HiScreen™ prepacked columns

HiScreen columns are part of the process development platform available from GE Healthcare (Fig 1). The columns are prepacked with a range of BioProcess™ chromatography media (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 media 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 media 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 media using the same linear flow velocity

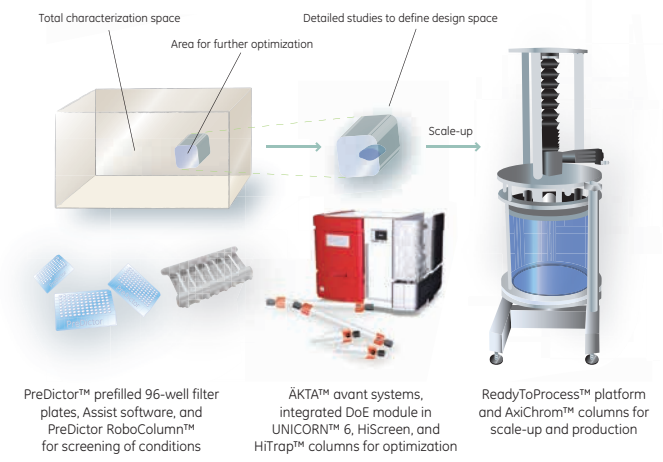


Fig 1. GE Healthcare's process development platform.

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

Use in process development

Prepacked HiScreen columns have many attributes that make them ideal for general process development work. Eight different examples of their use are shown later. A number of selected aspects are highlighted below.

Robustness

All columns are packed with robust BioProcess chromatography media 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 ten runs on the same column. The results described later in this Data file illustrate that this key parameter remains very stable throughout, thus demonstrating the robustness of the HiScreen format.

Method optimization and parameter screening

When developing a new process, parameters such as selectivity, capacity, 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 also prefilled with BioProcess media.

Once these conditions are identified, equivalent prepacked HiScreen columns are conveniently used for fine-tuning and verification. Screening of different HIC media in HiScreen columns is described later in this Data file.

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 can be found in Figures 9 and 13.

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.

Affinity chromatography (AC)

HiScreen Ni FF
HiScreen IMAC FF
HiScreen Capto™ Blue
HiScreen Blue FF
HiScreen MabSelect SuRe™ LX
HiScreen MabSelect SuRe
HiScreen MabSelect™
HiScreen MabSelect Xtra
HiScreen Capto L

Ion exchange chromatography (IEX)

HiScreen Capto Q ImpRes
HiScreen Capto SP ImpRes
HiScreen Capto Q
HiScreen Capto S
HiScreen Capto S ImpAct
HiScreen Capto DEAE
HiScreen Q FF
HiScreen SP FF
HiScreen DEAE FF
HiScreen Q HP
HiScreen SP HP

Multimodal chromatography

HiScreen Capto Core 700
HiScreen Capto MMC
HiScreen Capto adhere
HiScreen Capto MMC ImpRes
HiScreen Capto adhere ImpRes

Hydrophobic interaction chromatography (HIC)

Hiscreen Capto Phenyl (high sub)
HiScreen Capto Butyl
HiScreen Butyl FF
HiScreen Butyl-S FF
HiScreen Butyl HP
HiScreen Octyl FF
HiScreen Phenyl FF (high sub)
HiScreen Phenyl FF (low sub)
HiScreen Phenyl HP
HiScreen Capto Phenyl ImpRes

Column characteristics

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

Note: HiScreen columns cannot be opened or repacked.

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 medium and the column tubing used.

Chromatography media characteristics

Affinity chromatography

Capto L

HiScreen Capto L is prepacked with Capto L medium for capturing antibody fragments such as Fab, scFv, and Dab. 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, and MabSelect SuRe LX are columns prepacked with MabSelect protein A-derived media for the capture of MAbs. HiScreen MabSelect media characteristics are described below and in Table 2.

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

MabSelect Xtra is designed for maximum binding capacity, allowing binding from samples with high expression levels of the MAb.

