GE Healthcare
Life Sciences

Instructions 28-9958-80 AC **Core beads**

Capto™ Core 700

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

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1 BioProcess™ Media

BioProcess media are developed and supported for production scale chromatography. All BioProcess media are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of media for production scale. Regulatory Support Files (RSF) are available to assist process validation and submissions to regulatory authorities. BioProcess media cover all purification steps from capture to polishing.

2 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 flow-through fraction. A schematic representation of Capto Core 700 is given in Figure 1. The octylamine ligand (see Table 1) 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.

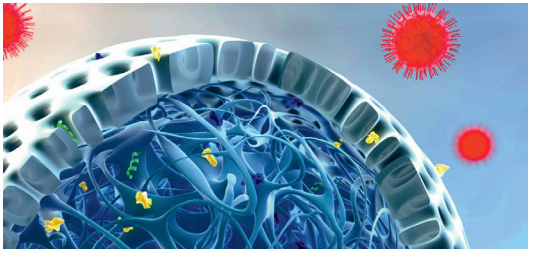

Fig 1. Schematic representation of the principle for Capto Core 700. Large targets are excluded from the beads, whereas smaller impurities can enter into the core where they are being captured by the ligands.

The product is based on a high flow agarose base matrix, which gives good pressure-flow properties (Fig 2).

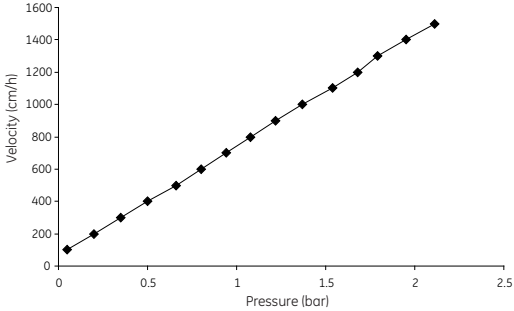

Fig 2. Example of pressure flow curve for Capto Core 700 in packed bed. Running conditions: AxiChrom™ 300 (30 cm i.d.), 20 cm bed height with Packing Factor 1.15 in water at 20ºC. Pressure contribution from system, tubings and column is excluded.

The construction 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 medium are found in Table 1.

Table 1. Characteristics of Capto Core 700.

Matrix	High flow agarose
Size cut-off of outer layer	$M_r \approx 700\,000$
pKa of protonated octylamine[1]	10.65
Functional group in the core	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2NH$-
Total ionic capacity/ml medium	40 to 85 µmol (Cl^-)
Particle size (d_{50v})[2]	90 µm
Maximum operational flow velocity[3]	500 cm/h in columns with 20 cm bed height at <2 bar (0.2 MPa)
Binding capacity[4]/ml medium	≈13 mg ovalbumin
pH - Cleaning-in-Place stability - pH working range	 2 to 14 3 to 13
Working temperature	4°C to 30°C
Chemical stability	All commonly used aqueous buffers, 1 M sodium hydroxide[5], 6 M guanidine hydrochloride, 30% isopropanol and 70% ethanol
Avoid	Oxidizing agents, anionic detergents
Storage	20% ethanol

[1] pKa of protonated octylamine before attachment to the resin. After attachment the pKa may be slightly different.
[2] d_{50v} is the average particle size of the cumulative volume distribution.
[3] Maximum flow velocity that has been verified for long-time use.
[4] Dynamic binding capacities were measured at 10% breakthrough with a residence time of 3 minutes (1.6 ml/min = 200 cm/h) in HiScreen columns. The mobile phase was 20 mM Tris/HCl with 0.15 M NaCl at pH 7.5.
[5] No significant change in ionic capacity and carbon content after 1 week storage in 1 M NaOH at 40°C.

3 Method optimization

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.

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 3.

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 1) 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

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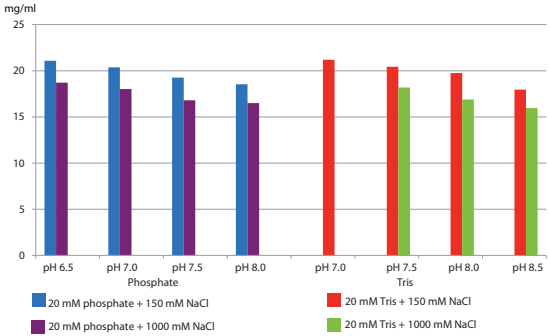

Fig 3. Static binding capacities for ovalbumin in phosphate and Tris buffers at different pH and salt concentrations.

