Purification of influenza A/H1N1 using Capto Core 700

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GE Healthcare
Life Sciences

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Purification of influenza A/H1N1 using Capto™ Core 700

Capto Core 700 is a chromatography medium (resin) optimized for purification of viruses and other large biomolecules. The medium is designed to be used in flowthrough mode for large targets (> M_r 700 000) while scavenging smaller contaminants. Capto Core 700 is built on the core bead technology which allows for dual functionality combining size separation with binding chromatography. The size separating mode of operation could be compared to gel filtration (size exclusion chromatography), which is a common approach for polishing steps in vaccine processes. Here, the performance of Capto Core 700 in three different processes for the purification of influenza H1N1 virus from infected mammalian cells was evaluated and compared. This study shows that while offering the same purity as gel filtration, Capto Core 700 enables significant improvements in productivity and process economy.

Introduction

Influenza vaccine has historically and is today primarily produced in embryonated chicken eggs. However, to meet the needs for pandemic preparedness and scalability of vaccine productions, cell-based processes are being developed and implemented to a greater extent in the industry. The methods for purification processes have typically involved a combination of sucrose density gradient ultracentrifugation, ultrafiltration/diafiltration (UF/DF) with hollow-fiber membranes, and chromatography using affinity-, ion exchange-, and/or gel filtration (GF) media. In these processes, both sucrose density gradient ultracentrifugation and GF have limitations in, for example, scalability and productivity. In GF, the low productivity relates to low flow rates and limited sample loads.

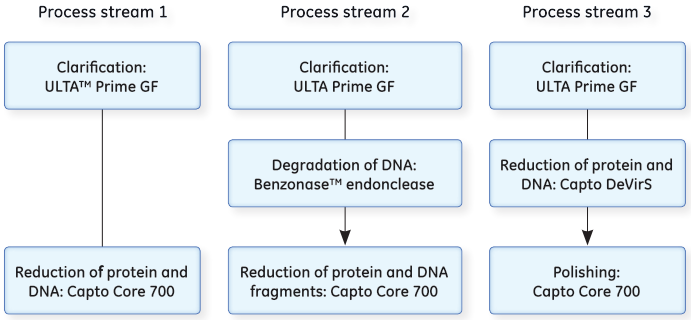


Fig 1. Three alternative process streams for the purification of influenza A/H1N1 were evaluated in this study.

The Capto Core 700 medium has a core bead design and consists of an inactive shell and a ligand-activated core. Small contaminant molecules enter into the beads where they are captured. Viruses and other large entities with a molecular mass (M_r) greater than approximately 700 000 (700 kDa) are excluded and are collected in the chromatography flowthrough. The octylamine ligands in the core of the bead are multimodal, being both hydrophobic and positively charged. These internalized ligands bind various contaminants strongly over a wide range of pH and salt concentrations.

Using Capto Core 700 allows higher flow rates (1) and significantly higher sample loads than traditional GF (typically several column volumes compared with 0.1 to 0.3 column volumes in GF).

The aim of this work was to evaluate Capto Core 700 chromatography medium as an alternative to current chromatography technologies used in vaccine processes. An experiment to determine the binding capacity and window of operation with Capto Core 700 for Madin-Darby canine kidney (MDCK) cell protein was performed using PreDictor™ 96-well plates for high-throughput process development. The approach taken for designing a purification process for flu vaccine is also described (Fig 1).

Finally, the process economy impact of Capto Core 700 compared with Sepharose™ 4 Fast Flow, a GF medium often used in the vaccine manufacturing industry today, was also investigated.

Materials and methods

Cell culture and infection

MDCK cells (inoculation concentration of 500 000 cells/mL) were grown on Cytodex™ 3 microcarriers for 48 h in an Applikon™ Bioreactor (Applikon Technology). The final cell density was approximately 2 500 000 cells/mL at which point cells were infected with influenza A/Solomon Islands/3/2006 (H1N1) and harvested at 72 h post infection.

Preliminary studies

Investigation of operating window (binding study) for Capto Core 700

The binding capacity of Capto Core 700 for host cell protein (HCP) from MDCK cell lysate was evaluated in buffers containing sodium phosphate and Tris, 150–1000 mM NaCl, pH 6.5–8.0. PreDictor 96-well plates were filled with 10 µL of Capto Core 700 for the binding study. Clarified MDCK cell lysate (200 µL) was applied to the wells of the plate and incubated in the various equilibration buffers for 60 min. After incubation, unbound sample was removed by centrifugation and the medium was washed with buffer. Collected fractions were analyzed for total protein using the Bradford protein assay.