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

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

	Capto L	MabSelect	MabSelect Xtra	MabSelect SuRe, MabSelect SuRe LX
Matrix	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose
Ligand	Recombinant protein L *(E. coli)*	Recombinant protein A *(E. coli)*	Recombinant protein A *(E. coli)*	Alkali-tolerant protein A-derived *(E. coli)*
Average particle size, d_{50v}*	85 µm	85 µm	75 µm	85 µm
Dynamic binding capacity/ml medium	~ 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‡
Recommended flow velocity**	120 to 150 cm/h	120 to 250 cm/h	50 to 150 cm/h	75 to 150 cm/h (MabSelect SuRe LX) 120 to 150 cm/h (MabSelect SuRe)
Maximum flow velocity¶	500 cm/h	500 cm/h	300 cm/h	500 cm/h
pH stability††				
Cleaning-in-place (CIP)	15 mM NaOH	2 to 12	2 to 12	2 to 14
Working	2 to 10	3 to 10	3 to 10	3 to 12
Storage	2°C to 8°C in 20% ethanol	2°C to 8°C in 20% ethanol	2°C to 8°C in 20% ethanol	2°C to 8°C in 20% ethanol

* d_{50v} is the medium particle size of the cumulative volume distribution.
† Determined at 10% breakthrough by frontal analysis 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 very low pH.
Working range: pH interval where the medium can be operated without significant change in function.
Cleaning-in-place: pH stability where the medium can be subjected to cleaning- or sanitization-in-place without significant change in function.

Ni Sepharose 6 Fast Flow

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

IMAC Sepharose 6 Fast Flow

Immobilized metal ion affinity chromatography (IMAC) is a widely used separation method for purifying proteins and peptides that have an affinity for metal ions. The method continues to grow in popularity, in particular for purifying histidine-tagged proteins but 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 medium can easily be charged with Ni^{2+}, Co^{2+}, Zn^{2+}, Cu^{2+}, Fe^{3+} or other metal ions. HiScreen IMAC FF medium characteristics are shown in Table 3.

Blue Sepharose 6 Fast Flow

HiScreen Blue FF is prepacked with the BioProcess chromatography medium Blue Sepharose 6 Fast Flow. This medium has Cibacron Blue 3G 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. It also binds several enzymes including kinases, dehydrogenases, and most enzymes requiring adenyl-containing cofactors, such as NAD^+. HiScreen Blue FF medium characteristics are shown in Table 3.

Capto Blue

HiScreen Capto Blue is prepacked with the base matrix Capto, to which Cibacron Blue 3G 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. This allows 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	Highly cross-linked 6% agarose	Highly cross-linked agarose	Highly cross-linked 6% agarose
Average particle size, d_{50v}*	90 µm	75 µm	90 µm
Dynamic binding capacity/ml medium	Approx. 40 mg $(histidine)_6$-tagged protein (Ni^{2+}-charged).† Untagged protein: Approx. 25 mg (Cu^{2+}-charged), or approx. 15 mg (Zn^{2+}- or Ni^{2+}-charged).†	≈ 25 mg HSA	18 mg HSA
Metal ion capacity/ml medium	Approx. 15 µmol Ni^{2+}	N/A	N/A
Ligand density	N/A	11 to 16 µmol Cibacron Blue 3G	Approx. 7 µmol Cibacron Blue 3G/ml of drained medium
Recommended flow velocity‡	30 to 300 cm/h	150 to 300 cm/h	30 to 300 cm/h
Maximum flow velocity‡	450 cm/h	600 cm/h	450 cm/h
pH stability (without metal ion)§			
Cleaning-in-place (CIP)	2 to 14	2 to 13	4 to 12
Working	3 to 12	2 to 13	3 to 13
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 seven days in: 70% ethanol, 6 M guanidine hydrochloride, 8 M urea	40°C for seven days 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 average particle size of the cumulative volume distribution.

† Dynamic binding capacity conditions:
Samples: $(Histidine)_6$-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.

§ Working range: pH interval where the medium can be operated without significant change in function.
Cleaning-in-place: pH stability where the medium 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 highly-rigid agarose, offers outstanding pressure/flow properties plus an optimized pore structure. Capto media thus combine high capacity with high flow rate and low backpressure to reduce process cycle times and increase productivity. The full range is available in HiScreen columns.

