HiScreen prepacked columns

PROCESS DEVELOPMENT

HiScreen™ columns are part of the process development platform available from Cytiva (Fig 1). The columns are prepacked with a range of BioProcess™ chromatography resins and are designed for method optimization and parameter screening (Fig 2). HiScreen columns have small bed volumes (4.7 mL), reducing the cost of sample and buffer consumption. The resins used in HiScreen columns are also available in other prepacked formats and as bulk packs, for all scales of work from development and pilot studies to routine production.

Key benefits:

- Prepacked with different BioProcess resins for convenient process development
- Excellent for method optimization and parameter screening due to the 10 cm bed height
- Easily connected in series to achieve 20 cm bed height
- Small bed volume for fast results and minimal sample/buffer consumption
- Reproducible results, scalable to BioProcess columns packed with the same resins using the same linear flow velocity

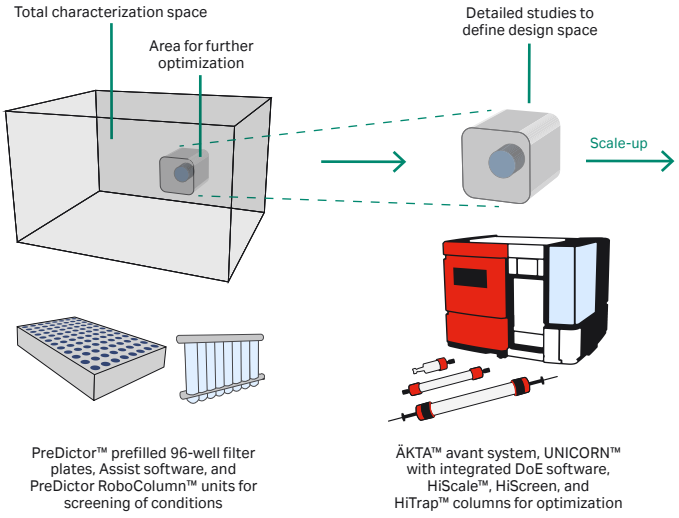


Fig 1. Conceptual visualization of a workflow for process development.

Fig 2. HiScreen columns prepacked with BioProcess resins for convenient and reproducible method optimization in process development work.

Use in process development

Prepacked HiScreen columns have many attributes that make them suitable for general process development work. Eight different examples of their use are shown in the section *Examples of HiScreen column* use later in this document. A number of selected aspects are highlighted below.

Robustness

All columns are packed with robust BioProcess chromatography resins that allow repeated use with reproducible results. For example, the stability of HiScreen MabSelect Xtra™ was tested by measuring its dynamic binding capacity (DBC) after 10 runs on the same column. The results described in the first example in *Examples of HiScreen column use* illustrate that this key parameter remains stable throughout the study, demonstrating the robustness of the HiScreen format.

Method optimization and parameter screening

When developing a new process, parameters such as selectivity, capacity, and binding and elution conditions have to be screened at smaller scale to save time and money. Initially, high-throughput parallel screening of process conditions is easily performed using 96-well PreDictor plates, which are prefilled with BioProcess resin.

Once these conditions are identified, equivalent prepacked HiScreen columns are conveniently used for fine-tuning and verification. Screening of different hydrophobic interaction chromatography (HIC) resins in HiScreen columns is described in the eigth example in *Examples of HiScreen column use*.

Scale-up

Scale-up of a chromatography step can be as straightforward as increasing column diameter to accommodate a larger feed volume while keeping the bed height and the linear flow velocity constant. The 10 cm bed height of HiScreen columns gives sufficient residence time to serve as the basis for linear process scale-up. If necessary, however, two columns can be connected in series with a union (see *Accessories*) to give a bed height of 20 cm. Successful scale-up from HiScreen to larger column formats is described in the third and sixth examples in *Examples of HiScreen column use*.

Cover major chromatography techniques

The range of HiScreen columns covers four main separation techniques — affinity, ion exchange, multimodal (mixed mode), and hydrophobic interaction chromatography — commonly used when developing purification processes for biomolecules intended for large-scale production.

Column characteristics

HiScreen columns are made of biocompatible polypropylene that does not interact with biomolecules. The columns can be run on peristaltic pumps or chromatography systems, such as the ÄKTA systems. The columns are delivered with stoppers at the inlet and outlet. Table 1 lists the characteristics of HiScreen columns.

Table 1. Characteristics of HiScreen columns

Column volume	4.7 mL
Column dimensions	0.77 × 10 cm
Column hardware pressure limit*	8 bar (0.8 MPa, 117 psi)

* Note: The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography resin and the column tubing used. Note also that HiScreen columns cannot be opened or repacked.

Chromatography resin characteristics

Affinity chromatography

Capto L

HiScreen Capto™ L is prepacked with Capto L resin for capturing antibody fragments such as Fab, scFv, and dAb (Table 2). The protein L ligand is produced in *E. coli*. Fermentation and subsequent purification are performed in the absence of animal products. Protein L provides highly specific binding to the variable region of the kappa light chain of antibodies.

MabSelect

HiScreen MabSelect™, MabSelect Xtra, MabSelect SuRe™, MabSelect SuRe LX, and MabSelect PrismA are columns prepacked with MabSelect protein A-derived resins for the capture of mAbs. HiScreen MabSelect resin characteristics are summarized in Table 2.

MabSelect resin is designed for fast purification of mAbs from large sample volumes. The resin is compatible with high flow rates and high pressure when scaling up.

MabSelect Xtra resin is designed for high binding capacity, allowing binding from samples with high mAb expression levels.

MabSelect SuRe resin is designed with an alkaline-tolerant, protein A-derived ligand, allowing the use of 0.1 to 0.5 M sodium hydroxide for cleaning-in-place (CIP).

MabSelect SuRe LX resin has high dynamic binding capacity for high-titer cultures of antibodies. MabSelect SuRe LX is designed with the same alkaline-tolerant, protein A-derived ligand as used in MabSelect SuRe resin, which makes the MabSelect SuRe LX stable in sodium hydroxide concentrations as high as 0.5 M.

MabSelect PrismA is a next-generation protein A chromatography resin that offers significantly enhanced alkaline stability (up to 1.0 M NaOH) and binding capacity for improved process economy in mAb processing.

Ni Sepharose 6 Fast Flow

Purifying histidine-tagged (his-tagged) recombinant proteins by immobilized metal affinity chromatography (IMAC) continues to grow in popularity. Nickel (Ni^{2+}) is the most used metal ion in such IMAC purifications. HiScreen Ni FF is prepacked with Ni Sepharose™ 6 Fast Flow, consisting of 90 µm beads of highly cross-linked agarose to which a chelating ligand has been immobilized. The chelating ligand is immobilized to the Sepharose 6 Fast Flow matrix at a density such that charging it with Ni^{2+} ions ensures a high binding capacity for proteins. Furthermore, leakage of Ni^{2+} ions is minimized.

The resin is compatible with a wide range of additives commonly used in the purification of his-tagged proteins. HiScreen Ni FF characteristics are shown in Table 3.

IMAC Sepharose 6 Fast Flow

IMAC is a widely used separation method for purifying proteins and peptides that have an affinity for metal ions. In addition to his-tagged proteins, the method continues to grow in popularity also for untagged or native proteins.

HiScreen IMAC FF is prepacked with IMAC Sepharose 6 Fast Flow, the uncharged version of Ni Sepharose 6 Fast Flow, which consists of 90 µm, highly cross-linked agarose beads with a covalently immobilized chelating group. The resin can easily be charged with Ni^{2+}, Co^{2+}, Zn^{2+}, Cu^{2+}, Fe^{3+} or other metal ions. HiScreen IMAC FF characteristics are shown in Table 3.

Table 2. Characteristics of Capto L and different MabSelect resins packed in HiScreen columns

	Capto L	MabSelect	MabSelect Xtra	MabSelect SuRe, MabSelect SuRe LX	MabSelect PrismA
Matrix	Highly cross-linked agarose, spherical				
Ligand	Recombinant protein L (*E. coli*)	Recombinant protein A (*E. coli*)	Recombinant protein A (*E. coli*)	Alkali-tolerant protein A-derived (*E. coli*)	Alkaline-stabilized protein A-derived (*E. coli*)
Particle size, d_{50v}*	85 µm	85 µm	75 µm	85 µm	~ 60 µm
Dynamic binding capacity (Q_{B10})/mL resin	~ 25 mg Fab†	~ 30 mg human IgG‡	~ 40 mg human IgG§	~ 60 mg human IgG/mL MabSelect SuRe LX¶ ~ 30 mg human IgG/mL MabSelect SuRe‡	~ 80 mg human IgG/mL¶ resin at 6 min residence time ~ 65 mg human IgG/mL resin at 4 min residence time
Recommended operating flow velocity**	100 to 300 cm/h	75 to 300 cm/h	75 to 300 cm/h	75 to 300 cm/h (MabSelect SuRe LX) 75 to 300 cm/h (MabSelect SuRe)	75 to 300 cm/h
Maximum operating flow velocity**	500 cm/h	500 cm/h	300 cm/h	500 cm/h	500 cm/h‡‡
pH stability††					
Operational	2 to 10	3 to 10	3 to 10	3 to 12	3 to 12
Cleaning-in-place (CIP)	N/A (CIP performed in 15 mM NaOH)	2 to 12	2 to 12	2 to 13.7	2 to 14
Storage	2°C to 8°C in 20% ethanol				

* d_{50v} is the median particle size of the cumulative volume distribution

† Determined at 10% breakthrough by frontal analysis (displacement chromatography) at a mobile phase velocity of 100 cm/h in a column with a bed height of 10 cm, i.e., residence time is 6.0 min. Residence time is equal to bed height (cm) divided by nominal flow velocity (cm/h) during sample loading. Nominal flow velocity is equal to volumetric flow rate (mL/h) divided by column cross-sectional area (cm^2).