Benchmarking of purification performance: Capto Core 700 vs Sepharose 4 Fast Flow

The performance of Capto Core 700 was compared to that of Sepharose 4 Fast Flow. Capto Core 700 was packed in Tricorn™ 5/50 column (column volume [CV], 1 mL) and 10 CV of clarified and concentrated virus material was loaded. Sepharose 4 Fast Flow was packed in Tricorn 10/600 (CV, 47 mL) and 0.1 CV of virus feed was loaded. Recovery of virus as measured by the quantitation of hemagglutinin (HA) and reduction of HCP was compared between the two media.

Process development

Clarification

Clarification of harvested cells was achieved by microfiltration (MF) using ULTA Prime GF normal-flow filter capsules. A 2.0 µm (4" membrane, 0.10 m^2 effective filtration area) rating was used initially followed by a 0.6 µm (4" membrane, 0.11 m^2 effective filtration area) rating. The ULTA Prime GF filters were washed with buffer (20 mM sodium phosphate, 150 mM NaCl, 0.05% sodium azide, pH 7.2) before use.

Degradation of DNA

Benzonase endonuclease treatment was applied for the removal of DNA in process stream 2 (Fig 1). Degradation of DNA using Benzonase results in small oligonucleotide fragments that enter through the inactive layer and into the core where they are captured by the internalized ligands. Benzonase was applied after MF and before column purification with Capto Core 700 in process stream 2 (Fig 1).

Capto DeVirS: binding/elution of influenza virus

Capto DeVirS is a chromatography medium designed for the capture and intermediate purification of viruses. The Capto DeVirS ligand is dextran sulfate, which allows for pseudo affinity for several virus types including influenza and was therefore selected for virus capture in process stream 3.

Chromatography runs were performed in a HiScale™ 50/20 column packed with 202 mL of Capto DeVirS. ÄKTAexplorer™ 100 chromatography system was used for chromatography runs on Capto DeVirS.

Capto Core 700: polishing step

In process stream 1–3, chromatography runs were performed in an XK 16/20 column packed with 25 mL of Capto Core 700. ÄKTAexplorer 10 chromatography system was used for chromatography runs on Capto Core 700.

Analytical methods

Virus quantitation

In this study, Biacore™ T200 system and Sensor Chip CM5 were used to measure HA content according to a previously described method (2). The potency of influenza vaccines are mainly determined by quantitation of HA using the single radial immunodiffusion (SRID) assay. This method, although approved by both FDA and EMEA, is labor-intensive and suffers from low precision and sensitivity. Biacore biosensor assays offer greater precision in the quantitation of influenza HA and faster analysis than SRID in vaccine development and manufacturing.

HCP quantitation

HCP is usually quantitated as total protein with, for example, the Bradford protein assay. This method is not sensitive or specific enough to detect levels below the regulatory critical limits. Therefore, Biacore biosensor assay was used for the quantitation of HCP using the same instrumentation as mentioned above. In house produced polyclonal anti-MDCK HCP antibodies were immobilized on the Biacore chip for MDCK HCP protein binding. The HCP standard was set using Bradford to estimate protein content.

As reference, the total protein content (including HA) was also measured with the Bradford protein assay. The assay was performed according to the manufacturer's recommended methods (3).

Determination of infectious particles

The median Tissue Culture Infectious Dose ($TCID_{50}$) assay measures dilution that generates cytopathic effect in 50% of the cell culture and is an infectivity method that is one of the most commonly used methods for detection of the infective virus. The $TCID_{50}$ assay is simple to perform and requires no specific instrumentation for result interpretation. The outcome is either presented as a log10 titer ($10^{x.x}$ $TCID_{50}$ units/mL) or a dilution ($10^{-x.x}$/mL).

Results and discussion

Investigation of operating window (binding study) for Capto Core 700

The performance of Capto Core 700 was robust in both 20 mM sodium phosphate and Tris buffer containing up to 1 M NaCl and in pH from 6.5 to 7.5 for sodium phosphate and pH 7.5 to 8.0 for Tris, respectively (Fig 2). In general, the low NaCl concentration of 150 mM resulted in higher binding capacity while lower pH yielded higher binding capacity for the MDCK cell lysate proteins.