HiScreen Capto S, Capto Q, Capto DEAE, Capto MMC, and Capto adhere are an excellent choice for capture and initial purification. Their media 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 media, respectively. The charged groups are linked to the high-flow 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 ImpRes

HiScreen Capto Q ImpRes and HiScreen Capto SP ImpRes are columns packed with Capto ImpRes chromatography media for efficient intermediate purification and polishing in robust purification processes. Capto Q ImpRes and Capto SP ImpRes are strong anion and strong cation exchange BioProcess media, respectively. The combination of high-flow agarose plus small particle size adds high resolution to the well-established Capto platform. Media characteristics are summarized in Table 5.

Capto S ImpAct

HiScreen Capto S ImpAct columns are prepacked with Capto S ImpAct, a strong cation exchanger chromatography medium for intermediate purification and polishing of a wide range of biomolecules, especially monoclonal antibodies. The high binding capacity and the high flow 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 media packed in HiScreen columns

	Capto Q	Capto S	Capto DEAE
Matrix	High-flow agarose with a dextran surface extender		
Average particle size, d_{50v}*	90 µm	90 µm	90 µm
Charged group	$-N^+(CH_3)_3$	$-SO_3^-$	$-N^+H(CH_2CH_3)_2$
Total ionic capacity (mmol/ml medium)	0.16 to 0.22 (Cl^-)	0.11 to 0.14 (Na^+)	0.29 to 0.35 (Cl^-)
Dynamic binding capacity/ml medium	> 100 mg BSA	> 140 mg lysozyme	> 90 mg ovalbumin
Recommended flow velocity†	150 to 350 cm/h	150 to 350 cm/h	150 to 350 cm/h
Maximum flow velocity†	700 cm/h	700 cm/h	700 cm/h
pH stability‡			
Cleaning-in-place (CIP) Working	2 to 14 2 to 12	3 to 14 4 to 12	2 to 14 2 to 12
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 medium particle size of the cumulative volume distribution.
† Water at room temperature.
‡ Working range: pH interval where the medium can be operated without significant change in function.
Cleaning-in-place: pH stability where the medium 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% highly cross-linked agarose with an average bead size of 90 µm. This medium 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% highly cross-linked agarose with an average bead size of 90 µm. This medium has good flow properties and high loading capacities.

HiScreen SP FF is prepacked with SP Sepharose Fast Flow. This strong cation exchange medium is based on 6% highly cross-linked agarose with an average bead size of 90 µm. The medium 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 an average bead 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 an average bead 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 to traditional ion exchange media, making Capto MMC a weak multimodal cation exchanger.

Capto adhere is a strong multimodal anion exchanger, offering alternative selectivity compared to 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 contaminants remain bound.

Capto Core 700 beads have a core activated with octylamine ligands and an inert outer layer with no 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 beads where they are captured. The molecular size cut-off for proteins is approximately M_r 700 000. Targets larger than this 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 bead size and selectivity of the ligand enable effective removal of contaminants such as DNA, host cell proteins (HCP), leached protein A, aggregates, and viruses in MAb production processes. The media 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 media are based on the highly cross-linked beaded agarose matrices Capto, Sepharose High Performance, or Sepharose Fast Flow, with different immobilized hydrophobic ligands. These HIC media are designed for capture and intermediate purification, with excellent flow properties and high physical and chemical stability.

They are alkali-resistant media, allowing the use of 0.5 to 1.0 M sodium hydroxide for CIP. Capto HIC media 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 media. HiScreen HIC media characteristics are shown in Table 8.

