HiScreen™ Capto™ Core 700 HiTrap™ Capto Core 700, 1 ml

Capto Core 700 chromatography medium is aimed at intermediate purification and polishing of viruses and other large biomolecules in flow-through mode. The product is based on the core bead concept. Each bead has a ligand-activated core and an outer layer without ligands. The layer prevents the large targets from entering into the beads whereas smaller proteins and impurities enter into the core where they bind to the hydrophobic and positively charged octylamine ligands.

Due to the core beads concept, Capto Core 700 offers high purity, improved process economy and increased productivity compared to gel filtration in group separation mode by enabling:

- Up to 100 times higher load
- Significantly higher flow rates
- Straightforward optimization and scale up

HiScreen Capto Core 700 (4.7 ml) and HiTrap Capto Core 700 (1 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 Core 700 and HiTrap Capto Core 700 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.

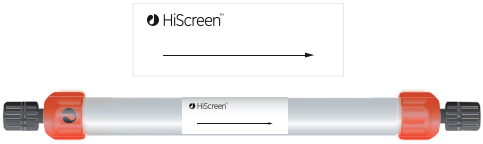

Fig 1. HiScreen column

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.

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	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 Core 700

Capto Core 700 is based on the core bead concept. Each bead has a core activated with octylamine ligands. The core is surrounded by a layer without ligands. This outer layer prevents large targets from binding to the ligands whereas smaller impurities can enter freely into the beads where they are being captured.

The molecular size cut-off for proteins is approximately M_r = 700 000 which means that targets that are larger than this will pass through the column in the flowthrough fraction.

A schematic representation of Capto Core 700 is given in Figure 3. The octylamine ligand (see Table 3) is both hydrophobic and positively charged in order to interact strongly with most of the impurities over a wide range of pH and salt concentrations.

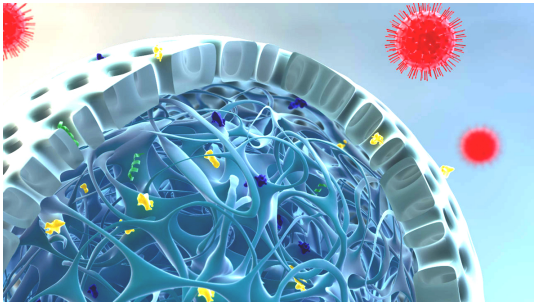

Fig 3. Schematic representation of the principle for Capto Core 700. Large targets are excluded from the beads, whereas smaller impurities can enter into the core where they are being captured by the ligands.

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

The design of the bead, with a layer that prevents binding of the large target, allows for high resolution at high flow rates. Thus Capto Core 700 provides excellent productivity and process economy. Further characteristics of the chromatography medium are found in Table 3.

Table 3. Characteristics of Capto Core 700

Matrix	High flow agarose
Size cut-off of outer layer	M_r ~ 700 000
pKa of protonated octylamine [1]	10.65
Functional group in the core	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2NH$-
Total ionic capacity	40 to 85 µmol (Cl^-/ml medium)
Average particle size (d_{50v}) [2]	85 µm
Dynamic binding capacity [3]	~13 mg ovalbumin/ml medium
Maximum flow velocity [4]	500 cm/h
pH stability [5]	
Working range	2 to 14
Cleaning-in-place	3 to 13
Working temperature	4°C to 30°C
Chemical stability	All commonly used aqueous buffers, 1 M sodium hydroxide [6], 6 M guanidine hydrochloride, 30% isopropanol, 70% ethanol
Avoid	Oxidizing agents, anionic detergents
Storage	4°C to 30°C in 20% ethanol

1 pKa of protonated octylamine before attachment to the resin. After attachment the pKa might be slightly different.

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

3 Determined 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.

4 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.

5 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.

6 No significant change in ionic capacity and carbon content after 1 week storage in 1 M NaOH at 40°C.

2 Optimization

General

The aim of designing and optimizing a separation process with Capto Core 700 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 start in process development it is also necessary to consider process cost, cleaning of the media and environmental constraints.

The HiScreen column format is ideal to use for method optimization when developing a new purification process. The small column volume, 4.7 ml, and the 10 cm bed height make 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*).

Note: *The HiTrap column format is suited for initial screening of binding and elution conditions. Further optimization is however preferably done on a larger column such as HiScreen Capto Core 700.*

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 Figure 4.

Capto Core 700 is compatible with most of the commonly used buffers for gel filtration and ion exchange chromatography (e.g., phosphate or Tris buffer). Citrate buffer is not recommended since it may give lower binding capacity.

Most host cell proteins are negatively charged above pH 7. Since the ligands are positively charged (for pH under the pKa of the ligand, see Table 3) it is advised to use a pH of 7 to 9 to ensure good binding of the host cell proteins to the ligands.