‡ Determined at 10% breakthrough by frontal analysis at a mobile phase velocity of 500 cm/h in a column with a bed height of 20 cm, i.e., residence time is 2.4 min

§ Determined at 10% breakthrough by frontal analysis at a nominal flow velocity of 250 cm/h in a column with a bed height of 10 cm, i.e., residence time is 2.4 min

¶ Determined at 10% breakthrough by frontal analysis at a nominal flow velocity of 100 cm/h in a column with a bed height of 10 cm, i.e., residence time is 6 min

** Water at room temperature

†† pH below 3 is sometimes required to elute strongly bound antibody species. However, protein ligands can hydrolyze at low pH
Operational range: pH interval where the resin can be operated without significant change in function
Cleaning-in-place: pH stability where the resin can be subjected to cleaning- or sanitization-in-place without significant change in function

‡‡ The maximum flow rate in HiScreen PrismA. For MabSelect PrismA packed in other column formats, refer to MabSelect PrismA instructions, 29262586

Blue Sepharose 6 Fast Flow

HiScreen Blue FF is prepacked with the BioProcess chromatography resin Blue Sepharose 6 Fast Flow. This resin has Cibacron Blue 3G-A covalently attached to the Sepharose 6 Fast Flow matrix by the triazine coupling method. The blue dye binds many proteins, such as albumin, interferon, lipoproteins, and blood coagulation factors. The dye also binds several enzymes including kinases, dehydrogenases, and most enzymes requiring adenyl-containing cofactors, such as NAD^+. HiScreen Blue FF characteristics are shown in Table 3.

Capto Blue

HiScreen Capto Blue is prepacked with the base matrix Capto, to which Cibacron Blue 3G-A is covalently bound via a hydrophilic spacer immobilized with a stable amine bond. Capto Blue is highly chemically stable and has a more rigid agarose base matrix than Blue Sepharose 6 Fast Flow allowing the use of faster flow rates and larger sample volumes, leading to higher throughput and improved process economy. The application area is the same as for Blue Sepharose Fast Flow. HiScreen Capto Blue characteristics are shown in Table 3.

Table 3. Characteristics of Ni Sepharose 6 Fast Flow, IMAC Sepharose 6 Fast Flow, Capto Blue, and Blue Sepharose 6 Fast Flow packed in HiScreen columns

	Ni Sepharose 6 Fast Flow and IMAC Sepharose 6 Fast Flow	Capto Blue	Blue Sepharose 6 Fast Flow
Matrix		Cross-linked agarose, 6%, spherical	
Particle size, d_{50v}*	90 µm	75 µm	90 µm
Metal ion capacity/mL resin	Approx. 15 µmol Ni^{2+}	N/A	N/A
Ligand density	N/A	11 to 16 µmol Cibacron Blue 3G-A	Approx. 7 µmol Cibacron Blue 3G-A/mL of drained resin
Dynamic binding capacity (Q_{B10})/mL resin†	Approx. 40 mg $(his)_6$-tagged protein (Ni^{2+}-charged).† Untagged protein: approx. 25 mg (Cu^{2+}-charged), or approx. 15 mg (Zn^{2+}- or Ni^{2+}-charged)	≥ 18 mg HSA	≥ 18 mg HSA
Recommended operating flow velocity‡		30 to 300 cm/h	
Maximum operating flow velocity‡	450 cm/h	600 cm/h	410 cm/h
pH stability (without metal ion)§			
Operational	3 to 12	2 to 13	3 to 13
Cleaning-in-place (CIP)	2 to 14	2 to 13	4 to 12
Chemical stability	Without metal ion: 1 M NaOH, 70% acetic acid, tested for 12 h; 2% SDS, tested for 1 h. 30% 2-propanol, tested for 30 min.	40°C for 7 d in: 70% ethanol, 6 M guanidine hydrochloride, 8 M urea	40°C for 7 d in: 70% ethanol, 6 M guanidine hydrochloride, 8 M urea
Avoid in buffers	Chelating agents, e.g., EDTA, EGTA, and citrate	N/A	N/A
Storage	4°C to 30°C in 20% ethanol	2°C to 8°C in 0.1 M potassium phosphate and 20% ethanol	2°C to 8°C in 0.1 M potassium phosphate and 20% ethanol

* d_{50v} is the median particle size of the cumulative volume distribution

† Determined at 10% breakthrough by frontal analysis (displacement chromatography). Dynamic binding capacity conditions:

Samples: $(his)_6$-tagged proteins: Capacity data were obtained for a protein (M_r 28 000) bound from an *E. coli* extract, and a pure protein (M_r 43 000; applied at 1 mg/mL in binding buffer; capacity at 10% breakthrough).

Untagged protein: capacity determined at 10% breakthrough for human apo-transferrin applied at 1 mg/mL in binding buffer

Column volumes: 0.25 or 1 mL

Flow rates: 0.25 or 1 mL/min, respectively

Binding buffer: 20 mM sodium phosphate, 500 mM NaCl, 5 mM imidazole, (1 mM imidazole for untagged protein), pH 7.4

Elution buffer: 20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, (50 mM imidazole for untagged protein), pH 7.4

‡ Water at room temperature

§ Operational: pH interval where the resin can be operated without significant change in function. Cleaning-in-place: pH stability where the resin can be subjected to cleaning- or sanitization-in-place without significant change in function

Ion exchange chromatography

Capto

Capto ion exchangers have many uses in large-scale bioprocessing. Their matrix, based on a rigid, highly cross-linked agarose base matrix, offers outstanding pressure/flow properties in addition to an optimized pore structure. Capto resins thus combine high capacity with high flow rate and low backpressure to reduce process cycle times and increase productivity. The full range of Capto resins is available in HiScreen columns.

HiScreen Capto S, Capto Q, Capto DEAE, Capto MMC, and Capto adhere are excellent choices for capture and initial purification. Their characteristics are summarized in Table 4. HiScreen Capto Q ImpRes, HiScreen Capto SP ImpRes, and HiScreen Capto S ImpAct extend the range to cover high-resolution intermediate purification and polishing. Table 5 summarizes their main characteristics.

Capto S, Capto Q, and Capto DEAE are strong cation, strong anion, and weak anion exchange resins, respectively. The charged groups are linked to the highly cross-linked agarose base matrix modified with a dextran surface extender that further increases capacities and mass transfer properties. As strong ion exchangers, Capto S and Capto Q maintain charge and function over a wide pH range, whereas Capto DEAE, a weak ion exchanger, has a pH-dependent ion exchange capacity. The two anion exchangers, Capto Q and Capto DEAE, also differ in selectivity.

Capto Q ImpRes and Capto SP ImpRes are strong anion and strong cation exchange BioProcess resins, respectively. The resins are intended for efficient intermediate purification and polishing in robust purification processes. The combination of a highly cross-linked agarose base matrix and a small particle size adds high resolution to the well-established Capto platform.

Capto S ImpAct is a strong cation exchange chromatography resin for intermediate purification and polishing of a wide range of biomolecules, especially monoclonal antibodies. The high binding capacity and the highly cross-linked agarose base matrix in combination with the small particle size of Capto S ImpAct results in excellent pressure-flow properties as well as impressive resolution.

Table 4. Characteristics of Capto IEX resins packed in HiScreen columns

	Capto Q	Capto S	Capto DEAE
Matrix		Highly cross-linked agarose, spherical	
Particle size, d_{50v} *		90 µm	
Charged group	$-N^+(CH_3)_3$	$-SO_3^-$	$-N^+H(CH_2CH_3)_2$
Total ionic capacity (mmol/mL resin)	0.16 to 0.22 (Cl^-)	0.11 to 0.14 (Na^+)	0.29 to 0.35 (Cl^-)
Dynamic binding capacity (Q_{B10})/mL resin†	> 100 mg BSA	> 120 mg lysozyme	> 90 mg ovalbumin
Recommended operating flow velocity‡		150 to 300 cm/h	
Maximum operating flow velocity‡		600 cm/h	
pH stability§			
Operational	2 to 12	4 to 12	2 to 12
Cleaning-in-place (CIP)	2 to 14	3 to 14	2 to 14
Storage	4°C to 30°C in 20% ethanol	4°C to 30°C in 0.2 M sodium acetate and 20% ethanol	4°C to 30°C in 20% ethanol

* d_{50v} is the median particle size of the cumulative volume distribution

† Determined at 10% breakthrough by frontal analysis (displacement chromatography)

‡ Water at room temperature

§ Operational: pH interval where the resin can be operated without significant change in function
Cleaning-in-place: pH stability where the resin can be subjected to cleaning- or sanitization-in-place without significant change in function

Sepharose Fast Flow

HiScreen Q FF is prepacked with Q Sepharose Fast Flow, a strong anion exchanger based on 6% cross-linked agarose with a particle size of 90 µm. This resin has good flow properties and high loading capacities.

HiScreen DEAE FF is prepacked with DEAE Sepharose Fast Flow, a weak anion exchanger based on 6% cross-linked agarose with a particle size of 90 µm. This resin has good flow properties and high loading capacities.

HiScreen SP FF is prepacked with SP Sepharose Fast Flow. This strong cation exchange resin is based on 6% cross-linked agarose with a particle size of 90 µm. The resin has good flow properties and high loading capacities. Sepharose Fast Flow characteristics are shown in Table 6.

Sepharose High Performance

HiScreen Q HP is prepacked with Q Sepharose High Performance, which is a strong anion exchanger based on rigid, highly cross-linked, 6% agarose with a particle size of 34 µm. The smaller bead size will result in higher resolution and sharper peaks.

HiScreen SP HP is prepacked with SP Sepharose High Performance, a strong cation exchanger based on rigid, highly cross-linked 6% agarose with a particle size of 34 µm. The smaller bead size results in higher resolution and sharper peaks.

Sepharose High Performance characteristics are shown in Table 6.

Multimodal chromatography

Capto MMC has an innovative salt-tolerant ligand that allows binding of proteins at the conductivity of the feed material. The diversity of interactions with the target molecules offers alternative selectivity compared with traditional ion exchange resins, making Capto MMC a weak multimodal cation exchanger.