Capto Phenyl ImpRes is a medium 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 media in HiScreen columns

	Capto SP ImpRes	Capto Q ImpRes	Capto S ImpAct
Matrix	High-flow agarose	High-flow agarose	High-flow agarose
Average 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 medium	0.15 to 0.18 (Cl^-) mmol/ml medium	37 to 63 µmol (H^+)/ml medium
Dynamic binding capacity/ml medium†	> 70 mg lysozyme > 95 mg BSA	> 55 mg BSA > 48 mg β-Lactoglobulin	> 90 mg lysozyme > 85 mg BSA > 100 mg IgG
Recommended flow velocity‡	100 to 300 cm/h	100 to 300 cm/h	100 to 300 cm/h
Maximum flow velocity‡	300 cm/h	300 cm/h	300 cm/h
pH stability§			
Cleaning-in-place (CIP)	3 to 14	2 to 14	3 to 14
Working	4 to 12	2 to 12	4 to 12
Chemical stability	All 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 medium particle size of the cumulative volume distribution.

† Dynamic binding capacity at 10% breakthrough measured 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.

§ Working range: pH interval where the medium can be operated without significant change in function.
Cleaning-in-place: pH stability where the medium 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 week storage in 1 M NaOH at 40°C.

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

	Q Sepharose FF	DEAE Sepharose FF	SP Sepharose FF	Q Sepharose HP	SP Sepharose HP
Matrix	6% highly cross-linked agarose	6% highly cross-linked agarose	6% highly cross-linked agarose	6% highly cross-linked agarose	6% highly cross-linked agarose
Average 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 medium)	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/ml medium†	120 mg HSA	110 mg HSA	70 mg ribonuclease A	50 mg HSA	55 mg ribonuclease A
Recommended flow velocity‡	300 cm/h	300 cm/h	300 cm/h	30 to 150 cm/h	30 to 150 cm/h
Maximum flow velocity‡	450 cm/h	450 cm/h	450 cm/h	150 cm/h	150 cm/h
pH stability§					
Cleaning-in-place (CIP)	1 to 14	1 to 14	3 to 14	1 to 14	3 to 14
Working	2 to 12	2 to 12	4 to 13	2 to 12	4 to 13
Chemical stability	All 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)				

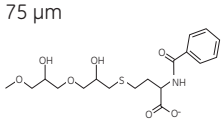
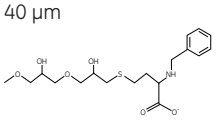
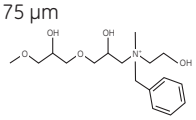
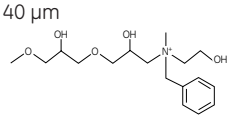
* d_{50v} is the average particle size of the cumulative volume distribution.

† 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.

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

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

	Capto MMC	Capto MMC ImpRes	Capto adhere	Capto adhere ImpRes	Capto Core 700
Matrix	High-flow agarose	High-flow agarose	High-flow agarose	High-flow agarose	High-flow agarose
Average 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 medium)	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/ml medium	45 mg BSA (at 30 mS/cm)	60–90 mg MAb	Not available	45–85 mg MAb	≈ 13 mg ovalbumin
Recommended flow velocity†	150 to 300 cm/h	Up to 220 cm/h	150 to 300 cm/h	Up to 220 cm/h	100 to 300 cm/h
Maximum flow velocity†	600 cm/h	500 cm/h	600 cm/h	500 cm/h	500 cm/h
pH stability‡					
Cleaning-in-place (CIP)	2 to 14	2 to 14	2 to 14	2 to 14	2 to 14
Working	2 to 12	3 to 12	3 to 12	3 to 12	3 to 13
Storage	4°C to 30°C in 20% ethanol	4°C to 30°C in 20% ethanol	4°C to 30°C in 20% ethanol	4°C to 30°C in 20% ethanol	4°C to 30°C in 20% ethanol

* d_{50v} is the medium particle size of the cumulative volume distribution.
† Water at room temperature.
‡ Working range: pH interval where the medium can be operated without significant change in function.
Cleaning-in-place: pH stability where the medium can be subjected to cleaning- or sanitization-in-place without significant change in function.

