Multimodal chromatography

Capto™ Core 400
Capto Core 700

Capto Core 400 and Capto Core 700 multimodal chromatography resins are designed for intermediate purification and polishing of viruses and other large biomolecules. The core bead technology and multimodal, octylamine ligand give the resins dual-functionality: size separation and binding chromatography in one chromatography resin. These features make Capto Core 400 and Capto Core 700 excellent alternatives to size-exclusion chromatography (SEC) resins that are typically employed in the final stages of virus purification in vaccine manufacture. Capto Core 400 and Capto Core 700 offer a range of performance advantages over SEC, which is often regarded as a productivity bottleneck in the polishing process due to low flow rates and limited sample loads.

Key performance characteristics include:

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

Resin characteristics

Core bead technology

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 400 000 for Capto Core 400 and ~ M_r 700 000 for Capto Core 700) 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 impurities 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 can be removed from the beads by cleaning-in-place (CIP) procedures using NaOH and in most cases, a solvent. The main characteristics of Capto Core 400 and Capto Core 700 are summarized in Table 1.

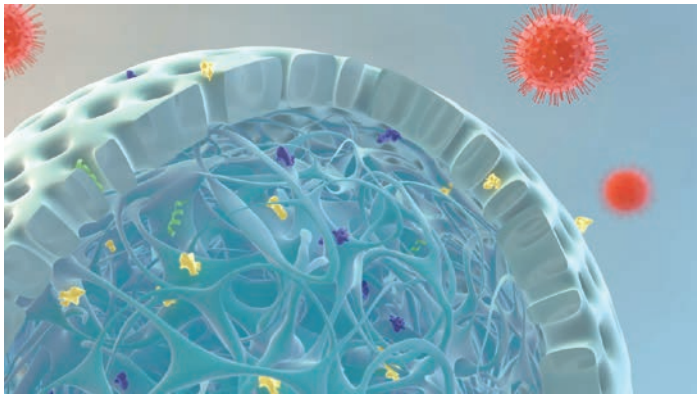

Fig 1. Schematic representation of the principle for core bead technology, showing a bead with the inactive shell, pores in the shell, and the ligand-activated core. Small proteins and impurities (colored green, yellow, and purple) penetrate the core, while target viruses (red) and larger proteins are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

Group separation with high loads

The core bead technology of Capto Core 400 and 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 90 µm high flow agarose matrix, flow velocities as high as 700 cm/h are possible. The short residence times, high flow velocities, and high loading enable a larger operational window than traditional SEC. 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 400 and Capto Core 700 allows greater freedom of process design. This is exemplified in Figure 2 that schematically illustrates the greater load capacity and flow rates enabled with Capto Core 700 relative to that of Sepharose™ 4 Fast Flow, which is a SEC resin typically used in large-scale polishing processes.

Table 1. Characteristics of Capto Core 400 and Capto Core 700

	Capto Core 400	Capto Core 700
Matrix	Highly cross-linked agarose	
Average particle size (d_{50v})	90 µm	85 µm
Ligand	Octylamine	
Binding capacity*	22 mg ovalbumin/mL resin	13 mg ovalbumin/mL resin
Average molecular weight cutoff	M_r 400 000	M_r 700 000
Maximum flow velocity	700 cm/h in column with 20 cm bed height at < 2 bar (0.2 MPa)	500 cm/h in column with 20 cm bed height at < 2 bar (0.2 MPa)
pH stability Operational† CIP‡	 3 to 13 3 to 14	
Chemical stability	All commonly used aqueous buffers, 1 M sodium hydroxide (NaOH)§, 6 M guanidine hydrochloride, 30% isopropanol, and 70% ethanol.	
Avoid	Oxidizing agents, citrate buffers	
Storage	20% ethanol at 4°C to 30°C	

* 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.

