GE Healthcare
Life Sciences

Capto™ Core 700

Capto Core 700 is designed for intermediate purification and polishing of viruses and other large biomolecules. The novel core bead technology and multimodal, octylamine ligand give Capto Core 700 dual-functionality, size separation, and binding chromatography in one chromatography medium (resin). These features make Capto Core 700 an excellent alternative to gel filtration (size-exclusion chromatography) media that are typically employed in the final stages of virus purification in vaccine manufacture. Capto Core 700 offers a range of performance advantages over gel filtration (GF), which is often regarded as a productivity bottleneck in the polishing process due to low flow rates and limited sample loads.

Key performance characteristics of Capto Core 700 include:

- Significantly improved productivity enabled by up to 100-fold higher sample load and significantly higher flow rates compared with GF
- Core bead technology with ligand-activated core and inactive shell allows efficient capture of contaminants while target molecules are collected in the flowthrough
- Straightforward optimization due to flowthrough chromatography and robust performance allowing for a wide window of operation

Characteristics of the medium

Core bead technology

Capto Core 700 is composed of a ligand-activated core and inactive shell. The inactive shell excludes large molecules (cut off ~ M_r 700 000 [700 kDa]) from entering the core through the pores of the shell (Fig 1). These larger molecules are collected in the column flowthrough 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 contaminants small enough to enter the core. The multimodal ligands ensure strong binding with most impurities over a wide range of pH and salt concentrations. Bound impurities are removed from the beads by cleaning-in-place (CIP) procedures using NaOH and in most cases, a solvent. The characteristics of Capto Core 700 are summarized in Table 1.

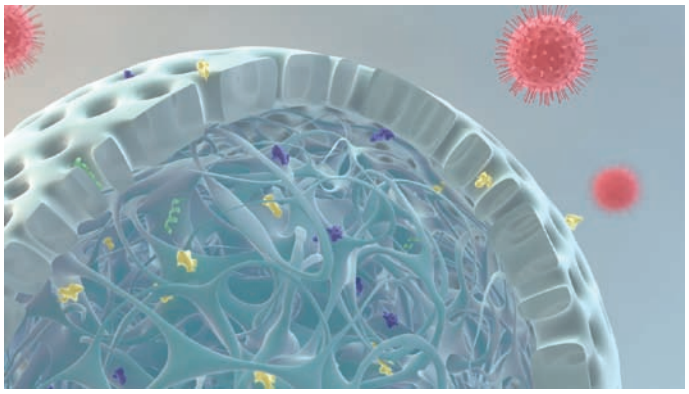

Fig 1. Schematic representation of the principle for Capto Core 700 showing a bead with the inactive shell, pores in the shell, and the ligand-activated core. Small proteins and contaminants (colored green, yellow, and purple) penetrate the core while target viruses (red) and larger proteins are excluded from the medium and are collected in the flowthrough.

Table 1. Characteristics of Capto Core 700

Matrix	Highly cross-linked agarose
Average particle size (d_{50v})	85 µm
Ligand	Octylamine
Binding capacity[1]	13 mg ovalbumin/mL medium
Molecular weight cutoff	M_r 700 000
Maximum flow velocity	500 cm/h in column with 20 cm bed height at < 2 bar (0.2 MPa)
pH stability	
Working	3 to 13
Short term	2 to 14
Working temperature	4°C to 30°C
Chemical stability	All commonly used aqueous buffers, 1 M sodium hydroxide (NaOH)[2], 6 M guanidine hydrochloride, 30% isopropanol, and 70% ethanol
Avoid	Oxidizing agents, citrate buffers
Storage	20% ethanol at 4°C to 30°C

[1] Dynamic binding capacity measured at 10% breakthrough with a residence time of 3 min (200 cm/h) on HiScreen™ columns. The buffer was 20 mM Tris-HCl, 150 mM NaCl, pH 7.5

[2] No significant change in ionic capacity and carbon content after 1 week storage in 1 M NaOH at 40°C

Group separation with high loads

The core bead technology in Capto Core 700 enables high-load during group separation of molecules. The core bead technology also allows for short residence times (sometimes as low as 1 min) and in combination with the large 85 µm high flow agarose matrix, flow velocities as high as 500 cm/h are possible. The short residence times, high flow velocities, and high loading enables a larger operational window than traditional GF. The larger operational window allows for increased volume throughput and smaller equipment with reduced footprint. The large bead size also contributes to reducing back pressure during purification of highly viscous samples.