Capto adhere is a strong multimodal anion exchanger, offering alternative selectivity compared with traditional anion exchangers. Capto adhere is designed for post-protein A purification of mAbs. Removal of leached protein A, aggregates, host cell proteins, nucleic acids, and viruses from mAbs is performed in flowthrough mode to allow antibodies to pass directly through the column, while the impurities remain bound.

Capto Core 700 particles have a core activated with octylamine ligands and an inert outer layer without ligands. The octylamine ligand of Capto Core 700 is multimodal, being both hydrophobic and positively charged in order to interact strongly with impurities over a wide range of pH and salt concentrations. The outer layer prevents large targets from binding to the ligands, while smaller impurities can enter freely into the particles where they are captured. The molecular size cut-off for proteins is approximately M_r 700 000. Larger targets will pass through the column in the flowthrough fraction.

Capto MMC ImpRes and Capto adhere ImpRes are multimodal cation and anion exchangers, respectively. Both are designed for high-resolution polishing of mAbs, domain antibodies (dAbs), mAb charged variants, and other biomolecules. The small particle size and selectivity of the ligand enable effective removal of impurities such as DNA, host cell proteins (HCP), leached protein A, aggregates, and viruses in mAb production processes. The resins are designed to allow effective, high-resolution polishing of mAb in the second or third step of a purification scheme after the protein A capture step.

Characteristics of HiScreen multimodal chromatography columns are shown in Table 7.

Hydrophobic interaction chromatography

HiScreen Capto Phenyl (high sub), Capto Phenyl ImpRes, Capto Butyl, Phenyl HP, Phenyl FF (high sub), Phenyl FF (low sub), Butyl HP, Butyl FF, Butyl-S FF, and Octyl FF are the HIC columns available. The resins are based on Capto, Sepharose High Performance, or Sepharose Fast Flow matrices, with different immobilized hydrophobic ligands. These HIC resins are designed for capture and intermediate purification, with excellent flow properties and high physical and chemical stability.

The HIC resins are alkaline-resistant, allowing the use of 0.5 to 1.0 M sodium hydroxide for CIP. Capto HIC resins are based on a highly cross-linked agarose matrix with high physical and chemical stabilities that provide excellent flow properties. Such high flow rates permit rapid processing of large sample volumes with only moderate reductions in binding capacity. The differences in selectivities for model proteins are due to greater cross-linking of the agarose base matrix of Capto resins.

HiScreen HIC column characteristics are shown in Table 8.

Capto Phenyl ImpRes is a resin designed for intermediate and polishing steps in downstream purification processes when medium to high hydrophobicity is required. The selectivity is similar to that of Phenyl Sepharose 6 Fast Flow (high sub). Higher flow rates can, however, be used with Capto Phenyl ImpRes, while the smaller bead size enables high-resolution polishing for effective removal of contaminants.

Table 5. Characteristics of Capto ImpRes and Capto S ImpAct IEX resins in HiScreen columns

	Capto SP ImpRes	**Capto Q ImpRes**	**Capto S ImpAct**
Matrix	Highly cross-linked agarose, spherical		
Particle size, d_{50v}*	40 µm	40 µm	50 µm
Charged group	$-CH_2CH_2CH_2SO_3^-$	$-CH_2N^+(CH_3)_3$	$-SO_3^-$
Total ionic capacity	0.13 to 0.16 (H^+) mmol/mL resin	0.15 to 0.18 (Cl^-) mmol/mL resin	37 to 63 µmol (H^+)/mL resin
Dynamic binding capacity (Q_{B10})/mL resin†	> 70 mg lysozyme > 95 mg BSA	> 55 mg BSA > 48 mg β-Lactoglobulin	> 90 mg lysozyme > 85 mg BSA > 100 mg IgG
Recommended operating flow velocity‡	100 to 300 cm/h		
Maximum operating flow velocity‡	300 cm/h		
pH stability§			
Operational	4 to 12	2 to 12	4 to 12
Cleaning-in-place (CIP)	3 to 14	2 to 14	3 to 14
Chemical stability	Commonly used aqueous buffers, 1 M sodium hydroxide¶, 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol, and 70% ethanol		
Storage	4°C to 30°C in 0.2 M sodium acetate and 20% ethanol	4°C to 30°C in 20% ethanol	4°C to 30°C in 0.2 M sodium acetate and 20% ethanol

* d_{50v} is the median particle size of the cumulative volume distribution

† Dynamic binding capacity at 10% breakthrough measured by frontal analysis (displacement chromatography) at a residence time of 4 min (150 cm/h) in a Tricorn™ 5/100 column with 10 cm bed height in 50 mM Tris, pH 8.0 (BSA on Capto Q ImpRes), 20 mM sodium phosphate, pH 7.2 (lysozyme), 50 mM sodium acetate, pH 4.75 (BSA on Capto SP ImpRes)

‡ Water at room temperature

§ Operational: pH interval where the resin can be operated without significant change in function
Cleaning-in-place: pH stability where the 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 1 wk storage in 1 M NaOH at 40°C

Table 6. Characteristics of Sepharose Fast Flow and Sepharose High Performance IEX resins packed in HiScreen columns

	Q Sepharose FF	**DEAE Sepharose FF**	**SP Sepharose FF**	**Q Sepharose HP**	**SP Sepharose HP**
Matrix	Cross-linked agarose, 6%, spherical				
Particle size, d_{50v}*	90 µm	90 µm	90 µm	34 µm	34 µm
Ion exchange type	Strong anion	Weak anion	Strong cation	Strong anion	Strong cation
Charged group	$-N^+(CH_3)_3$	$-N^+(C_2H_5)_2H$	$-CH_2CH_2CH_2SO_3^-$	$-N^+(CH_3)_3$	$-CH_2CH_2CH_2SO_3^-$
Total ionic capacity (mmol/mL resin)	0.18 to 0.25 (Cl^-)	0.11 to 0.16 (Cl^-)	0.18 to 0.25 (H^+)	0.14 to 0.20 (Cl^-)	0.15 to 0.20 (H^+)
Dynamic binding capacity (Q_{B10})/mL resin†	~ 42 mg BSA	110 mg HSA	70 mg ribonuclease A	~ 70 mg BSA	55 mg ribonuclease A
Recommended operating flow velocity‡	300 cm/h	300 cm/h	300 cm/h	30 to 150 cm/h	30 to 150 cm/h
Maximum operating flow velocity‡	450 cm/h	450 cm/h	450 cm/h	150 cm/h	150 cm/h
pH stability§					
Operational	2 to 12	2 to 12	4 to 13	2 to 12	4 to 13
Cleaning-in-place (CIP)	2 to 14	2 to 14	3 to 14	2 to 14	3 to 14
pH ligand fully charged¶	Entire pH range	Below 9	Entire pH range	Entire pH range	Entire pH range
Chemical stability	Commonly used aqueous buffers, 1 M NaOH, 8 M urea, 6 M guanidine hydrochloride, 70% ethanol				
Storage	4°C to 30°C in 20% ethanol, 0.2 M sodium acetate (SP) 4°C to 30°C in 20% ethanol (Q, DEAE)				

* d_{50v} is the median particle size of the cumulative volume distribution

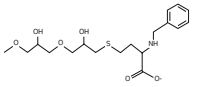
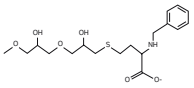
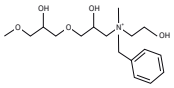
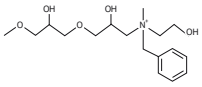
† Dynamic binding capacity at 10% breakthrough measured by frontal analysis (displacement chromatography). Running conditions: Q Sepharose FF and DEAE Sepharose FF: 0.05 M Tris-HCl, pH 7.5 at 75 cm/h. SP Sepharose FF: 0.1 M sodium acetate, pH 5.0 at 75 cm/h. Q Sepharose HP: 0.02 M Tris-HCl, pH 8.2 at 156 cm/h. SP Sepharose HP: 0.1 M sodium acetate, pH 6.0 at 156 cm/h.

‡ Water at room temperature

§ Operational: pH interval where the resin can be operated without significant change in function
Cleaning-in-place: pH stability where the resin can be subjected to cleaning- or sanitization-in-place without significant change in function

¶ pH range where ligand is fully charged; although the ligand is fully charged throughout the range stated, only use the resin within the stated stability ranges

Table 7. Characteristics of multimodal chromatography resins packed in HiScreen columns

	Capto MMC	Capto MMC ImpRes	Capto adhere	Capto adhere ImpRes	Capto Core 700
Matrix	Highly cross-linked agarose, spherical				
Particle size, d_{50v}*	75 µm	40 µm	75 µm	40 µm	85 µm
Charged group					$CH_3CH_2CH_2CH_2CH_2$ $CH_2CH_2CH_2NH$—
Total ionic capacity (mmol/mL resin)	0.07 to 0.09 (H^+)	0.03 to 0.04 (H^+)	0.09 to 0.12 (Cl^-)	0.08 to 0.11 mmol (Cl^-)	0.04 to 0.09 (Cl^-)
Dynamic binding capacity (Q_{B10})/mL resin†	45 mg BSA (at 30 mS/cm)	60 to 90 mg mAb	N/A	45 to 85 mg mAb	≈ 13 mg ovalbumin
Recommended operating flow velocity‡	150 to 300 cm/h	100 to 200 cm/h	150 to 300 cm/h	100 to 200 cm/h	100 to 300 cm/h
Maximum operating flow velocity‡	600 cm/h	300 cm/h	600 cm/h	300 cm/h	500 cm/h
pH stability§					
Operational			3 to 12		
Cleaning-in-place (CIP)	3 to 14	2 to 14	2 to 14	2 to 14	2 to 14
pH ligand fully charged¶	N/A	N/A	Entire pH range	Entire pH range	N/A
Storage	4°C to 30°C in 20% ethanol				

* d_{50v} is the median particle size of the cumulative volume distribution

† Dynamic binding capacity at 10% breakthrough measured by frontal analysis (displacement chromatography)