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

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

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

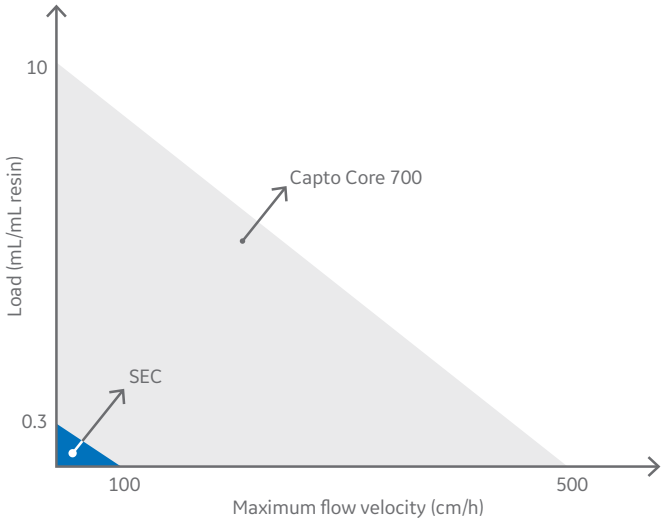

Fig 2. Schematic illustration of the significantly greater sample load and flow velocity possible with Capto Core 700 in comparison to conventional SEC resins (note, schematic is not to scale).

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 resins are shown in Figure 3. Similar recovery of influenza virus HA and reduction of HCP were observed for both resins (see Application note 29000334). 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 Mr 500 000 hollow-fiber filter to 20 mM Tris, pH 7.5 + 150 mM NaCl
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, pH 7.5 + 150 mM NaCl
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

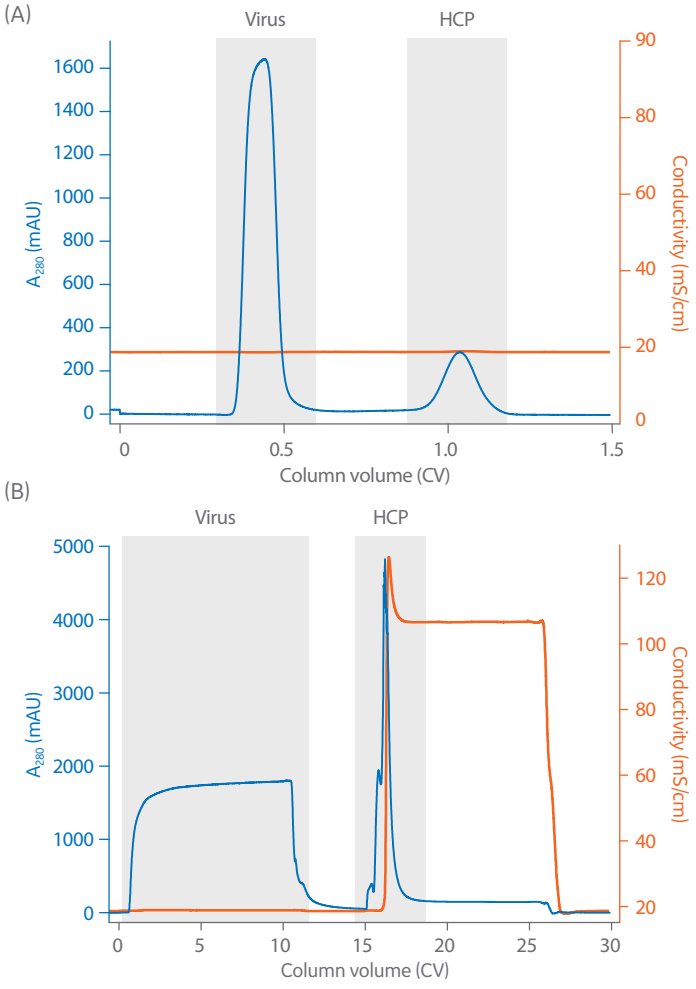

Fig 3. Chromatograms from the purification of influenza virus HA in the size range 80–120 nm and removal of HCP from virus material run on A) the conventional SEC resin, Sepharose 4 Fast Flow and B) Capto Core 700 resin.

Robust binding performance

The octylamine ligand chosen for Capto Core 400 and 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, as citrate ions can interact with the ligand.