The improved window of operation provided by Capto Core 700 allows greater freedom of process design.

Figure 2 illustrates schematically the greater load capacity and flow rates enabled with Capto Core 700 relative to that of Sepharose™ 4 Fast Flow, which is a GF medium typically used in large-scale polishing processes.

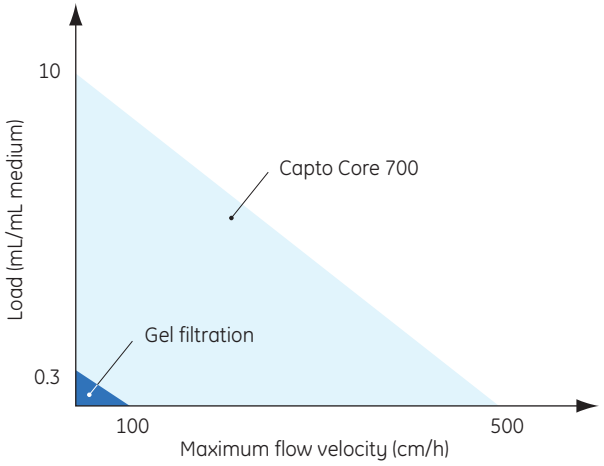

Fig 2. Schematic illustration of the significantly greater sample load and flow velocity possible with Capto Core 700 in comparison to conventional GF media (note, schematic is not to scale).

To evaluate the load capacity of Capto Core 700, a comparison of influenza virus hemagglutinin (HA) purification and removal of host cell proteins (HCP) was performed with conventional Sepharose 4 Fast Flow. The Sepharose 4 Fast Flow was packed in Tricorn™ 10/600 columns to a bed height of 60 cm, which gave a column volume (CV) of 47 mL. The sample load for the Sepharose 4 Fast Flow packed column was 0.1 CV. The equivalent load for the Capto Core 700 (packed in Tricorn 5/50, CV 1 mL) was 10 CV.

Chromatograms showing the separation of virus and HCP on both media are shown in Figure 3. Similar recovery of HA and reduction of HCP were observed for both media (see Application note 29-0003-34). However, Capto Core 700 allowed up to 100-fold more sample to be processed in one run than by group separation on Sepharose 4 Fast Flow.

Columns:	Tricorn 10/600 packed with Sepharose 4 Fast Flow, CV 47 mL Tricorn 5/50 packed with Capto Core 700, CV 1 mL
Sample:	Influenza H1N1 cultivated in MDCK cells, concentrated, and diafiltrated on an M_r 500 000 hollow-fiber filter to 20 mM Tris, 150 mM NaCl, pH 7.5
Sample loads:	Sepharose 4 Fast Flow, 0.1 CV (4.7 mL feed); Capto Core 700, 10 CV (10 mL feed)
Buffer:	20 mM Tris, 150 mM NaCl, pH 7.5
Flow velocities:	Sepharose 4 Fast Flow, 30 cm/h; Capto Core 700, 100 cm/h
Cleaning-in-place (CIP)/elution:	Capto Core 700, 30% 2-propanol in 1 M NaOH
System:	ÄKTAexplorer 10S