Table 8. Characteristics of HIC media 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 beads	High-flow agarose	6% cross-linked agarose beads	6% cross-linked agarose beads	6% cross-linked agarose beads
Hydrophobic ligand	Phenyl —O—	Phenyl —O—	Phenyl —O—	Phenyl —O—	Phenyl —O—
Ligand density/ml medium	≈ 27 µmol	≈ 9 µmol	40 µmol	25 µmol	25 µmol
Average particle size, d_{50v}*	75 µm	40 µm	90 µm	90 µm	34 µm
Recommended flow velocities†	150 to 350 cm/h	Up to 220 cm/h	300 cm/h	300 cm/h	30 to 150 cm/h
Maximum flow velocities†	600 cm/h	500 cm/h	450 cm/h	450 cm/h	150 cm/h
pH stability‡					
Cleaning-in-place (CIP)	2 to 14	2 to 14	2 to 14	2 to 14	2 to 14
Working	3 to 13	3 to 13	3 to 13	3 to 13	3 to 13
Storage	4°C to 30°C in 20% ethanol	4°C to 30°C in 20% ethanol	4°C to 30°C in 20% ethanol	4°C to 30°C in 20% ethanol	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 beads	6% cross-linked agarose beads	4% cross-linked agarose beads	6% cross-linked agarose beads	4% cross-linked agarose beads
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 medium	≈53 µmol	50 µmol	40 µmol	10 µmol	5 µmol
Average particle size, d_{50v}*	75 µm	34 µm	90 µm	90 µm	90 µm
Recommended flow velocities†	150 to 350 cm/h	30 to 150 cm/h	150 cm/h	300 cm/h	150 cm/h
Maximum flow velocities†	600 cm/h	150 cm/h	240 cm/h	450 cm/h	240 cm/h
pH stability‡					
Cleaning-in-place (CIP)	2 to 14	2 to 14	2 to 14	2 to 14	2 to 14
Working	3 to 13	3 to 13	3 to 13	3 to 13	3 to 13
Storage	4°C to 30°C in 20% ethanol	4°C to 30°C in 20% ethanol	4°C to 30°C in 20% ethanol	4°C to 30°C in 20% ethanol	4°C to 30°C in 20% ethanol

* d_{50v} is the average particle size of the cumulative volume distribution.
† Water at room temperature.
‡ Working range: pH interval where the medium can be operated without significant change in function.
Cleaning-in-place: pH stability where the medium can be subjected to cleaning- or sanitization-in-place without significant change in function.

Capto Phenyl (high sub) is designed for initial and intermediate step purifications requiring a chromatography medium 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 step purifications requiring a chromatography medium 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 media are excellent for purifications requiring medium to high hydrophobicity.

Butyl Sepharose High Performance is based on a 34 µm matrix. The small beads give high resolution and make the product excellent for polishing steps. Even though the ligand concentration is higher than for other butyl media, 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 medium is the least hydrophobic in the GE Healthcare HIC portfolio.

Butyl Sepharose 4 Fast Flow and Octyl Sepharose 4 Fast Flow are intended for purifications requiring media 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 columns in use

1. Robustness study of HiScreen MabSelect Xtra

The robustness of HiScreen MabSelect Xtra was tested by ten 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.

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

HiScreen MabSelect Xtra	DBC for hIgG [mg/ml medium]	Difference [%]
New column	37	-
After cycles 1 to 5	39	+5
After cycles 6 to 10	39	+4

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 medium 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

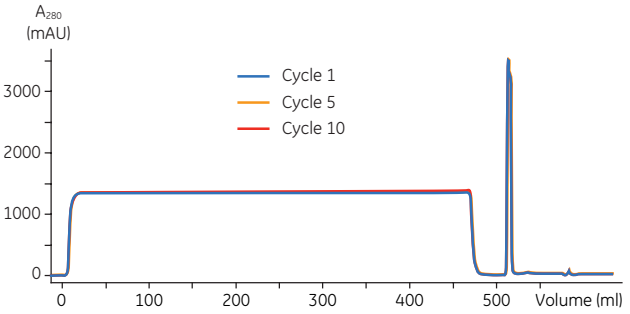


Fig 3. Overlay of chromatograms for cycles 1, 5, and 10 on HiScreen MabSelect Xtra.