Effective removal of HCP and DNA

Most HCPs are negatively charged above pH 7. The expected pKa for the octylamine ligand of Capto Core 400 and Capto Core 700 is in the range of 10.5–11.5, implying that the ligand is positively charged below this pH-range. 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. However, it is recommended that DNA/RNA levels are reduced prior to the purification step using either Capto Core 400 or Capto Core 700, 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 for Capto Core 700, the dynamic binding capacity (DBC) 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 columns. The protein samples were loaded until 1% and 10% breakthroughs were achieved (Fig 5). As shown, Capto Core 700 allows proteins of up to M_r 660 000 to enter the bead core and bind to the resin. While impurities of molecular weights lower than the cutoff values can enter the bead core and get trapped by the resin, entities with higher molecular weights, such as the target virus, are excluded from the beads and can pass through the column in the flowthrough. Capto Core 400 allows proteins of up to M_r 350 000 to enter the bead core and bind to the resin, whereas larger entities are excluded (data not shown).

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

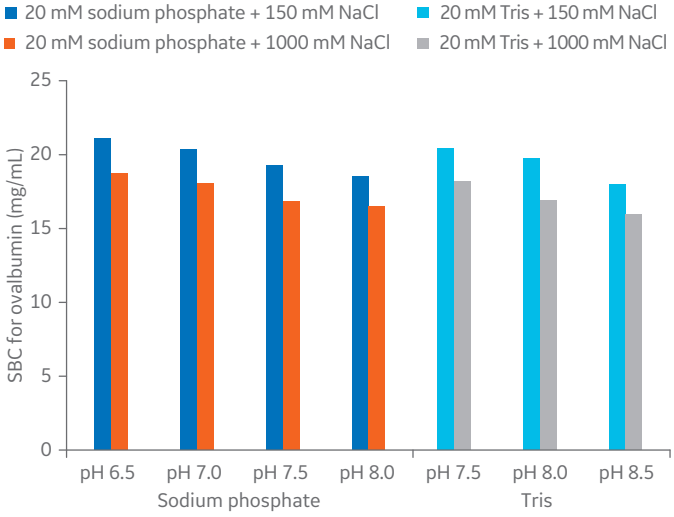

Fig 4. Static binding capacity (SBC) of Capto Core 700 for ovalbumin in sodium phosphate and Tris buffers with different NaCl concentrations and a range of pH.

Columns:	HiScreen Capto Core 700, 4.7 mL
Sample:	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, pH 7.5 + 150 mM NaCl
Flow velocities:	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

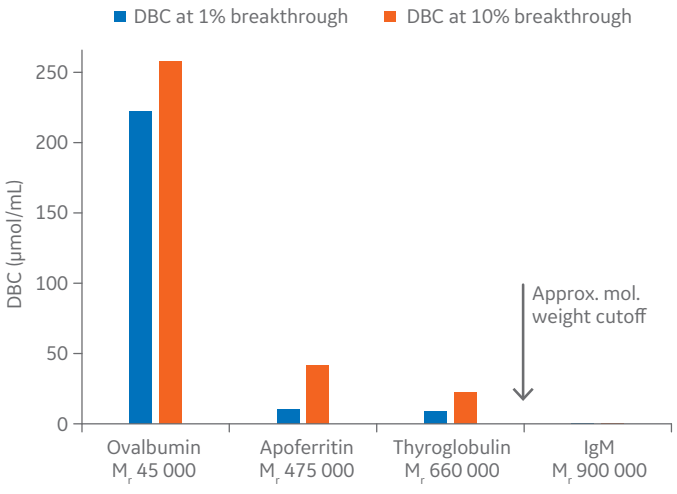

Fig 5. DBC at 1% and 10% breakthrough for large proteins on Capto Core 700. Note that DBC was measured in µmol/mL.

Table 2. Characteristics of the virus samples and operating condition

	Retroviridae	Flaviviridae	Picornaviridae
Size	100 nm	50 nm	30 nm
Type	Enveloped, RNA	Enveloped, RNA	Naked, RNA
Sample tested	Clarified harvest	Clarified harvest	Purified virus suspension
Columns	HiScreen 4.7 mL (0.77 cm i.d, 10 cm bed height)		
Sample and load	Clarified harvest, 10 CV	Clarified harvest, 20 CV	Purified virus, 6 CV
Equilibration/wash	50 mM Tris, pH 7.3 + 50 mM NaCl	50 mM Tris, pH 7.5 + 150 mM NaCl	35 mM phosphate buffer, pH 7
Flow velocity during loading	150 cm/h (Capto Core 400) 300 cm/h (Capto Core 700)	150 cm/h (Capto Core 400) 400 cm/h (Capto Core 700)	150, 325, and 500 cm/h (Capto Core 400 and Capto Core 700)
Elution	50 mM Tris, pH 7.3 + 1.2 M NaCl	50 mM Tris, pH 7.5 + 1 M NaCl	35 mM Phosphate buffer, pH 7
CIP (cleaning in place)	1 N NaOH, 30% isopropanol		
System	ÄKTA™ pure		