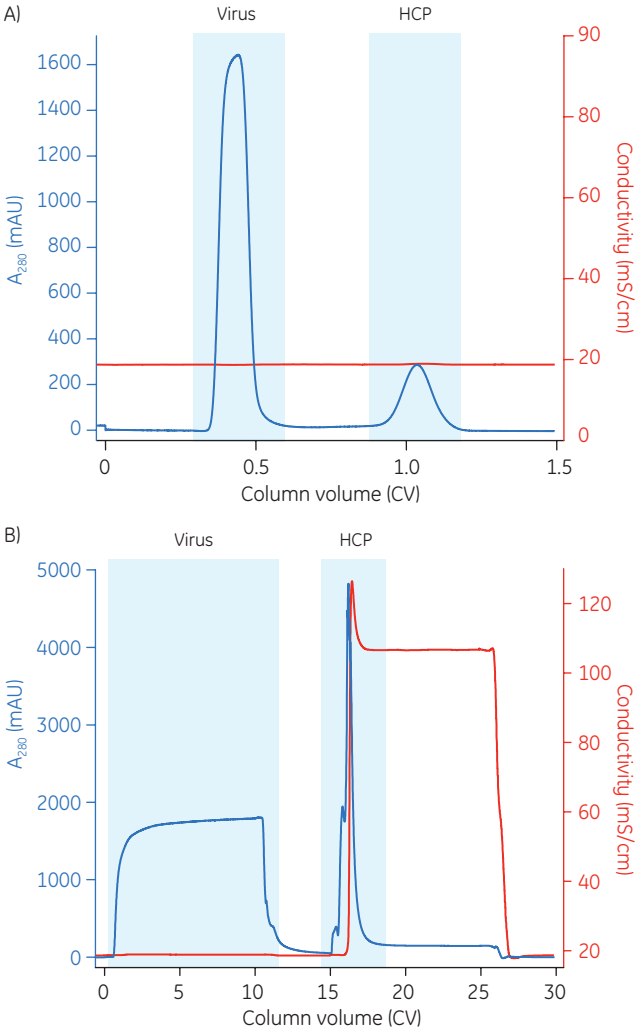

Fig 3. Chromatograms from the purification of influenza virus HA and removal of HCP from virus material run on **A)** a conventional GF medium, Sepharose 4 Fast Flow and **B)** Capto Core 700 medium.

Robust binding performance

The octylamine ligand chosen for Capto Core 700 is multimodal, giving a broad window of operation with excellent binding capacity in a range of buffers. The ligand is functional in sodium phosphate and Tris buffers containing up to 1 M NaCl (Fig 4). Citrate buffer is, for example, not recommended since citrate ions can interact with the ligand.

Amount of medium: 10 µL of Capto Core 700 in HTPD 96-well filter plate
Sample: Ovalbumin (1.5 mg/mL) in equilibration buffers
Sample load: 200 µL of ovalbumin (60 min incubation)
Equilibration buffers: 20 mM sodium phosphate, 150–1000 mM NaCl, pH 6.5–8.0; 20 mM Tris, 150–1000 mM NaCl, pH 7.5–8.5
Equilibration: 3 × 200 µL of equilibration buffer
Wash: 200 µL of equilibration buffer

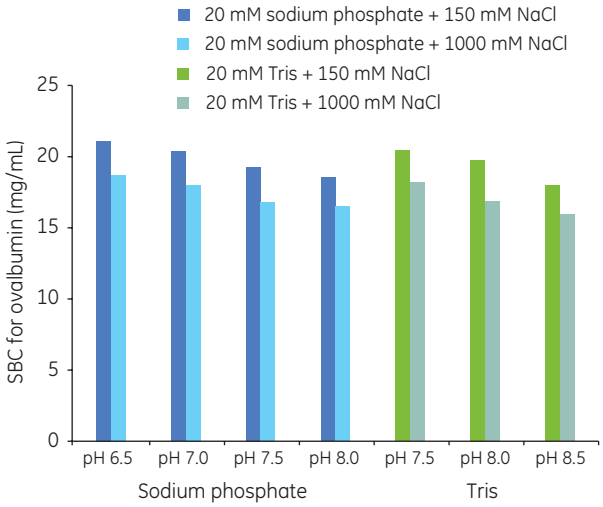

Fig 4. Static binding capacity (SBC) of Capto Core 700 for ovalbumin in sodium phosphate and Tris buffers with different NaCl concentrations and a range of pH.