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 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 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 7 Maintenance.

4 Scale-up

Scale-up is typically performed by keeping bed height and flow velocity constant while increasing bed diameter and volumetric flow rate. However, since optimization is preferentially performed with small column volumes, in order to save sample and buffer, some parameters such as the sample load may be optimized using shorter bed heights than those being used in the final scale. As long as the residence time is kept constant, the binding capacity for the impurities remains the same. The residence time is approximated as the bed height (cm) divided by the linear flow velocity (cm/h).

Procedure

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. 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.

5 Column packing

Recommended columns

Table 2. Recommended columns for Capto Core 700.

Column	Inner diameter (mm)	Bed volume[1]	Bed height (cm)
Lab scale			
HiScale™ 16/20	16	20–40 ml	max 20
HiScale 16/40	16	20–70 ml	max 35
HiScale 26/20	26	53–106 ml	max 20
HiScale 26/40	26	53–186 ml	max 35
HiScale 50/20	50	196–393 ml	max 20
HiScale 50/40	50	196–687 ml	max 35
Production scale[2]			
AxiChrom[3]	50–200	0.2–12.5 l	max 40
AxiChrom[3]	300–1000	7–314 l	max 40
BPG™[4]	100–300	1–28 l	max 40

[1] Bed volume range calculated from 10 cm bed height to maximum bed height.
[2] For other columns, please contact GE Healthcare.
[3] Intelligent packing method according to Capto Core 700 can be used.
[4] The pressure rating of BPG 450 is too low to use with Capto Core 700 media.

Note: *AxiChrom 300-1000 columns can be equipped with either acrylic or stainless steel column tubes. Use stainless steel columns only with Capto Core 700, as this material is compatible with the recommended CIP solutions (see Section 7 Maintenance).*

For more details about packing HiScale columns, see instructions *HiScale™ columns (16, 26, 50) and accessories* (28-9674-70) and the video *How to pack a HiScale™ column* (www.gelifesciences.com/HiScale).

For information on packing of process scale columns, please contact your local GE Healthcare representative.

Packing HiScale columns

Packing preparations

Materials needed

- Capto Core 700
- 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 $CF = L_{settled}/L_{packed}$
PF	Packing factor $PF = L_{cons}/L_{packed}$
A_C	Cross sectional area of the column
V_C	Column volume $V_C = L_{packed} \times A_C$
C_{slurry}	Concentration of the slurry

Preparation of the slurry

To measure the slurry concentration, let the media settle in 20% ethanol at least overnight in a measuring cylinder.

For calculating the amount of medium to fill into the column, use the following equation: $V = (A_C \times L_{packed} \times CF) / C_{slurry}$

CF for Capto Core 700 when using the packing methods described in Table 4, 5 and 6 are found in Table 3.

Table 3. Compression factors for Capto Core 700 in 20% ethanol during gravity settling.

Column	**HiScale 16, 26, 50**	
Bed height (cm)	10	35
Compression factor	1.16	1.08

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:

- Wash 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 the column

Table 4. Main features of the packing method for HiScale 16/20 and HiScale 16/40.

Column	HiScale 16/20	HiScale 16/40
Bed height (cm)	10	35
Slurry/ packing solution	20% ethanol with 0.4 M NaCl	
Slurry concentration (%)	50	50
Packing factor (PF)	1.15	1.06
Packing velocity (cm/h)	300	300
Packing flow rate (ml/min)	10	10
Flow condition (cm/h)	750	260
Flow condition (ml/min)	25	8.6

Table 5. Main features of the packing method for 26/20 and HiScale 26/40.

Column	HiScale 26/20	HiScale 26/40
Bed height (cm)	10	35
Slurry/ packing solution	20% ethanol with 0.4 M NaCl	
Slurry concentration (%)	50	50
Packing factor (PF)	1.15	1.06
Packing velocity (cm/h)	300	300
Packing flow rate (ml/min)	27	27
Flow condition (cm/h)	750	260
Flow condition (ml/min)	66	23

Table 6. Main features of the packing method for HiScale 50/20 and HiScale 50/40.