Case study on virus purification

The purification performance of Capto Core 400 and Capto Core 700 was investigated using viruses from three different sizes and families (Table 2). Virus samples were loaded on chromatography columns packed with either Capto Core 400 or Capto Core 700. Columns were operated in flow-through mode for impurity removal. For the retrovirus and flavivirus samples, a lower flow velocity was selected for the purification step with Capto Core 400 resin to facilitate observation of diffusion of virus into the beads.

As shown from the results in Figure 6, the larger retrovirus and flavivirus particles pass in the flowthrough from both Capto Core 400 and Capto Core 700, while the larger part of the smaller picornavirus particles are trapped in the bead core of Capto Core 700. Figure 7 shows recovery of picornavirus from both Capto Core 400 and Capto Core 700 at three different flow velocities, to demonstrate impact of selected flow on resin performance. For maximal performance, flow velocity should be optimized with regard to recovery and purity of the target product.

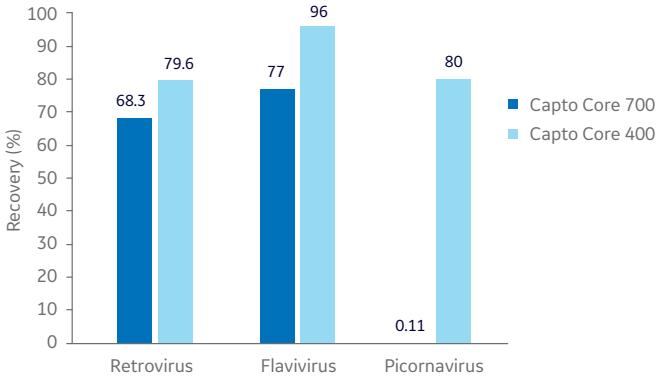

Fig 6. ELISA results from study with Capto Core 400 and Capto Core 700 on flavivirus, retrovirus, and picornavirus.

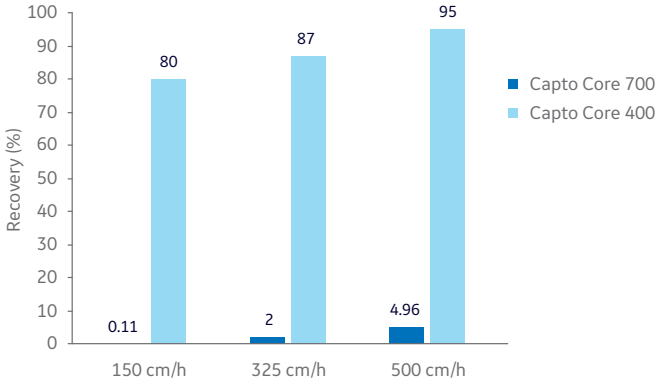

Fig 7. ELISA results from study with Capto Core 400 and Capto Core 700 on picornavirus at three different flow velocities: 150, 325, and 500 cm/h.

Cleaning-in-place and sanitization

Regular CIP is necessary to remove captured impurities and allow re-use of Capto Core 400 and Capto Core 700 with maintained capacity. Use of 1 M NaOH in 27% 1-propanol is recommended for effective CIP and sanitization of the resin after every cycle. Due to the strong binding of a wide range of impurities to the ligand, an organic solvent will be needed for CIP with most samples. However, this will be sample-dependent and it might 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 lifetime study of Capto Core 700 resin, the DBC of pure ovalbumin remained stable over the tested 45 cycles (Fig 8). 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 resin over many purification cycles, which can improve overall process economy. Recovery and purity of the vaccine remained high throughout the test (data not shown).