Effective removal of HCP and DNA

Most HCP are negatively charged above pH 7. The ligands of Capto Core 700 are positively charged below a pKa of 10.6, and a pH of 7 to 9 is therefore recommended to ensure good binding of HCP. DNA is negatively charged over a wider pH range and the efficiency of the DNA removal is thus less dependent on pH. It is, however, recommended that DNA/RNA levels are reduced prior to the Capto Core 700 purification step, for example, by use of an anion exchange step or by treatment with Benzonase™ endonuclease. Degradation of DNA using Benzonase yields small oligonucleotide fragments that enter the core of the beads where these bind to the internal ligands. Benzonase will also enter the core and bind, and will thus be removed from the virus flowthrough fraction.

Molecular weight cutoff

To investigate the molecular weight cutoff, the dynamic binding capacity of Capto Core 700 was determined for a range of proteins of different sizes. The proteins investigated were ovalbumin (M_r 45 000), apoferritin (M_r 475 000), thyroglobulin (M_r 660 000), and bovine IgM (approx. M_r 900 000). Protein samples were diluted to a final concentration between 1.0 and 4.0 mg/mL and loaded on prepacked HiScreen Capto Core 700 column. The protein samples were loaded until 1% and 10% breakthrough of the protein, respectively, were achieved (Fig 5).

Capto Core 700 binds proteins up to M_r 660 000 while proteins with higher molecular weight, such as IgM, are excluded from the beads and pass through the column in the flowthrough (Fig 5).

Column: HiScreen Capto Core 700, 4.7 mL
Samples: Ovalbumin (3.3 mg/mL); apoferritin (2.5 mg/mL); thyroglobulin (4.0 mg/mL); bovine IgM (1.0 mg/mL)
Buffer: 20 mM Tris-HCl, 150 mM NaCl, pH 7.5
Flow rate: 200 cm/h (1.6 mL/min, 3 min residence time)
Cleaning-in-place (CIP)/elution: 1 M NaOH, 20% 1-propanol at 0.5 mL/min
System: ÄKTAexplorer™ 10S
Detection: UV, 280 nm

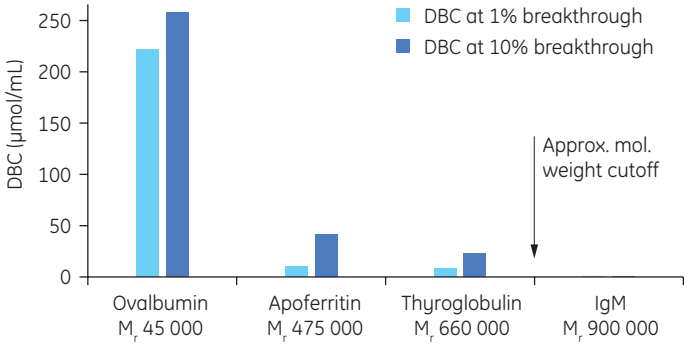

Fig 5. Dynamic binding capacity (DBC) at 1% and 10% breakthrough for large proteins on Capto Core 700. Note that DBC was measured in µmol/mL.

Cleaning-in-place and sanitization

Regular CIP is necessary to remove captured contaminants and allow re-use of Capto Core 700 with maintained capacity. Use of 1 M NaOH in 27% 1-propanol is recommended for effective CIP and sanitization of the medium after every cycle. Due to the strong binding of a wide range of contaminants to the ligand, an organic solvent will be needed for CIP with most samples. However, this will be sample dependent and it may be possible to use CIP solutions without organic solvents. CIP protocols are dependent on the feed material and running conditions and optimization is therefore recommended for the chosen application.

In a medium lifetime study, the dynamic binding capacity of pure ovalbumin remained stable over the tested 45 cycles (Fig 6). This study tested DBC every eleventh cycle with 10 cycles of fouling using 10 CV of MDCK cell lysate and CIP in between. The results clearly demonstrate the stability of the medium over many purification cycles, which may improve overall process economy. Recovery and purity of the vaccine remained high throughout the test (data not shown).