Column	HiScale 50/20	HiScale 50/40
Bed height (cm)	10	35
Slurry/ packing solution	20% ethanol with 0.4 M NaCl	
Slurry concentration (%)	50	50
Packing factor (PF)	1.15	1.06
Packing velocity (cm/h)	300	300
Packing flow rate (ml/min)	100	100
Flow condition (cm/h)	750	260
Flow condition (ml/min)	245	85

Packing procedure

1. Assemble the column according to the column instructions (*HiScale columns (16, 26, 50) and accessories*, code no 28-9674-70).
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.
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 Table 4, 5 and 6.
11. Let the flow run until the bed has consolidated.
12. Use the scale on the column to measure the bed height (L_{cons}). 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.
    $L_{packed} = L_{cons}/PF$. See Table 4, 5 and 6.
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.

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 Table 4, 5 and 6.

21 Let the flow run for about 10 column volumes.
The column is ready to be tested.

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 *Column efficiency testing* (28-9372-07).

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 liquid 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:

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

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

L = bed height (cm)

N = number of theoretical plates

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:

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

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

Figure 4 shows a UV trace for acetone in a typical test chromatogram from which the HETP and A_S values are calculated.

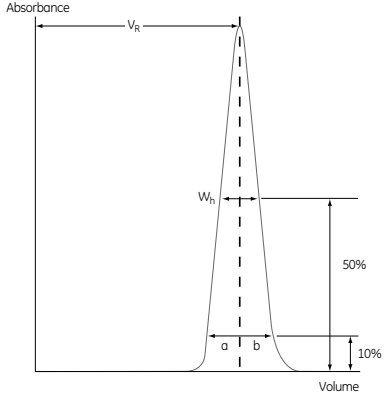

Fig 4. A typical test chromatogram showing the parameters used for HETP and A_S calculations.

7 Maintenance

For best performance from Capto Core 700 and to maximize the life time of the media, follow the procedures described below.

Equilibration

After packing, and before a chromatographic run, equilibrate with start buffer by washing with five bed volumes or until the column effluent shows stable conductivity and pH 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 start buffer until the conductivity and pH values are stable.

Cleaning-In-Place (CIP)

Regular CIP prevents the build-up of contaminants in the packed bed and helps to maintain capacity, flow properties and general performance of the medium. CIP should be performed after each run to ensure reproducible results.

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.

Note: *Make sure that columns, tubing and other equipment are compatible with the selected CIP solution.*

Note: *AxiChrom 300-1000 columns can be equipped with either acrylic or stainless steel column tubes. Use stainless steel columns only with Capto Core 700, as this material is compatible with the recommended CIP solutions.*

The nature of the sample will ultimately determine the final CIP protocol so the CIP procedures described above 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.

Sanitization

To reduce microbial contamination in the packed column, sanitization using 0.5 to 1 M NaOH with a contact time of 1 h is recommended. The CIP protocol described above with 1 h contact time sanitizes the medium effectively.

Storage

Store unused medium in the container at a temperature of 4°C to 30°C. Ensure that the cap is fully tightened. Packed chromatography media and bulk media should be stored in 20% ethanol. After storage, equilibrate with at least five column volumes of start buffer.

8 Ordering information

Product	Pack size	Code No
Capto Core 700	25 ml	17-5481-01
	100 ml	17-5481-02
	1 l	17-5481-03
	5 l	17-5481-04

Capto Core 700 is supplied in suspension in 20% ethanol. For additional information, including a data File, please contact your local GE Healthcare representative.

Related products

Related product	Quantity	Code No
HiTrap™ Capto Core 700	5 × 1 ml	17-5481-51
HiScreen™ Capto Core 700	1 × 4.7 ml	17-5481-15
HiScale 16/20	1	28-9644-41
HiScale 16/40	1	28-9644-24
HiScale 26/20	1	28-9645-14
HiScale 26/40	1	28-9645-13
HiScale 50/20	1	28-9644-45
HiScale 50/40	1	28-9644-44

Accessories

Product	Quantity	Code No
HiScale Packing tube 16/20	1	28-9868-16
HiScale Packing tube 16/40	1	28-9868-15
HiScale Packing tube 26/20	1	28-9803-83
HiScale Packing tube 26/40	1	28-9645-05
HiScale Packing tube 50/20	1	28-9802-51
HiScale Packing tube 50/40	1	28-9645-06

Literature

Literature		Code No
Data file	Capto Core 700	28-9961-82
Video	How to pack a HiScale™ column www.gelifesciences.com/HiScale	NA
Instructions	HiScale columns (16, 26, 50) and accessories	28-9674-70
Application note	Column efficiency testing	28-9372-07

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