solutions with higher viscosities, for example, 20% ethanol, use lower flow rates.

² See also [Chapter 4 Cleaning-In-Place \(CIP\), on page 15](#).

Purification

Flow rate: See the preceding two sections for the recommended flow rates for different operations. Collect fractions throughout the separation.

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

Step	Action
1	Before connecting the column to a system: <ol style="list-style-type: none">Remove the two stoppers from the HiScreen column. Or:Remove the stopper from the inlet and the snap-off end at the HiTrap column outlet.
2	Connect the column to the system with fingertight 1/16" connectors (28401081). <p>Note: <i>Make a drop-to-drop connection to prevent air from entering the column.</i></p> <p>Note: <i>Make sure that the connectors are tight to prevent leakage.</i></p>

Step Action

- 3 Wash with 1 CV of distilled water to remove the ethanol. This prevents precipitation of buffer salts at exposure to ethanol.

Note:

The viscosity for 20% ethanol is higher than that for water. For this step, do not use a higher flow rate than mentioned below:

- 1.9 mL/min for HiScreen (250 cm/h)
 - 1.6 mL/min for HiTrap (250 cm/h)
- 4 Equilibrate the column with at least 5 CV binding buffer or until the UV baseline, eluent pH, and conductivity are stable.
- 5 Adjust the sample to the chosen starting pH and conductivity, see [Prepare the sample, on page 10](#).
- 6 Load approximately 5 to 20 CV of the sample onto the column. Collect fractions for analysis of purity and recovery of target, see [Chapter 2 Optimization, on page 7](#).
- 7 Wash with 5 to 10 CV binding buffer or until the UV trace of the flowthrough returns to near baseline.
- Note:**
The target is in the flowthrough.
- 8 Perform a CIP to elute and clean the column, see [Chapter 4 Cleaning-In-Place \(CIP\), on page 15](#).

Step	Action
------	--------

- | | |
|---|--|
| 9 | Equilibrate with 5 to 10 CV binding buffer or until the UV baseline, pH, and conductivity reach the required values. |
|---|--|

Note:

Equilibration can be shortened by washing with a high concentration buffer first to roughly obtain the desired pH value. Then wash with binding buffer until the conductivity and pH values are stable.

Note:

Do not exceed the maximum flow rate (see [Table 5, on page 12](#) and [Table 6, on page 12](#)) or the column hardware pressure limit (see [Table 1, on page 3](#) and [Table 2, on page 4](#)).

4 Cleaning-In-Place (CIP)

General description

Regular CIP prevents the buildup of contaminants and helps maintaining the capacity, flow properties, and performance of the chromatography resin and prepacked columns.

It is recommended to perform a CIP:

- after each run with live feed
- when an increase in back pressure is seen
- if reduced column performance is observed
- before first-time use or after long term storage

CIP protocol

For increased contact time and due to the viscosity of the CIP solutions, it is recommended to use a lower flow rate than during purification, see [Table 5, on page 12](#) and [Table 6, on page 12](#).

Wash with a solution of 1 M NaOH in 30% isopropanol or in 27% 1-propanol with reversed flow direction.

Use a total contact time of 30 to 60 minutes, depending on the sample. For more effective cleaning, a pause of 15 to 30 minutes can be included.

The characteristics of the sample determine the final CIP protocol, so the protocol might require optimization. The main parameters to vary during CIP optimization are:

- NaOH and solvent concentrations
- contact time and volume of the CIP solution

5 Scale-up

After method optimization at laboratory scale, the process is ready for scale-up. For quick and small scale-up, two HiTrap or HiScreen columns can be connected in series with a union (18112093).

Note: *The back pressure increases when columns are connected in series. This can be addressed by decreasing the flow rate.*

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

Scale-up is typically performed by keeping bed height and flow velocity (cm/h) constant while increasing bed diameter and flow rate (mL/min or L/h).

For further scale-up, bulk resins are available, see [Chapter 9 Ordering information, on page 21](#).

Procedure

Step	Action
1	Select bed volume according to required sample load. Keep sample concentration constant.
2	Select column diameter to obtain the desired bed height. The excellent rigidity of the high flow base matrix allows for flexibility in choice of bed heights.
3	Large equipment used during scale-up can cause deviations from the method optimized at small-scale. In such cases, check the buffer delivery and monitoring systems for time delays or volume changes.