‡ Water at room temperature

§ Operational: pH interval where the resin can be operated without significant change in function.
Cleaning-in-place: pH stability where the resin can be subjected to cleaning- or sanitization-in-place without significant change in function

¶ pH range where ligand is fully charged; although the ligand is fully charged throughout the entire pH range, only use the resin within the stated stability range

Table 8. Characteristics of HIC resins packed in HiScreen columns

	Capto Phenyl (high sub)	Capto Phenyl ImpRes	Phenyl Sepharose 6 Fast Flow (high sub)	Phenyl Sepharose 6 Fast Flow (low sub)	Phenyl Sepharose High Performance
Matrix	Highly cross-linked agarose, spherical	Highly cross-linked agarose, spherical	Cross-linked agarose, 6%	Cross-linked agarose, 6%	Cross-linked agarose, spherical
Hydrophobic ligand			Phenyl —O— 		
Ligand density/mL resin	≈ 27 µmol	≈ 9 µmol	40 µmol	25 µmol	25 µmol
Particle size, d_{50v}*	75 µm	40 µm	90 µm	90 µm	34 µm
Dynamic binding capacity (Q_{B10})/mL resin†	27 mg BSA	19 mg BSA	N/A	N/A	N/A
Recommended operating flow velocity‡	150 to 350 cm/h	Up to 220 cm/h	300 cm/h	300 cm/h	75 cm/h
Maximum operating flow velocity‡	600 cm/h	500 cm/h	450 cm/h	450 cm/h	150 cm/h
pH stability§					
Operational			3 to 13		
Cleaning-in-place (CIP)			2 to 14		
Storage	4°C to 30°C in 20% ethanol				

	Capto Butyl	Butyl Sepharose High Performance	Butyl Sepharose 4 Fast Flow	Butyl-S Sepharose 6 Fast Flow	Octyl Sepharose 4 Fast Flow
Matrix	Highly cross-linked agarose, spherical	Cross-linked agarose, spherical	Cross-linked agarose, 4%, spherical	Cross-linked agarose, 6%, spherical	Cross-linked agarose, 4%, spherical
Hydrophobic ligand	Butyl —O—$(CH_2)_3$—CH_3	Butyl —O—$(CH_2)_3$—CH_3	Butyl —O—$(CH_2)_3$—CH_3	Butyl -S —S—$(CH_2)_3$—CH_3	Octyl —O—$(CH_2)_7$—CH_3
Ligand density/mL resin	≈ 53 µmol	50 µmol	40 µmol	10 µmol	5 µmol
Particle size, d_{50v}*	75 µm	34 µm	90 µm	90 µm	90 µm
Dynamic binding capacity (Q_{B10})/mL resin†	27 mg BSA	N/A	N/A	N/A	N/A
Recommended operating flow velocity‡	150 to 350 cm/h	75 cm/h	150 cm/h	300 cm/h	150 cm/h
Maximum operating flow velocity‡	600 cm/h	150 cm/h	230 cm/h	450 cm/h	240 cm/h
pH stability§					
Operational			3 to 13		
Cleaning-in-place (CIP)			2 to 14		
Storage	4°C to 30°C in 20% ethanol				

* d_{50v} is the median particle size of the cumulative volume distribution

† Dynamic binding capacity at 10% breakthrough measured by frontal analysis (displacement chromatography)

‡ Water at room temperature

§ Operational: pH interval where the resin can be operated without significant change in function. Cleaning-in-place: pH stability where the resin can be subjected to cleaning- or sanitization-in-place without significant change in function

Capto Phenyl (high sub) is designed for initial and intermediate purification steps, requiring a chromatography resin with medium to high hydrophobicity. Performance and selectivity are similar to that of Phenyl Sepharose 6 Fast Flow (high sub). Higher flow rates can be used with Capto Phenyl (high sub) during equilibration, wash, and regeneration.

Capto Butyl is intended for initial and intermediate purification steps, requiring a chromatography resin with low to medium hydrophobicity. Capto Butyl often works efficiently with relatively low salt concentrations. The mechanism of binding and elution onto the butyl ligand is different from that onto the phenyl ligand, giving a different selectivity. Performance during sample run and selectivity are similar to that of Butyl Sepharose 4 Fast Flow. Higher flow rates can be used with Capto Butyl during equilibration, wash, and regeneration.

Phenyl Sepharose High Performance is based on a 34 µm matrix and is suited for laboratory and intermediate process-scale separations and for final step purifications where high resolution is needed. The degree of substitution gives Phenyl Sepharose High Performance a selectivity similar to that of Phenyl Sepharose 6 Fast Flow (low sub).

Phenyl Sepharose 6 Fast Flow is available with high or low levels of phenyl substitution differing in selectivity, efficiency, and binding capacity. These resins are excellent for purifications requiring medium to high hydrophobicity.

Butyl Sepharose High Performance is based on a 34 µm matrix. The small particles give high resolution and make the product excellent for polishing steps. Even though the ligand concentration is higher than for other butyl resins, Butyl Sepharose High Performance shows a similar selectivity for the test proteins used in the functional test.

Butyl-S Sepharose 6 Fast Flow is designed for purification of relatively strong hydrophobic molecules at low salt concentrations, for example to remove lipids, lipoproteins, and pigments from biological samples. This resin is the least hydrophobic in Cytiva's HIC resin portfolio.

Butyl Sepharose 4 Fast Flow and Octyl Sepharose 4 Fast Flow are intended for purifications requiring resins with low to medium hydrophobicity, and often work efficiently at relatively low salt concentrations. Octyl Sepharose 4 Fast Flow has a different hydrophobic character from the phenyl and butyl ligands and is an important complement to the other hydrophobic matrices.

Examples of HiScreen column use

1. Robustness study of HiScreen MabSelect Xtra

The robustness of HiScreen MabSelect Xtra was tested in 10 repeated runs on the same column. Chinese hamster ovary (CHO) cell supernatant containing IgG was used as sample (Fig 3). Each run was followed by CIP with 50 mM NaOH in 1 M NaCl. The performance was measured by comparing dynamic binding capacity (DBC) before and after every fifth run (Table 9). The yield from each run was measured by UV absorbance at 280 nm.

Running conditions

Column:	HiScreen MabSelect Xtra, 4.7 mL
Sample:	470 mL CHO cell supernatant, IgG
Equilibration and wash 1:	25 mM sodium phosphate, 500 mM NaCl, pH 7.4
Buffer exchange and wash 2:	25 mM sodium phosphate, 25 mM sodium acetate, pH 7.5
Elution:	25 mM sodium phosphate, 25 mM sodium acetate, pH 3.5
CIP:	50 mM NaOH, 1 M NaCl at 1 mL/min (130 cm/h)
Flow rate:	2.3 mL/min (300 cm/h), 2 min residence time
System:	ÄKTAexplorer 100

Dynamic binding capacity (DBC)*

Sample:	3.6 mg/mL, human IgG, gammanorm (Octapharma)
Equilibration and wash :	20 mM sodium phosphate, 150 mM NaCl, pH 7.4
Elution:	100 mM sodium citrate, pH 3.0
CIP:	50 mM NaOH, 1 M NaCl at 1 mL/min (130 cm/h)
Flow rate:	2.3 mL/min (300 cm/h), 2 min residence time
System:	ÄKTAexplorer 10

* DBC is defined as milligram hIgG applied per milliliter resin at the point where the concentration of hIgG in the column effluent reaches a value of 10% of the concentration in the sample

UNICORN method

Equilibration:	10 column volumes (CV)
Sample loading:	100 CV
Wash 1:	6 CV
Buffer exchange and wash 2:	3 CV
Elution:	Until absorbance is below 200 mAU
Equilibration:	10 CV
CIP:	10 mL (10 min contact time)
Equilibration:	10 CV

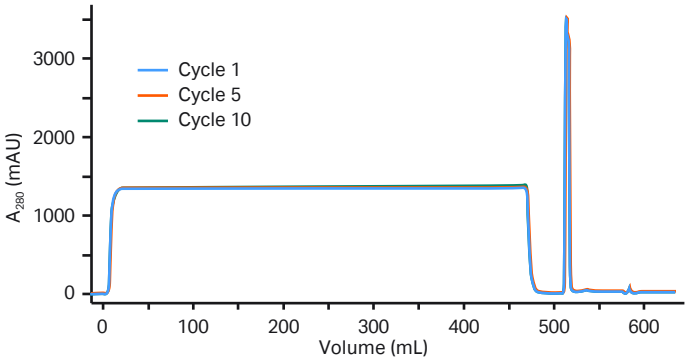


Fig 3. Overlay of chromatograms for cycles 1, 5, and 10 on HiScreen MabSelect Xtra

Table 9. DBC results at start, and after cycles 5 and 10

HiScreen MabSelect Xtra	DBC for hIgG (mg/mL resin)	Difference (%)
New column	37	-
After cycles 1 to 5	39	+ 5
After cycles 6 to 10	39	+ 4

The DBC varied < 5% compared with the initial result. The yield was approximately 110 mg and stable over all 10 runs (Fig 4). The results show no significant change in yield or DBC, and a high purity (Fig 5).

In summary, HiScreen MabSelect Xtra is suitable for method optimization and gives the process considerable robustness.