The robust performance of the multimodal octylamine ligand in a relatively wide range of NaCl concentration and pH gives Capto Core 700 a wide window of operation. This reduces the need for optimization such as buffer exchange or dilution between steps, even with different feed materials when working with Capto Core 700.

Amount of medium:	10 µL of Capto Core 700 in PreDictor 96-well filter plate
Sample:	Clarified MDCK cell lysate in different equilibration buffers
Sample load:	200 µL of cell lysate (60 min incubation)
Equilibration buffers:	20 mM sodium phosphate, 150–1000 mM NaCl, pH 6.5–7.5 20 mM Tris, 150–1000 mM NaCl, pH 7.5–8.0
Equilibration:	3 × 200 µL of equilibration buffer
Wash:	200 µL of equilibration buffer

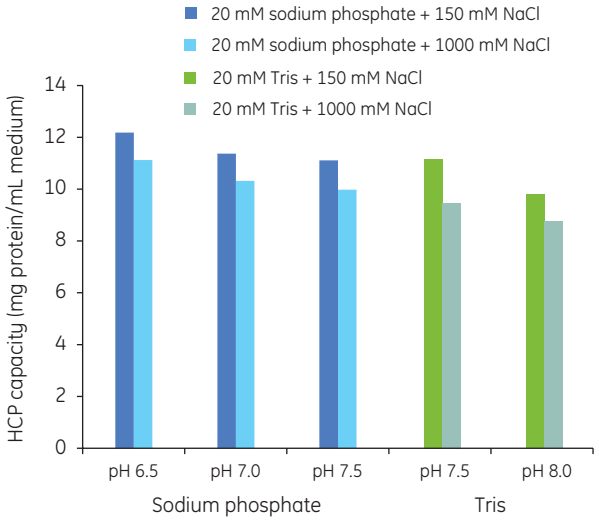


Fig 2. Protein binding capacity of Capto Core 700 in sodium phosphate and Tris buffers with different NaCl concentrations and a range of pH. The performance of Capto Core 700 is robust in terms of buffer component and NaCl concentration. At higher pH, the ligand gradually loses charge, which consequently decreases binding capacity.

Benchmarking of purification performance: Capto Core 700 vs Sepharose 4 Fast Flow

The purification performance of Capto Core 700 was compared to that of Sepharose 4 Fast Flow, which is a chromatography medium typically used for GF purification of a range of viruses in vaccine processes. Both chromatography methods provided a similar yield of virus HA and reduction of HCP (Table 1).

Table 1. Recovery of HA and reduction of HCP on Capto Core 700 compared with Sepharose 4 Fast Flow*, a standard medium used for GF in vaccine processes

Media	Recovery HA (%)	Recovery normalized (%)	Reduction HCP (%)	Reduction normalized (%)
Capto Core 700†	85	99	32	104
Sepharose 4 Fast Flow†	86	100	31	100

**Chromatography conditions:*

Columns:	Tricorn 5/50 (CV, 1 mL) packed with Capto Core 700; Tricorn 10/600 (CV, 47 mL) packed with Sepharose 4 Fast Flow
Sample:	Clarified virus material
Sample load:	Capto Core 700, 10 CV; Sepharose 4 Fast Flow, 0.1 CV
Buffer:	20 mM Tris, 150 mM NaCl, pH 7.5

†Capto Core 700 runs were performed in triplicate, Sepharose 4 Fast Flow runs in duplicate

Process stream 1: MF and single-step purification using Capto Core 700

Figure 3 shows the broad flowthrough peak containing the virus and residual DNA/HCP obtained on Capto Core 700.