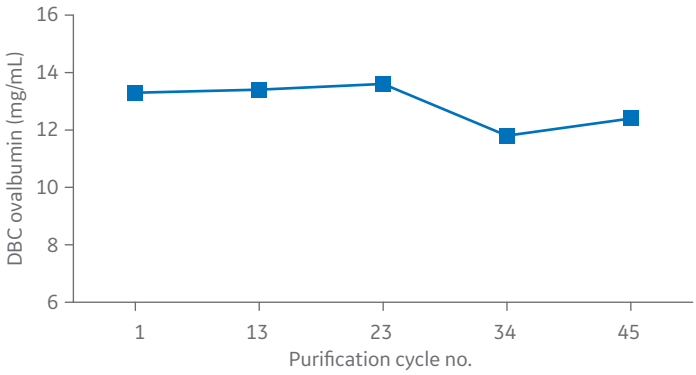

Fig 8. DBC of Capto Core 700 for ovalbumin measured over 45 purification cycles with 1 M NaOH in 27% 1-propanol as CIP agent. CIP protocol after every eleventh cycle was 5 CV CIP agent, 30 min pause, then 5 CV CIP agent. Fouling of the resin between DBC measurements was performed with MDCK cell lysate.

Small-scale formats for fast process development

Capto Core 400 and Capto Core 700 are 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 systems, HiTrap and HiScreen columns are convenient to use when developing an efficient and robust separation method. Further development and optimization using HiScale columns permits straightforward scale-up (for details of packing laboratory-scale columns, see the appropriate instructions).

Scale-up to production scale

Capto Core 400 and Capto Core 700 are available as bulk resins in a range of pack sizes from laboratory to production-scale, from 25 mL to 5 L. A wide range of process-scale columns for packing of Capto Core 400 and Capto Core 700 is also available (Table 3).

Scale-up is typically performed by keeping bed height and flow velocity constant, while increasing column bed diameter and flow rate. However, as conditions are often optimized in small column volumes, parameters such as DBC 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.

Capto Core 400 and Capto Core 700 are BioProcess™ resins with support for large-scale manufacture of biopharmaceuticals. This support includes validated manufacturing methods, secure long-term resin supply, and regulatory support files (RSF) to assist process validation and submissions to regulatory authorities.

Table 3. Recommended laboratory- and process-scale empty columns for packing of Capto Core 400 and Capto Core 700

Column family	Inner diameter (mm)	Max. bed height (cm)
Laboratory scale*		
HiScale™	16, 26, 50	35
Production scale†		
AxiChrom™‡	50–200	40
AxiChrom‡	300–1000	40
BPG§	100–300	40

* Visit gelifesciences.com/tricorn and gelifesciences.com/hiscale for the full range of HiTrap and HiScale columns.

† For other process-scale columns, please contact GE Healthcare or visit gelifesciences.com/bioprocess.

‡ Intelligent packing method for MabSelect SuRe resin can be used for Capto Core 700, whereas the intelligent packing method for Capto Q can be used for Capto Core 400. Visit gelifesciences.com/axichrom for details.

§ The pressure rating of BPG 450 is too low to use with Capto Core 400 or Capto Core 700.

Ordering information

Product	Quantity	Product code
Capto Core 400	25 mL	17372401
	100 mL	17372402
	1 L	17372403
	5 L	17372404
	60 L	17372460
Capto Core 700	25 mL	17548101
	100 mL	17548102
	1 L	17548103
	5 L	17548104
	60 L	17548160
Prepacked columns		
HiTrap Capto Core 400	5 × 1 mL	17372411
HiScreen Capto Core 400	1 × 4.7 mL	17372410
HiTrap Capto Core 700	5 × 1 mL	17548151
HiScreen Capto Core 700	1 × 4.7 mL	17548115

Related literature	Product code
Purification of influenza A/H1N1 using multimodal Capto Core 700, Application note	29000334
HiScreen prepacked columns, Data file	28930581
AxiChrom columns, Data file	28929041
BPG columns, Data file	18111523
Prepacked chromatography columns for ÄKTA systems, Selection guide	28931778

gelifesciences.com/captocore

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