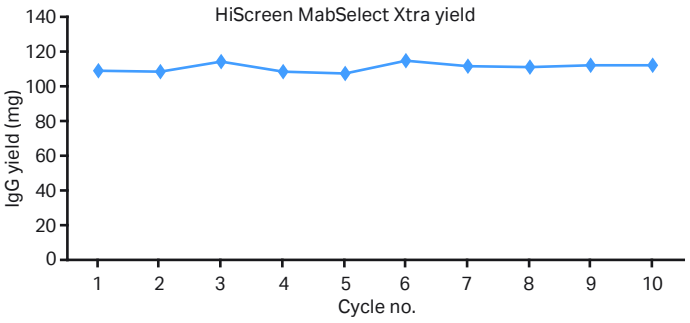
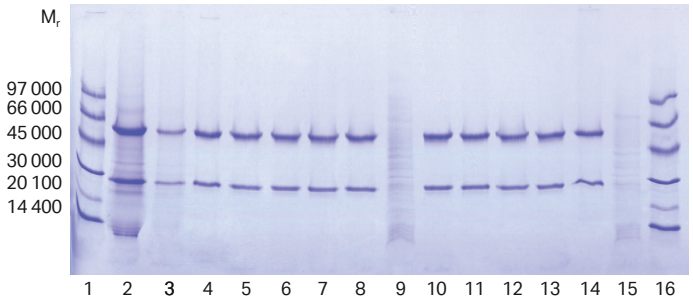


Fig 4. Yield for HiScreen MabSelect Xtra, cycles 1 to 10, in the robustness study



Lane	
1	Low Molecular Weight (LMW) marker (LMW-SDS Marker Kit)
2	Start material, diluted 1:1
3	Start material, diluted 1:5
4–8	Eluate, MabSelect Xtra, diluted 1:100, cycles 1 to 5
9	Flowthrough, MabSelect Xtra, diluted 1:5, cycles 1 to 5
10–14	Eluate, MabSelect Xtra, diluted 1:100, cycles 6 to 10
15	Flowthrough, MabSelect Xtra, diluted 1:5, cycles 6 to 10
16	LMW marker

Fig 5. SDS-PAGE under reducing conditions (ExcelGel™ SDS Gradient 8-18), Coomassie stained. The pools of the eluted peaks from the 10 runs on HiScreen MabSelect Xtra were analyzed and showed high purity.

2. Comparison of the binding capacity of different HiScreen MabSelect columns

Capacity is a key parameter in antibody purification. Measuring dynamic binding capacity (DBC) at different conditions for each individual antibody during process development will thus help find the best resin and binding/elution conditions for optimal production. Investigating DBC at different residence times is particularly important in this respect.

Figure 6 shows how DBC increases with residence time for HiScreen MabSelect, HiScreen MabSelect Xtra, and HiScreen MabSelect SuRe, and columns. As expected, the MabSelect Xtra resin clearly displays the best result.

Figure 7, shows DBC of MabSelect PrismA as compared with MabSelect SuRe and MabSelect SuRe LX resins at different residence times. MabSelect SuRe, MabSelect SuRe LX, and MabSelect PrismA all have an alkaline-tolerant, protein A-derived ligand, but the latter two have been optimized to give exceptionally high DBC for antibodies in high-titer cultures. Direct comparisons of the resins for a range of mAbs (data not shown here) reveal that MabSelect SuRe PrismA has the highest DBC of all three.

Running conditions

Columns:	HiScreen MabSelect SuRe, 4.7 mL HiScreen MabSelect, 4.7 mL HiScreen MabSelect Xtra, 4.7 mL
Sample:	3.6 mg/mL, human IgG, gammanorm (Octapharma)
Equilibration and wash:	20 mM sodium phosphate, 150 mM NaCl, pH 7.4
Elution:	100 mM sodium citrate, pH 3.0
CIP:	50 mM NaOH, 1 M NaCl (HiScreen MabSelect and HiScreen MabSelect Xtra) at 1 mL/min (130 cm/h) 0.5 M NaOH (HiScreen MabSelect SuRe) at 1 mL/min (130 cm/h)
Flow rate:	1.15 to 3.9 mL/min (150 to 500 cm/h)
System:	ÄKTAexplorer 10

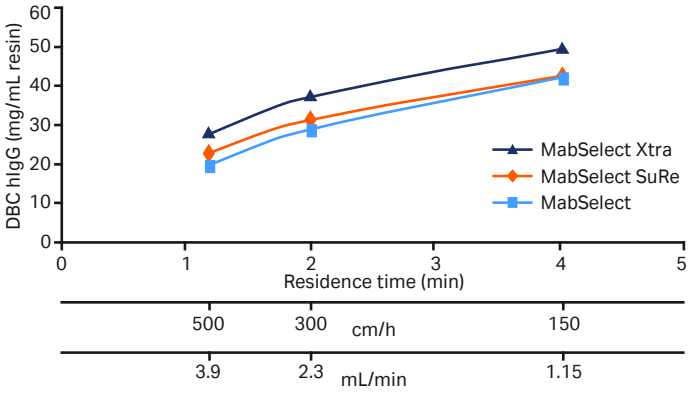


Fig 6. Comparison of DBC (Q_{B10}) for hIgG on HiScreen MabSelect, HiScreen MabSelect Xtra, and HiScreen MabSelect SuRe columns

Running conditions

Columns:	HiScreen MabSelect SuRe, 4.7 mL HiScreen MabSelect SuRe LX, 4.7 mL HiScreen MabSelect PrismA, 4.7 mL
Sample:	Gammanorm human IgG (Octapharma),5 mg/mL mAb from host cell-claried feed
Binding buffer:	PBS, pH 7.4
Elution buffer:	0.1 M acetic acid, pH 3.0
Flow rate:	0.46 to 4.65 mL/min , 60 to 600 cm/h (Residence times: 1 to 10 min)
System:	ÄKTAexplorer 10

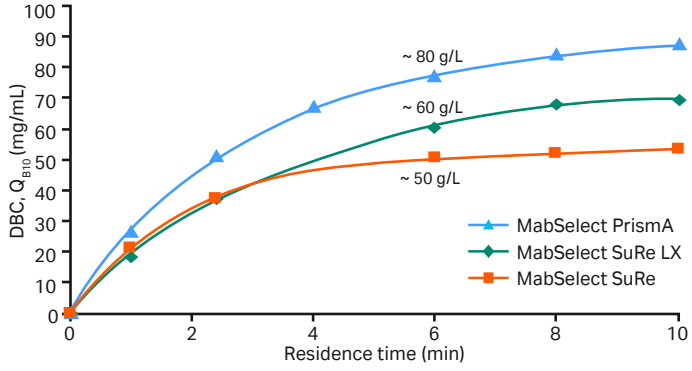


Fig 7. The DBC (Q_{B10}) of MabSelect SuRe, MabSelect SuRe LX, and MabSelect PrismA increases as a function of residence time in the column. The comparative data shows that MabSelect PrismA has the highest DBC of the resins at equivalent residence times.

3. Scale-up from HiScreen MabSelect SuRe to XK 16/20 column packed with MabSelect SuRe

This application shows a 4.6-fold scale-up from HiScreen MabSelect SuRe (4.7 mL) to a XK 16/20 column packed with MabSelect SuRe (21.5 mL, bed height 10.7 cm). The linear flow velocity was kept constant, and the sample volume was increased proportionally to the column volume. The same UNICORN method was used for both columns, increasing only the volumetric flow rate (mL/min). The results for the two columns were compared (Fig 8 and Fig 9).

Running conditions

Columns:	HiScreen MabSelect SuRe, 4.7 mL XK 16/20 packed with MabSelect SuRe, (1.6 × 10.7 cm), 21.5 mL
Sample:	349 mL or 1508 mL (75 CV), CHO cell supernatant, IgG
Equilibration and wash 1:	25 mM sodium phosphate, 500 mM NaCl, pH 7.4
Buffer exchange and wash 2:	25 mM sodium phosphate, 25 mM sodium acetate, pH 7.5
Elution:	25 mM sodium phosphate, 25 mM sodium acetate, pH 3.5
CIP:	0.5 M NaOH at 130 cm/h
Flow rate:	HiScreen MabSelect SuRe: 3.9 mL/min (500 cm/h), 1.2 min residence time XK 16/20 packed with MabSelect SuRe: 16.8 mL/min (500 cm/h), 1.2 min residence time
System:	ÄKTAexplorer 100

The eluted peaks were neutralized with 0.1 M NaOH

UNICORN method

Equilibration:	10 CV
Sample loading:	75 CV
Wash 1:	6 CV
Buffer exchange and wash 2:	3 CV
Elution:	Until absorbance is below 200 mAU
Equilibration:	10 CV
CIP:	10 mL or ~ 46 mL (10 min contact time)
Equilibration:	10 CV

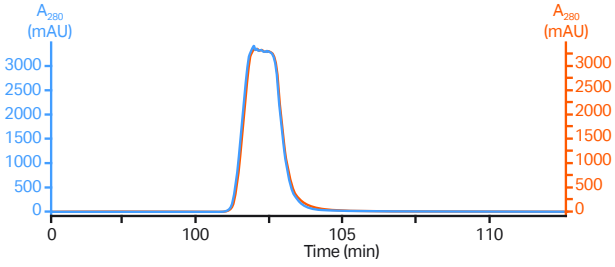


Fig 8. Overlay of UV absorbance curves (280 nm) for eluted peaks from HiScreen MabSelect SuRe (blue) and XK 16/20 packed with MabSelect SuRe (orange)

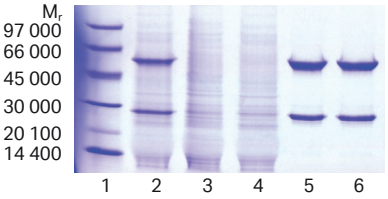


Fig 9. SDS-PAGE under reducing conditions (ExcelGel SDS Gradient 8–18), Coomassie stained. The sample volumes applied were adjusted according to the concentration in eluted pools. The eluted pool from HiScreen MabSelect SuRe was ~ 10% more concentrated than the eluted pool from XK 16/20 packed with MabSelect SuRe

Lane

1 LMW marker (LMW-SDS Marker Kit)
2 Start material, diluted 1:2
3 Flowthrough, HiScreen MabSelect SuRe, diluted 1:2
4 Flowthrough, XK column packed with MabSelect SuRe, diluted 1:2
5 Eluate, HiScreen MabSelect SuRe, diluted 1:30
6 Eluate, XK column packed with MabSelect SuRe, diluted 1:30

Both columns gave comparable results (Table 10) with high purity and similar recovery (97%). In summary, the chromatographic performance of the purification step was maintained as it was scaled up.