Column:	XK 16/20, packed with 25 mL of Capto Core 700
Sample and load:	Clarified virus material, 20 CV
Equilibration/wash:	20 mM sodium phosphate, 500 mM NaCl, 0.05% sodium azide, pH 7.2
Flow velocity during loading:	250 cm/h (3 min residence time)
CIP:	1 M NaOH, 27% 1-propanol (total contact time 60 min)
System:	ÄKTAexplorer 10

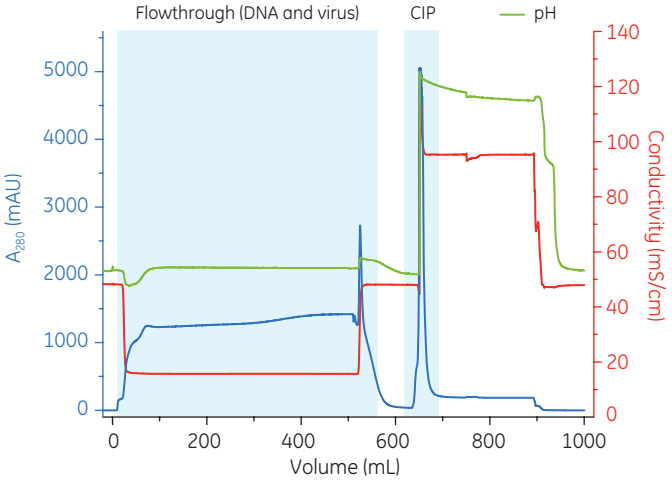


Fig 3. Purification of influenza A/H1N1 on Capto Core 700 following MF. Labels on the chromatogram indicate gDNA and virus in the flowthrough fraction and the peak of impurities obtained during CIP.

Results from the combination of MF with ULTA Prime GF and single-step purification with Capto Core 700 are shown in Table 2. Virus yield (measured in HA content) in fractions collected in the flowthrough was excellent and protein removal significant, while it was clear that a greater reduction of DNA would be required. Full-length genomic DNA (gDNA) will not enter the beads and hence not bind to the ligands. An expected result would therefore be low reduction of DNA in the column flowthrough.

Table 2. Virus HA yield, $TCID_{50}$, DNA, total protein, and HCP/HA quotient in a purification scheme incorporating MF and single-step chromatography using Capto Core 700

Step	HA yield (%)	Titer ($TCID_{50}$/mL)	DNA/HA (ng/µg)	Total protein/HA (µg/µg)	HCP/HA (µg/µg)
Microfiltration: ULTA Prime GF	64	9.7	2672	19.8	31.6
Chromatography: Capto Core 700	105	9.3	1459	4.2	11.2

Process stream 2: MF, DNA removal step, and single-step purification using Capto Core 700

In an attempt to decrease DNA concentration obtained in process 1 and potentially also decrease DNA associated HCPs, a DNA degrading step using Benzonase treatment was added to the workflow.

Results from this experiment are shown in Table 3. As expected, virus yield in flowthrough fractions in the Capto Core 700 chromatography step was excellent. DNA/HA content was effectively reduced by Benzonase treatment. Protein levels remained similar as process stream 1, that is a 3–5 fold reduction. Benzonase was also removed in the Capto Core 700 step as it entered the core and was bound. The $TCID_{50}$ titer remained high meaning that the infectivity of the virus was not affected either by filtration, Benzonase treatment, or chromatography.

Table 3. Virus HA yield, $TCID_{50}$, DNA, total protein, and HCP/HA quotient in a purification scheme incorporating MF, DNA reduction step using Benzonase endonuclease, and final chromatography step using Capto Core 700

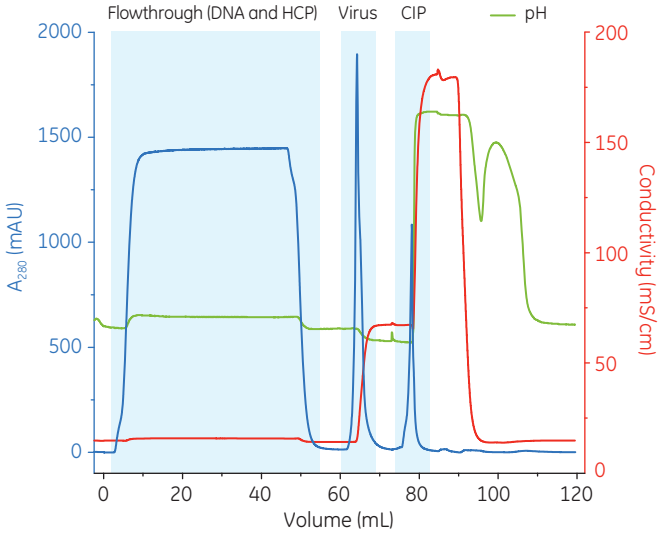
Step	HA yield (%)	Titer ($TCID_{50}$/mL)	DNA/HA (ng/µg)	Total protein/HA (µg/µg)	HCP/HA (µg/µg)
Microfiltration: ULTA Prime GF	64	9.7	2672	22.0	32.3
Benzonase endonuclease treatment			7.0	22.9	30.3
Chromatography: Capto Core 700	105	9.3	7.0	3.8	13.1