6 Adjusting pressure limits

Pressure generated by the flow through a column affects the packed bed and the column hardware, see image 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 maximum flow velocity (see [Table 5, on page 12](#) and [Table 6, on page 12](#)) can damage the column.

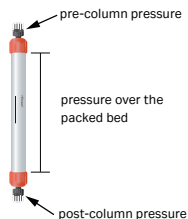


Fig 4. Pre-column and post-column measurements.

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 [Table 1, on page 3](#) and [Table 2, on page 4](#)).

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

Systems with a pressure sensor in the pump

For optimal functionality, adjust the pressure limit in the software 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.
 - Record 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.
 - Record the pressure as P2.
- 3
 - Calculate the new pressure limit as the sum of P2 and the column hardware pressure limit, see [Table 1, on page 3](#) and [Table 2, on page 4](#).
 - Replace the pressure limit in the software with the calculated value.

Result:

During a run, the actual pressure over the packed bed (Δp) is equal to the actual measured pressure minus the total system pressure (P1).

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

7 Storage

Store HiScreen and HiTrap columns equilibrated with 5 to 10 CV 20% ethanol at 4°C to 30°C. Do not freeze.

Make sure that the columns are sealed tightly, to prevent them from drying out.

8 Troubleshooting

Problem	Possible cause	Corrective action
High back pressure during the run	Solutions with high viscosity are used.	Decrease the flow rate.
	The column is clogged.	Clean the column, see Chapter 4 Cleaning-In-Place (CIP) , on page 15.
Unstable pressure curve during sample loading	Air bubbles trapped in the sample pump.	If possible, de-gas the sample using a vacuum de-gasser.
Gradual broadening of the eluate peak	Insufficient elution and CIP, caused by contaminant buildup in the column.	Optimize the elution conditions, the CIP protocol, or perform CIP more frequently.
Gradual decrease in yield	Insufficient elution and CIP.	Optimize the elution conditions, the CIP protocol, or perform CIP more frequently.
Gradual increase in CIP peaks	Insufficient elution and CIP.	Optimize the elution conditions, the CIP protocol, or perform CIP more frequently.
High back pressure during CIP	Protein precipitation in the column.	Make sure that all components are eluted from the column. Optimize elution conditions, or perform a CIP at a lower flow rate.
Decreased column performance despite optimized elution and CIP	Column longevity, which mainly depends on the sample type and sample preparation.	Use a new column.

9 Ordering information

Product	Quantity	Product code
HiScreen Capto Core 400	1 × 4.7 mL	17372410
HiScreen Capto Core 700	1 × 4.7 mL	17548115
HiTrap Capto Core 400	5 × 1 mL	17372411
HiTrap Capto Core 700	5 × 1 mL	17548151

Related product	Quantity	Product code
Capto Core 400	25 mL	17372401
	100 mL	17372402
	1 L	17372403
Capto Core 700	25 mL	17548101
	100 mL	17548102
	1 L	17548103
	5 L ¹	17548104

¹ Process-scale quantities are available. Visit [cytiva.com/bioprocess](https://www.cytiva.com/bioprocess), or contact your local Cytiva representative.

Related documentation	Reference
Datafile	
Capto Core 400, Capto Core 700	CY12286
Selection Guide	
Prepacked chromatography columns for ÄKTA systems	CY14019

Accessories HiScreen	Quantity	Product code
Fingertight connector 1/16" male, narrow <i>(For connecting HiTrap and HiPrep columns with 1/16" fittings to ÄKTA systems)</i>	8	28401081
Union 1/16" male - male i.d. 0.5 mm <i>(For connecting two columns with 1/16" fittings in series)</i>	2	28954326
Fingertight stop plug, 1/16" ¹ <i>(For sealing a HiScreen column)</i>	5	11000355

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

Accessories HiTrap	Quantity	Product code
Connector 1/16" male/luer female <i>(For connecting syringe to top of HiTrap column)</i>	2	18111251
Tubing connector flangeless/M6 female <i>(For connecting tubing to bottom of HiTrap column)</i>	2	18100368
Tubing connector flangeless/M6 male <i>(For connecting tubing to top of HiTrap column)</i>	2	18101798
Union 1/16" female/M6 male <i>(For connecting to original FPLC System through bottom of HiTrap column)</i>	6	18111257

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