Table 10. Yield calculated in milligram and percent

	Conc. (mg/mL)	Yield (mg)	Yield (%)
HiScreen MabSelect SuRe, 4.7 mL	10.2	81	97
XK 16/20 packed with MabSelect SuRe, 21.5 mL	9.2	353	97

4. Purification of GFP-$(His)_6$ expressed in *E. coli*

HiScreen Ni FF was used for purification of his-tagged green fluorescent protein, GFP-$(His)_6$, expressed in *E. coli.* The protein was eluted in two peaks using a 10 column volume (CV) linear gradient with imidazole up to 500 mM (Fig 10). SDS-PAGE analysis was performed for both peaks (Fig 11).

The first pool contained mostly contaminants whereas the second pool contained highly pure GFP-$(His)_6$, > 95%. The amount of GFP-$(His)_6$ in the second pool was 42 mg as determined by absorbance measurement at 490 nm, which is the specific wavelength for GFP.

Running conditions

Column:	HiScreen Ni FF, 4.7 mL
Sample:	40 mL of GFP-$(His)_6$ in *E. coli* lysate
Start and wash buffer:	20 mM sodium phosphate, 500 mM NaCl, 20 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4
Flow rate:	2.4 mL/min (300 cm/h)
Linear gradient:	0% to 100% elution buffer in 10 CV
System:	ÄKTA avant 25

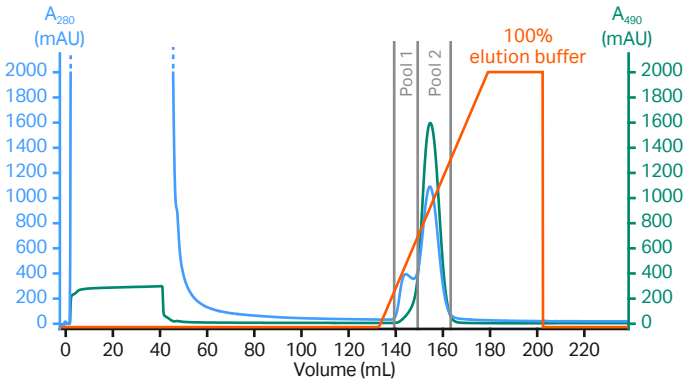
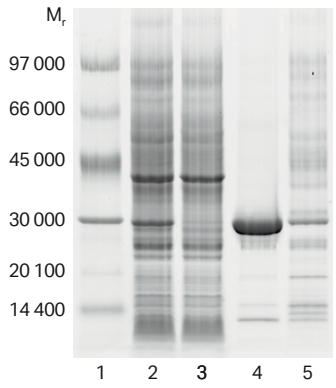


Fig 10. Purification of GFP-$(His)_6$ expressed in *E. coli* BL21 on HiScreen Ni FF. Indicated pools are analyzed by SDS-PAGE (Fig 11).



Lane

1 LMW marker (LMW-SDS Marker Kit)
2 Start material, diluted 1:10
3 Flowthrough, diluted 1:10
4 Eluted pool 2, HiScreen Ni FF, diluted 1:3
5 Eluted pool 1, HiScreen Ni FF

Fig 11. SDS-PAGE analysis (reducing conditions) of GFP-$(His)_6$ after purification on HiScreen Ni FF. ExcelGel SDS Gradient 8-18, Deep Purple Total Protein Stain.

5. Robustness study of HiScreen Capto Q

In this study, the robustness of two HiScreen Capto Q columns, connected in series to give 20 cm bed height, was tested by 10 repeated runs. Sonicated and clarified *E. coli* homogenate was used as sample. Each run was followed by CIP with 1 M NaOH. The performance was measured by comparing DBC before and after every fifth run.

The DBC varied < 6 % compared with the initial result showing that two columns connected in series give robust and reproducible results after repeated exposure to *E. coli* homogenate and cleaning procedures with 1 M NaOH (Fig 12 and Table 11).

Running conditions

Column:	2 × HiScreen Capto Q, 9.3 mL
Sample:	930 mL clarified *E. coli* homogenate
Equilibration and wash:	50 mM Tris, pH 8.0
Elution:	50 mM Tris, 1 M NaCl, pH 8.0
CIP:	1 M NaOH at 1.5 mL/min (190 cm/h)
Regeneration:	50 mM Tris, 3 M NaCl, pH 8.0
Flow rate:	3.5 mL/min (450 cm/h), 2.6 min residence time
System:	ÄKTAexplorer 100

Dynamic binding capacity (DBC)

Sample:	4 mg/mL, bovine serum albumin (BSA)
Equilibration and wash :	50 mM Tris, pH 8.0
Elution:	50 mM Tris, 1 M NaCl, pH 8.0
CIP:	1 M NaOH at 1 mL/min (130 cm/h)
Flow rate:	4.7 mL/min (600 cm/h), 2 min residence time
System:	ÄKTAexplorer 10

UNICORN method

Equilibration:	6 CV
Sample loading:	10 CV
Wash:	10 CV
Elution:	5 CV
CIP:	5 CV (15 min contact time)
Regeneration:	2 CV

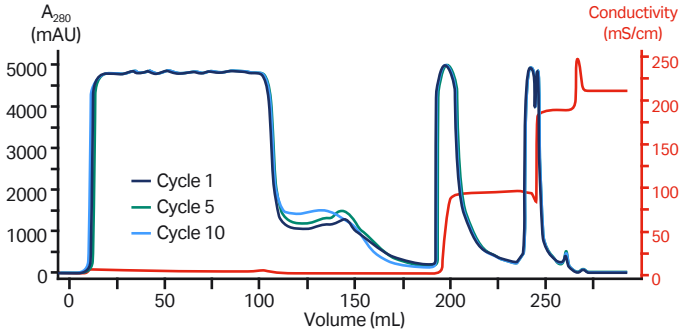


Fig 12. Two HiScreen Capto Q columns connected in series give a 20 cm bed height. Overlay of chromatograms for cycles 1, 5, and 10.

Table 11. DBC before and after cycles 5 and 10

2 × HiScreen Capto Q	DBC for BSA (mg/mL resin)	Difference (%)
New column	188	-
After cycles 1 to 5	176	-6
After cycles 6 to 10	179	-5

6. Scaling up from HiScreen to AxiChrom 50 column

This application shows the scale-up from two HiScreen Capto SP ImpRes columns connected in series (total 9.3 mL, 20 cm bed height) via HiScale 16/20 (40 mL, 20 cm bed height) to AxiChrom™ 50 (398 mL, 20.3 cm bed height), the latter two both packed with Capto SP ImpRes. Linear flow velocity was kept constant while sample volume was increased in proportion to the column volumes. Figure 13 shows the results.

Running conditions

Columns:	2 × HiScreen Capto SP ImpRes (0.77 × 20 cm), 9.3 mL total, HiScale 16/20 packed with Capto SP ImpRes (1.6 × 20 cm) 40 mL, AxiChrom 50 packed with Capto SP ImpRes (5 × 20.3 cm) 398 mL
Sample:	7.5 mg/mL BSA and 2.5 mg/mL lactoferrin
Sample volume:	1 CV
Start buffer:	50 mM acetate, pH 5
Elution buffer:	50 mM acetate, 1 M NaCl, pH 5
Flow velocities:	1.2 and 2.4 mL/min (150 and 300 cm/h)
Gradient:	0% to 100% elution buffer in 10 CV
Residence times:	4 or 8 min depending on flow rate
System:	ÄKTA avant 150

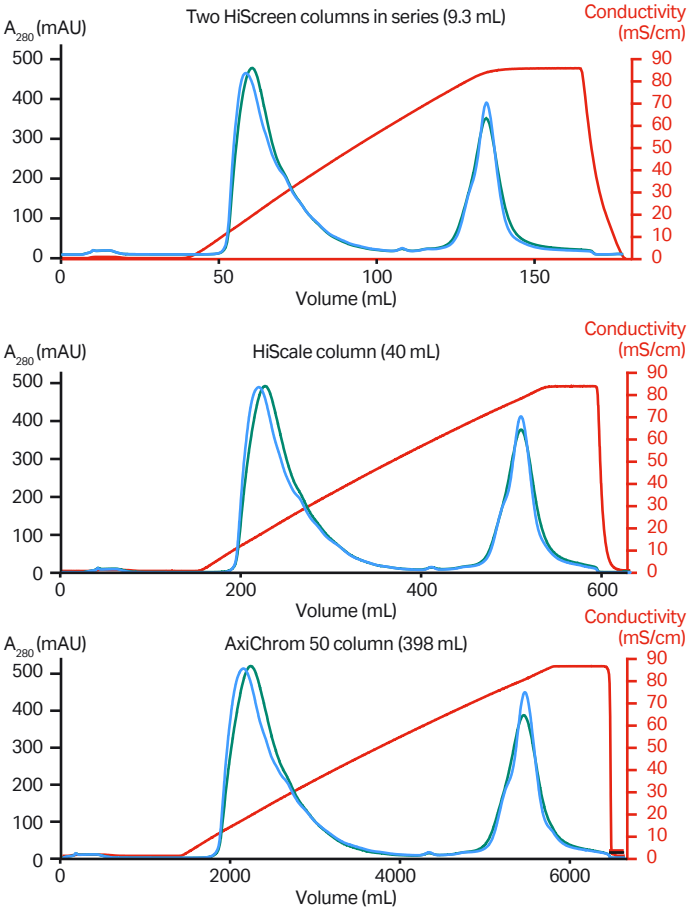
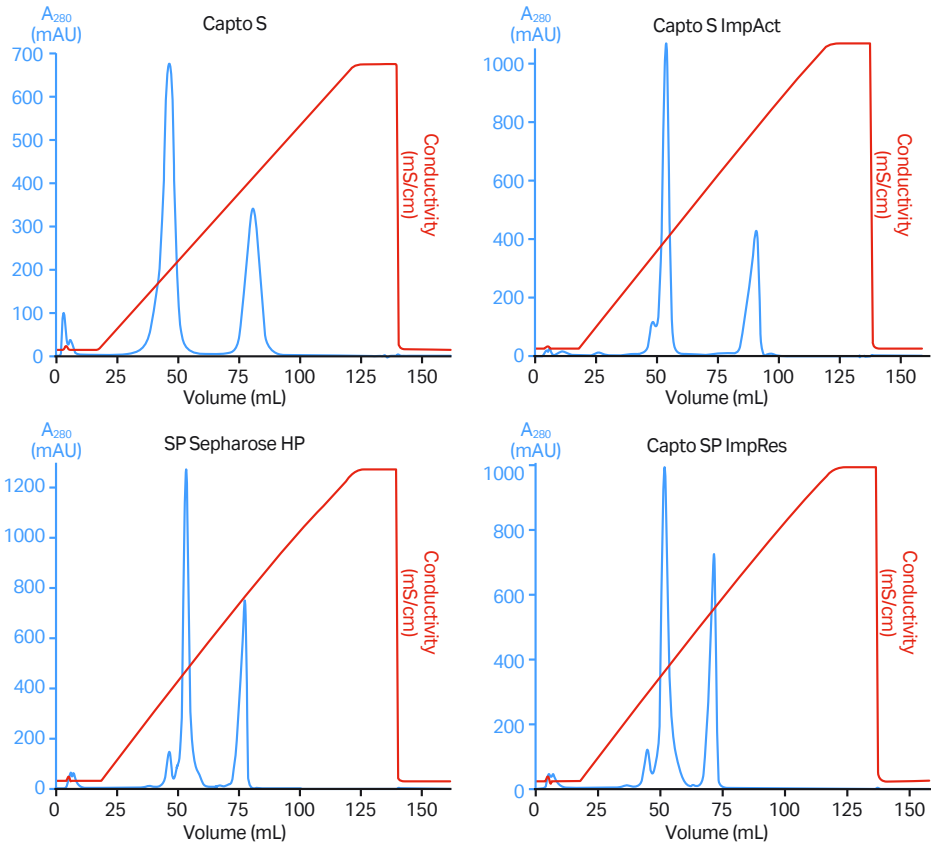