Process stream 3: MF and two-step chromatography using Capto DeVirS and Capto Core 700

Processes 1 and 2 both gave high yield of HA while insufficient DNA and HCP reduction was observed. Thus, it was considered desirable to further reduce both DNA and HCP content. For comparison to Process 1 and 2, a two-step chromatography process using Capto DeVirS for capture and Capto Core 700 for final purification of the virus was evaluated.

A)

Column: HiScale 50/20 column packed with 202 mL Capto DeVirS
Sample and sample load: Clarified virus material, 5 CV
Binding and wash buffer: 20 mM sodium phosphate, 150 mM NaCl, 0.05% sodium azide, pH 7.2
Elution buffer: 20 mM sodium phosphate, 750 mM NaCl, 0.05% sodium azide, pH 7.2 (giving ~500 mM NaCl in elution pool)
Flow velocity during loading: 60 cm/h
CIP: 1 M NaOH
System: ÄKTAexplorer 100



B)

Column: XK 16/20 packed with 25 mL of Capto Core 700
Sample: Eluted fractions from Capto DeVirS step
Sample load: 8 CV of Capto DeVirS eluate, 250 cm/h (3 min residence time)
Equilibration/ wash: 20 mM sodium phosphate, 500 mM NaCl, 0.05% sodium azide, pH 7.2
Flow velocity during loading: 250 cm/h
CIP: 1 M NaOH, 27% 1-propanol (total contact time 60 min)
System: ÄKTAexplorer 10S

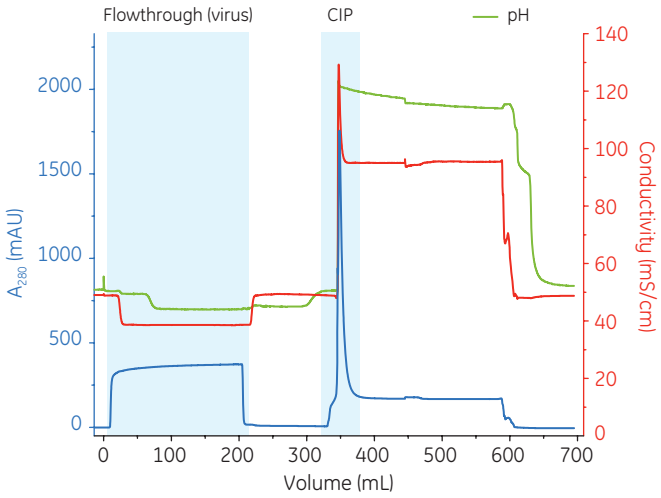


Fig 4. Two-step purification of influenza A/H1N1 virus after MF. Capture of the virus was achieved using A) Capto DeVirs and final purification using B) Capto Core 700. Labels on the chromatograms indicate the elution of virus, DNA, and HCP.

Chromatograms showing both chromatography steps after MF are shown in Figure 4. Capto DeVirS was effective for the capture of the virus (Fig 4A) and eluted fractions from this step were applied to the XK column packed with Capto Core 700 for final purification (Fig 4B). On account of the robust binding performance of Capto Core 700, equilibration of Capto Core 700 was achieved using the buffer used for elution in the Capto DeVirS step. The need for buffer exchange or dilution between steps was thereby eliminated, contributing to speeding up the chromatography process. This demonstrates the advantages from the wide window of operation that is enabled by Capto Core 700.

Table 4 shows the results in terms of HA recovery, $TCID_{50}$, DNA- and protein removal at each step of the process. In this case, good yield of virus HA as well as significant removal of HCP and DNA were observed. DNA was reduced 2.8 log and proteins 5–7 fold by Capto DeVirS. Capto Core 700 further reduced protein levels by 3–5 fold. The infectivity of the virus was retained throughout the process, as shown by the titer measured with $TCID_{50}$.