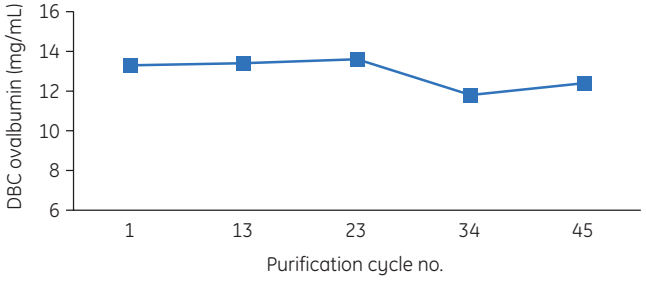

Fig 6. Dynamic binding capacity measurements of ovalbumin on Capto Core 700 over 45 purification cycles with 1 M NaOH in 27% 1-propanol as CIP agent. CIP protocol after every eleventh cycle was 5 CV CIP agent, 30 min pause, then 5 CV CIP agent. Fouling of the medium between DBC measurements was performed with MDCK cell lysate.

Small-scale formats for fast process development

Capto Core 700 is available in 1 mL prepacked HiTrap™ and 4.7 mL prepacked HiScreen formats. Combined with a chromatography system such as ÄKTA™ avant or other ÄKTA system, HiTrap and HiScreen columns are convenient to use when developing an efficient and robust separation method. Further development and optimization using HiScale columns (Table 2) then permits straightforward scale-up (for details of packing laboratory-scale columns, see the appropriate Instructions).

Scale-up to production scale

Capto Core 700 is available as a bulk medium in a range of pack sizes from lab- to production-scale, from 25 mL to 5 L. We also provide a wide range of process-scale columns for packing of Capto Core 700 (Table 2).

Scale-up is typically performed by keeping bed height and flow velocity constant while increasing column bed diameter and flow rate. However, since conditions are often optimized in small column volumes, parameters such as dynamic binding capacity can be optimized on shorter bed heights than those used at the final scale. By keeping the residence time and loading constant, the binding capacity and purity will be maintained. We recommend a maximum bed height of 40 cm with process-scale columns (Table 2).

Capto Core 700 is a BioProcess™ medium with support for large-scale manufacture of biopharmaceuticals. This support includes validated manufacturing methods, secure long-term media supply, and Regulatory Support Files (RSF) to assist process validation and submissions to regulatory authorities.

Table 2. Recommended laboratory- and process-scale empty columns for packing with Capto Core 700

Column family	Inner diameter (mm)	Max. bed height (cm)
Laboratory scale[1]		
HiScale™	16, 26, 50	35
Production scale[2]		
AxiChrom™[3]	50–200	40
AxiChrom[3]	300–1000	40
BPG™[4]	100–300	40

[1] Visit www.gelifesciences.com/tricorn and www.gelifesciences.com/hiscale for the full range of HiScale columns

[2] For other process-scale columns, please contact GE Healthcare or visit www.gelifesciences.com/bioprocess

[3] Intelligent packing method for Capto Core 700 can be used, visit www.gelifesciences.com/axichrom for details

[4] The pressure rating of BPG 450 is too low to use with Capto Core 700

Ordering information

Product	Quantity	Code number
Capto Core 700	25 mL	17-5481-01
Capto Core 700	100 mL	17-5481-02
Capto Core 700	1 L	17-5481-03
Capto Core 700	5 L	17-5481-04
Prepacked columns		
HiTrap Capto Core 700	5 × 1 mL	17-5481-51
HiScreen Capto Core 700	1 × 4.7 mL	17-5481-15

Related literature	Code number
Purification of influenza A/H1N1 using multimodal Capto™ Core 700, Application note	29-0003-34
HiScreen prepacked columns, Data file	28-9305-81
AxiChrom columns, Data file	28-9290-41
BPG columns, Data file	18-1115-23
Prepacked chromatography columns for ÄKTA systems, Selection guide	28-9317-78

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