Fig 13. Separation of BSA and lactoferrin at three increasing scales (9.3, 40 and 398 mL columns) and two flow velocities (150 cm/h [green curves] and 300 cm/h [blue curves]). Chromatographic performance was maintained during scale-up and at increased flow velocity.

The three column sizes all gave comparable purifications. Chromatograms also show that resolution at the higher flow rate was almost identical to that of the lower flow rate. Chromatographic performance was thus maintained during scale-up and increased flow rate.

7. High resolution for late-stage purification and polishing

Cytiva offers several "S-type" cation exchange resins. Even though the charged groups of the S-ligand are similar for these resins, differences in base matrix, ligand density, and surface extenders can lead to differences in selectivity and resolution. Figure 14 presents four ion exchangers — Capto S, Capto S ImpAct, SP Sepharose High Performance, and Capto SP ImpRes — in prepacked HiScreen columns. As can be seen by the sharper wpeaks, Capto S ImpAct, Capto SP ImpRes, and SP Sepharose High Performance deliver improved resolution compared with Capto S. This finding is due to the three former having smaller particle sizes, that is, 50 µm, 40 µm, and 34 µm, respectively, compared with Capto S (90 µm). The larger particle size of Capto S is designed for the capture step rather than the polishing step. The chromatograms in Figure 14 also indicate that the four resins have a high separation potential, as the selectivity of the two test proteins clearly differs.



Running conditions

Columns:	HiScreen Capto S, HiScreen Capto S ImpAct, HiScreen SP Sepharose HP, HiScreen Capto SP ImpRes
Sample:	3 mL protein mix (α-chymotrypsinogen A and lysozyme) in 20 mM sodium phosphate, pH 6.8
Sample load:	4.5 mg/mL α-chymotrypsinogen A and 3 mg/mL lysozyme
Start buffer:	20 mM sodium phosphate, pH 6.8
Elution buffer:	Start buffer + 500 mM NaCl
Flow rate:	0.9 mL/min , 116 cm/h (5.4 min residence time)
Gradient:	Linear, 0% to 100% elution buffer in 20 CV
System:	ÄKTA avant 25

Fig 14. Chromatograms showing different selectivity and resolution, comparing four cation ion exchange resins. Peaks (left to right) are α-chymotrypsinogen A and lysozyme. Compared with Capto S (90 µm), the smaller bead size gives Capto S ImpAct (50 µm), SP Sepharose HP (34 µm), and Capto SP ImpRes (40 µm) improved resolution.

8. Screening on different HIC resins

The following screening experiment shows how different hydrophobic characteristics affect selectivity for seven HiScreen HIC columns (Fig 15). A mixture of model proteins was separated using the same methods and buffers. A linear decreasing salt gradient over 10 CV was used to elute the bound proteins.

The matrix, ligand, and degree of ligand substitution contribute to the final hydrophobicity of the resin, and hence, also the selectivity. The binding capacity of HIC resins increases with the ligand density up to a certain level. Simultaneously, the strength of interaction increases, leading to more strongly bound components.

Other parameters that influence binding, resolution, selectivity, and recovery in HIC are:

- Type of base matrix
- Sample characteristics
- Flow rate
- Type of salt and concentration
- Temperature
- Additives

The choice of parameters, such as ligand, type, and concentration of salt, are all empirical and must be established by screening experiments for each separation. The results below confirm that empirical experiments are necessary when working with HIC resins.

Running conditions

Columns:	HiScreen Capto Butyl, HiScreen Butyl HP, HiScreen Butyl-S FF, HiScreen Butyl FF, HiScreen Capto Phenyl (high sub), HiScreen Phenyl HP, HiScreen Phenyl FF (high sub), HiScreen Phenyl FF (low sub) and HiScreen Octyl FF
Column volume:	4.7 mL
Sample:	Mixture of cytochrome C, ribonuclease A, α-lactoglobulin and α-chymotrypsinogen, 7 mg/mL (in proportions 1:2.5:2.5:1) dissolved in start buffer
Sample volume:	2 mL
Start and wash buffer:	100 mM Na_2PO_4, 1.7 M $(NH_4)_2SO_4$, pH 7.0
Elution buffer:	100 mM Na_2PO_4, pH 7.0
Flow rate:	HiScreen Butyl HP, HiScreen Phenyl HP: 0.6 mL/min (75 cm/h). HiScreen Butyl FF, HiScreen Octyl FF: 1.2 mL/min (150 cm/h). HiScreen Phenyl FF (high sub), HiScreen Phenyl FF (low sub), HiScreen Capto Phenyl (high sub), HiScreen Capto Butyl, HiScreen Butyl-S FF: 2.3 mL/min (300 cm/h)
linear gradient:	0% to 100% elution buffer in 10 CV
System:	ÄKTA avant 25

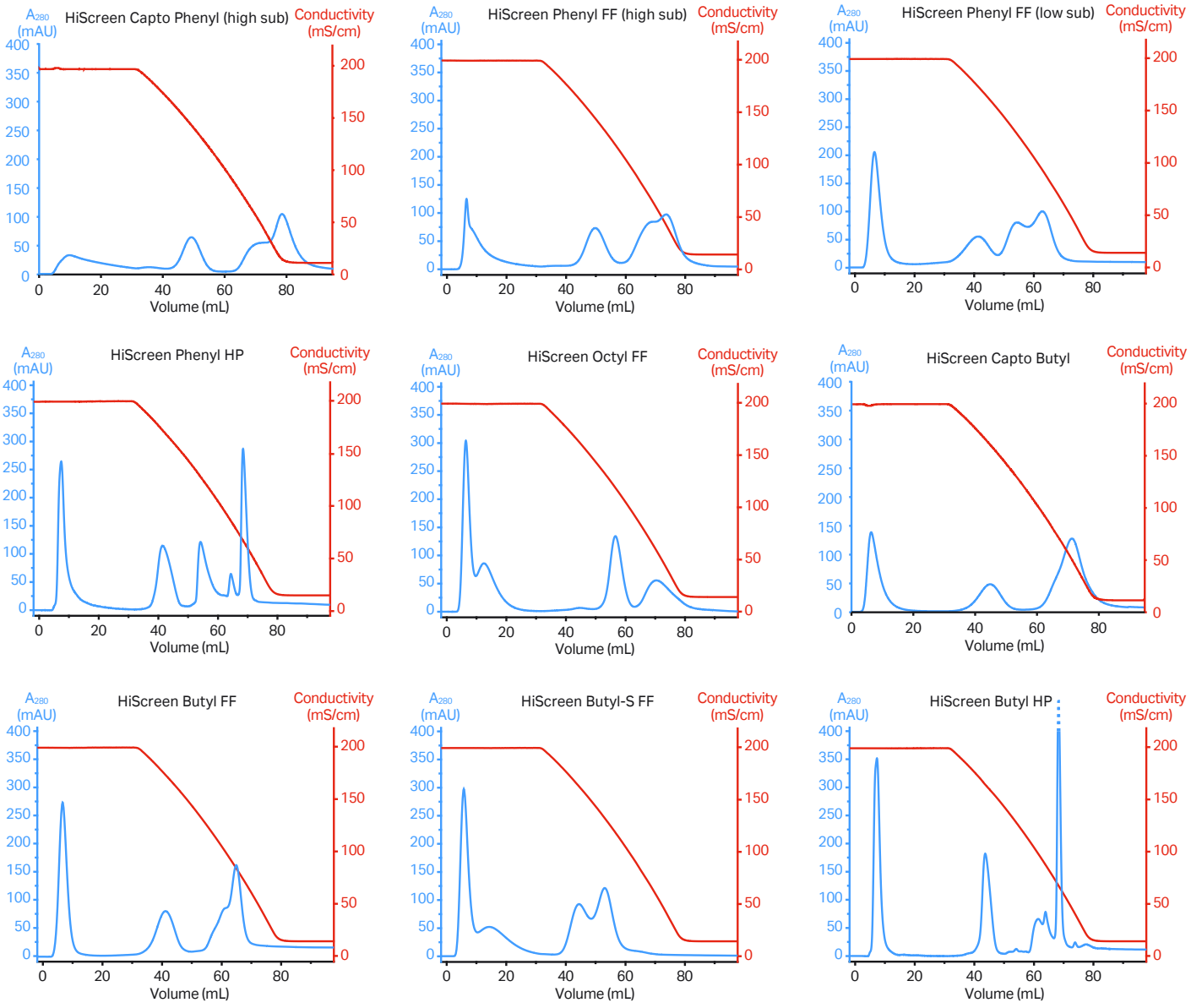


Fig 15. Comparison of the selectivity of nine different HiScreen HIC columns

Comparison of HiScreen with other column formats

Chromatography resins not yet available in the prepacked HiScreen format can also be candidates for process development and potential users thus have an interest in evaluating how they perform in screening and optimization. This can be achieved by packing and evaluating the candidate resins in, for example, Tricorn columns.