Table 4. Virus HA yield, $TCID_{50}$, DNA, total protein, and HCP/HA quotient in a purification scheme incorporating MF and two-step purification using Capto DeVirS and Capto Core 700

Step	HA yield (%)	Titer ($TCID_{50}$/mL)	DNA/HA (ng/µg)	Total protein/HA (µg/µg)	HCP/HA (µg/µg)
Microfiltration: ULTA Prime GF	64	9.7	2672	22.0	32.3
Chromatography first-step: Capto DeVirS	94		4.0	3.1	6.1
Chromatography second step: Capto Core 700	94	9.3	5.0	1.1	1.1

Cost analysis – comparison of Capto Core 700 approach vs GF

An economic evaluation of Capto Core 700 as an alternative to GF for polishing step was performed based on the example above. The comparison was made under the following conditions:

1. Calculation was based on a 1000 L fermentation volume
2. Same recoveries and purities of virus were assumed for Capto Core 700 and GF, as shown by previous experiments
3. Process conditions for Capto Core 700 step was the same as process 3 above, while the conditions for GF were chosen based on experience and presented as two different examples
4. Costs were calculated per batch and costs expected to be same for both techniques were excluded from the analysis
5. Labor costs included both salary and overhead costs relating to facility buildings etc. This was set to 800 USD/h but will vary from case to case

The comparison of Capto Core 700 and GF is shown in Table 5. The higher load volumes achieved with Capto Core 700 compared to GF resulted in smaller volumes of chromatography medium needed to process the same amount of material within a shorter timeframe (GF alternative 1). As a consequence, the labor costs, buffer costs, and costs for Capto Core 700 medium would be significantly lower than for traditional GF. The resulting total cost per batch for this step could be lowered by almost two-fold. Other benefits, not included in this simplified example, are that smaller buffer tanks and smaller columns could be used leading to reduced footprint, and the need for larger hardware investments is reduced.

Another alternative could be to use a smaller column for GF and compensate the low load volume by a larger number of cycles per batch (GF alternative 2, Table 5). The medium cost per batch would still be the same for GF as this calculation takes into account the complete lifetime of the medium. However, labor cost would increase further as a consequence of the longer process time, giving a three-fold higher total cost than for Capto Core 700. On the other hand, the benefits with reduced footprint and minimized hardware investments would in this case be the same as for the Capto Core 700 alternative.

Table 5. Key process parameters and cost comparison between an approach using Capto Core 700 and traditional GF

	Capto Core 700	GF alternative 1	GF alternative 2
Column volume (L)	25	200	50
Load volume (CV)	8	0.2	0.2
Number of cycles per batch	1	5	20
Medium cost (USD)	1850	6000	6000
Buffer cost (USD)	1000	2000	2000
Labor cost (USD)	4165	5100	12 750
Total cost (USD)	**7015**	**13 100**	**20 750**

Conclusions

Capto Core 700 allows a wide window of operation for pH and NaCl concentration, securing robust purification and often simplified process design in vaccine manufacturing. The combination of MF, capture of virus with Capto DeVirS, and final purification on Capto Core 700 showed excellent results in terms of virus purity and reduction of DNA and HCP. Using Capto Core 700 in this approach had the advantage of allowing direct transfer of eluted fractions containing target virus without the need for buffer exchange or dilution, which enables faster and simpler processing. Capto Core 700 combines scalability and high productivity with improved process economy, all common drawbacks of GF. Capto Core 700 enables chromatography processes with characteristics that the vaccine industry is looking for—scalability, high productivity, good process economy, and short start-up times.

References

1. Data file: Capto Core 700. GE Healthcare, 28-9983-07, Edition AA (2012).
2. Estmer Nilsson, C. *et al.* A novel assay for influenza virus quantification using surface plasmon resonance. *Vaccine* **28(3)**, 759–756 (2010).
3. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* ***72,*** 248-254 (1976).

Ordering information

Product	Code number
Cytodex 3, 2.5 kg	17-0485-25
ULTA Prime GF, 2.0 µm	28-9084-21
ULTA Prime GF, 0.6 µm	28-9083-33
Capto DeVirS, 1 L	17-5466-03
Capto Core 700, 1 L	17-5481-04
Tricorn 5/50 column	28-4064-09
Tricorn 10/600 column	28-4064-19
XK 16/20 column	28-9889-37
HiScale 50/20 column	28-9644-44
Sepharose 4 Fast Flow	17-0149-01

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