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

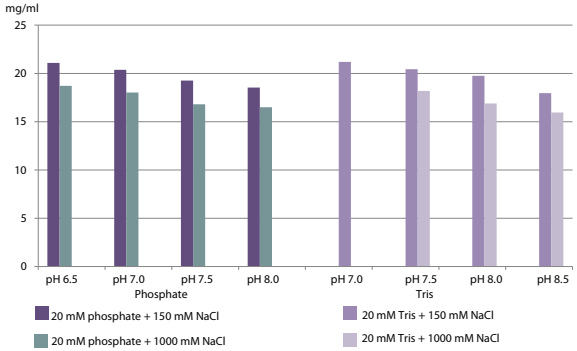

Fig 4. Static binding capacities for ovalbumin in phosphate and Tris buffers at different pH and salt concentrations.

Users of ÄKTA design chromatography systems with BufferPrep, or BufferPro functionality can select from a range of buffer recipes to conveniently screen media over a range of pH values and conductivities.

Reduction of DNA/RNA

High levels of DNA and RNA can in some cases impair the performance of Capto Core 700. It is therefore recommended that DNA/RNA levels are reduced prior to the Capto Core 700 purification step (e.g., by the use of 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 can enter into the core of Capto Core 700 where they are bound by the octylamine ligands. The Benzonase endonuclease itself can also be removed by Capto Core 700.

Sample load

Capto Core 700 works as a scavenger, meaning that the target will pass in the flowthrough 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 take out fractions during the sample application and analyze the fractions for recovery and purity of target. Typically about 5 to 20 column volumes can be loaded but this is very dependent on the actual composition of the sample.

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 starting buffer the bound impurities will be removed during the cleaning-in-place (CIP) of the medium, see Section *Cleaning-in-place (CIP)*.

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 proteins and fragmented DNA 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 and 1.0 ml/min for HiTrap (150 cm/h). During column equilibration and wash steps higher flow rates can be used, up to 3.9 ml/min for HiScreen and 3.2 ml/min for HiTrap (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 (ml/min)	Flow velocity (cm/h)
Equilibration [1]	up to 3.9	up to 3.2	up to 500
Wash[1]	up to 3.9	up to 3.2	up to 500
Load of sample	0.8 to 2.3	0.6 to 1.9	100 to 300
Cleaning-in-place [2]	0.16 to 0.31	0.13 to 0.26	20 to 40

[1] Stated flow rates are for buffers with the same viscosity as water at 20ºC. 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 (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 starting buffer or until the UV baseline, pH and conductivity are stable.
5	Adjust the sample to the chosen starting pH and conductivity, see Table 4.
6	Load about 5 to 20 CV of the sample onto the column. Take out fractions for analysis of purity and recovery of target, see Section *Optimization*.
7	Wash with 5 to 10 CV of starting 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 Section *Cleaning-in-Place (CIP)*.

Step	Action
9	Equilibrate with 5-10 CV of starting buffer or until the UV baseline, pH, and conductivity reach the required values. The equilibration step can be shortened by first washing with a high concentration buffer to obtain approximately the desired pH value and then washing with starting buffer until the conductivity and pH values are stable. ***Note:*** *Do not exceed the maximum recommended flow rate (see Table 5) or the column hardware pressure limit (see Tables 1 and 2).*

4 Cleaning-In-Place (CIP)

The HiScreen and HiTrap Capto Core 700 columns can be reused many times if the columns are eluted and cleaned properly in between runs. Regular CIP prevents the build-up of contaminants and helps to maintain capacity, flow properties and general performance of the chromatography medium and prepacked columns. CIP should be performed after each run to ensure reproducible results. It is also recommended to perform a CIP:

- When an increase in back pressure is seen.
- If reduced column performance is observed.
- Before first time use or after long term storage.

CIP of Capto Core 700 is performed by **using a combination** of solvent and sodium hydroxide:

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 min (sample dependent). A pause of 15 to 30 min could be included during the CIP for more effective cleaning.

The nature of the sample will ultimately determine the final CIP protocol so the CIP protocol may require optimization. NaOH and solvent concentrations along with contact time and volume of CIP solution are typically the main parameters to vary during the optimization of the CIP. For increased contact time and due to the viscosity of the CIP solutions it is recommended to use a lower flow rate than during the purification, see Table 5.

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*.

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.

Step	Action
3	The larger equipment used when scaling up may cause some 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 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.*

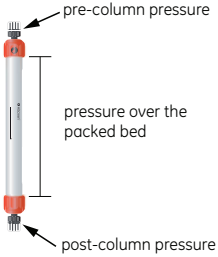

Fig 5. Pre-column and post-column measurements.

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 4°C to 30°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 Core 700	1 × 4.7 ml	17-5481-15
HiTrap Capto Core 700	5 × 1 ml	17-5481-51

Related product	Quantity	Code No
Capto Core 700	25 ml	17-5481-01
	100 ml	17-5478-02
	1 l	17-5478-03
	5 l [1]	17-5478-04

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

Related literature	Code No
Data File: Capto Core 700	28-9961-82
Prepacked chromatography columns for ÄKTA systems, Selection Guide	28-9317-78

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

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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