Studies show that a packed Tricorn column compares well with the equivalent prepacked HiScreen column in key aspects of process development. Results obtained from Tricorn studies can thus be compared with results obtained from HiScreen evaluations.

Ordering Information

Product	Quantity	Product code
HiScreen Capto L	1 × 4.7 mL	17547814
HiScreen Capto Q ImpRes	1 × 4.7 mL	17547015
HiScreen Capto SP ImpRes	1 × 4.7 mL	17546815
HiScreen Capto Q	1 × 4.7 mL	28926978
HiScreen Capto S	1 × 4.7 mL	28926979
HiScreen Capto S ImpAct	1 × 4.7 mL	17371747
HiScreen Capto Core 700	1 × 4.7 mL	17548115
HiScreen Capto MMC	1 × 4.7 mL	28926980
HiScreen Capto MMC ImpRes	1 × 4.7 mL	17371620
HiScreen Capto adhere	1 × 4.7 mL	28926981
HiScreen Capto adhere ImpRes	1 × 4.7 mL	17371520
HiScreen Capto DEAE	1 × 4.7 mL	28926982
HiScreen Capto Blue	1 × 4.7 mL	28992474
HiScreen Capto Phenyl (high sub)	1 × 4.7 mL	28992472
HiScreen Capto Phenyl ImpRes	1 × 4.7 mL	17548410
HiScreen Capto Butyl	1 × 4.7 mL	28992473
HiScreen MabSelect	1 × 4.7 mL	28926973
HiScreen MabSelect PrismA	1 × 4.7 mL	17549815
HiScreen MabSelect Xtra	1 × 4.7 mL	28926976
HiScreen MabSelect SuRe	1 × 4.7 mL	28926977
HiScreen MabSelect SuRe LX	1 × 4.7 mL	17547415
HiScreen Ni FF	1 × 4.7 mL	28978244
HiScreen IMAC FF	1 × 4.7 mL	28950517
HiScreen Q FF	1 × 4.7 mL	28950510
HiScreen DEAE FF	1 × 4.7 mL	28978245
HiScreen SP FF	1 × 4.7 mL	28950513
HiScreen Q HP	1 × 4.7 mL	28950511
HiScreen SP HP	1 × 4.7 mL	28950515
HiScreen Blue FF	1 × 4.7 mL	28978243
HiScreen Phenyl HP	1 × 4.7 mL	28950516
HiScreen Phenyl FF (high sub)	1 × 4.7 mL	28926988
HiScreen Phenyl FF (low sub)	1 × 4.7 mL	28926989
HiScreen Butyl HP	1 × 4.7 mL	28978242
HiScreen Butyl FF	1 × 4.7 mL	28926984
HiScreen Butyl-S FF	1 × 4.7 mL	28926985
HiScreen Octyl FF	1 × 4.7 mL	28926986

Related products

Product	Quantity	Product code
Ion exchange chromatography*		
HiTrap Capto Q ImpRes	5 × 1 mL	17547051
HiTrap Capto SP ImpRes	5 × 1 mL	17546851
HiTrap Capto Q	5 × 1 mL	11001302
HiTrap Capto S	5 × 1 mL	17544122
HiTrap Capto S ImpAct	5 × 1 mL	17371751
HiTrap Capto DEAE	5 × 1 mL	28916537
HiTrap Q FF	5 × 1 mL	17505301
HiTrap DEAE FF	5 × 1 mL	17505501
HiTrap SP FF	5 × 1 mL	17505401
HiTrap Q HP	5 × 1 mL	17115301
HiTrap SP HP	5 × 1 mL	17115101
HiPrep™ Q FF 16/10	1 × 20 mL	28936543
HiPrep DEAE FF 16/10	1 × 20 mL	28936541
HiPrep SP FF 16/10	1 × 20 mL	28936544
Capto Q ImpRes†	100 mL	17547002
Capto SP ImpRes†	100 mL	17546802
Capto Q†	100 mL	17531602
Capto S†	100 mL	17544101
Capto S ImpAct†	100 mL	17371702
Capto DEAE†	100 mL	17544301
Q Sepharose High Performance†	75 mL	17101401
SP Sepharose High Performance†	75 mL	17108701
Q Sepharose Fast Flow†	300 mL	17051001
SP Sepharose Fast Flow†	300 mL	17072901
DEAE Sepharose Fast Flow†	500 mL	17070901
Affinity chromatography*		
HiTrap Protein L	5 × 1 mL	17547851
HiTrap MabSelect SuRe	5 × 1 mL	11003493
HiTrap MabSelect	5 × 1 mL	28408253
HiTrap MabSelect Xtra	5 × 1 mL	28408258
HiTrap MabSelect PrismA	5 × 1 mL	17549852
HiTrap IMAC FF	5 × 1 mL	17092102
HisTrap™ FF	5 × 1 mL	17531901
HiTrap Blue HP	5 × 1 mL	17041201
Capto L	200 mL	17547802
Capto Blue†	25 mL	17544801
	500 mL	17544802
MabSelect SuRe†	200 mL	17543802
MabSelect PrismA	200 mL	17549802
MabSelect SuRe LX†	200 mL	17547402
MabSelect†	200 mL	17519902
MabSelect Xtra†	200 mL	17526902
Ni Sepharose 6 Fast Flow†	500 mL	17531803
IMAC Sepharose Fast Flow†	100 mL	17092108
Blue Sepharose 6 Fast Flow†	500 mL	17094802

Multimodal chromatography*		
HiTrap Capto Core 700	5 × 1 mL	17548151
HiTrap Capto MMC	5 × 1 mL	11003273
HiTrap Capto MMC ImpRes	5 × 1 mL	17371610
HiTrap Capto adhere	5 × 1 mL	28405844
HiTrap Capto adhere ImpRes	5 × 1 mL	17371510
Capto Core 700†	100 mL	17548102
Capto MMC†	100 mL	17531702
Capto MMC ImpRes†	25 mL	17371601
Capto adhere†	100 mL	17544401
Capto adhere ImpRes†	25 mL	17371501
Capto MMC ImpRes	100 mL	17371602
Capto adhere ImpRes	100 mL	17371502

Hydrophobic interaction chromatography*		
HiTrap HIC Selection Kit	70 × 1 mL	28411007
HiTrap Capto Phenyl ImpRes	5 × 1 mL	17548411
HiTrap Capto Phenyl ImpRes	5 × 5 mL	17548412
HiTrap Phenyl HP	5 × 1 mL	17135101
HiTrap Phenyl FF (high sub)	5 × 1 mL	17135501
HiTrap Phenyl FF (low sub)	5 × 1 mL	17135301
HiTrap Butyl HP	5 × 1 mL	28411001
HiTrap Butyl FF	5 × 1 mL	17135701
HiTrap Butyl-S FF	5 × 1 mL	17097813
HiTrap Octyl FF	5 × 1 mL	17135901
HiPrep Phenyl FF (high sub) 16/10	1 × 20 mL	28936545
HiPrep Phenyl FF (low sub) 16/10	1 × 20 mL	28936546
HiPrep Butyl FF 16/10	1 × 20 mL	28936547
HiPrep Octyl FF 16/10	1 × 20 mL	28936548
Capto Phenyl (high sub)†	25 mL	17545101
Capto Phenyl ImpRes†	25 mL	17548401
Capto Butyl†	25 mL	17545901
Phenyl Sepharose High Performance†	75 mL	17108201
Butyl Sepharose High Performance†	200 mL	17543202
Phenyl Sepharose 6 Fast Flow (low sub)†	200 mL	17086505
Phenyl Sepharose 6 Fast Flow (high sub)†	200 mL	17097305
Butyl-S Sepharose 6 Fast Flow†	200 mL	17097802
Butyl Sepharose 4 Fast Flow†	200 mL	17098001
Octyl Sepharose 4 Fast Flow†	200 mL	17094602

Desalting and buffer exchange		
HiTrap Desalting	5 × 5 mL	17140801
HiPrep 26/10 Desalting	1 × 53 mL	17508701
	4 × 53 mL	17508702

* For information about prefilled PreDictor plates for process development, visit cytiva.com/predictor

† Other quantities are also available. Please contact your local representative or visit cytiva.com/protein-purification or cytiva.com/bioprocess.

Column accessories	Use	Quantity	Product code
HiTrap/HiPrep, 1/16'' male connector for ÄKTA systems	Connection of columns with 1/16" fittings to ÄKTA systems	8	28401081
Union 1/16" male/1/16" male (0.5 mm i.d.)	Connecting two HiScreen columns in series	2	18112093
Fingertight stop plug, 1/16"	Sealing top and bottom of a HiScreen column	5	11000355

Related literature

Data file	
Capto adhere ImpRes	29034497

Handbooks	
Affinity chromatography vol. 1: Antibodies	18103746
Affinity chromatography vol. 2: Tagged proteins	18114275
Affinity chromatography vol. 3: Specific groups of biomolecules	18102229
Hydrophobic interaction chromatography and reversed phase chromatography	11001269
Ion exchange chromatography	11000421
Size exclusion chromatography	18102218

Selection guides	
Affinity chromatography, columns and resins	18112186
Antibody purification	28935197
Ion exchange chromatography, columns and resins	18112731
Ni Sepharose and IMAC Sepharose	28407092
Prepacked chromatography columns for ÄKTA systems	28931778

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