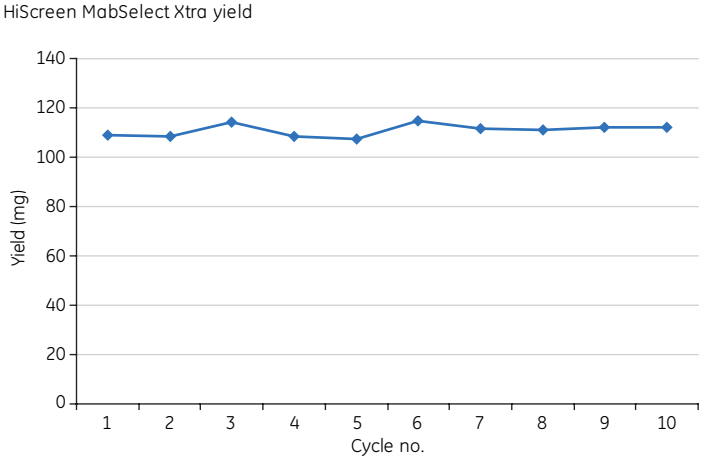
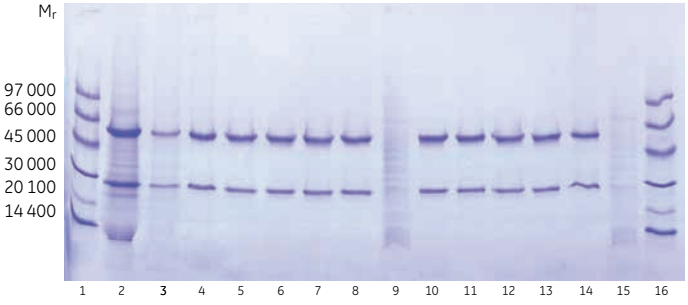


Fig 4. Yield for HiScreen MabSelect Xtra, cycles 1 to 10, in the robustness study.

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

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



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 ten 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 medium 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. As expected, MabSelect Xtra, the medium specifically designed for high capacity, clearly displays the best result.

Figure 7, which shows DBC data for two MAbs run on HiScreen MabSelect SuRe LX, confirms the observation that DBC increases with residence time. Both MabSelect SuRe and MabSelect SuRe LX have an alkali-tolerant, Protein A-derived ligand, but the latter has been optimized to give very high DBC for high-titer cultures of antibodies. Direct comparisons of both media for a range of MAbs (data not shown here) reveal that MabSelect SuRe LX has the highest DBC of the two.

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 rates:	1.15–3.9 ml/min (150–500 cm/h)
System:	ÄKTAexplorer 10

Dynamic binding capacity for different HiScreen MabSelect columns

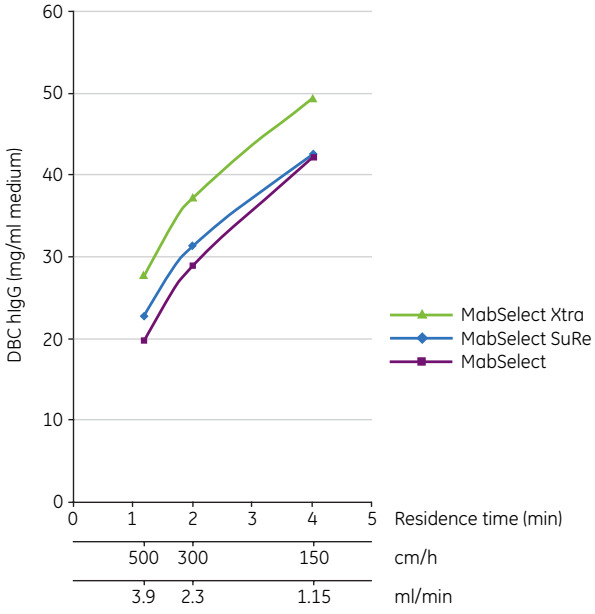


Fig 6. Comparison of DBC for hIgG on HiScreen MabSelect, HiScreen MabSelect Xtra, and HiScreen MabSelect SuRe.

Running conditions

Column:	HiScreen MabSelect SuRe LX, 4.7 ml
Sample:	Gammanorm human IgG (Octapharma), 5 mg/ml MAb from host cell-clarified feed
Binding buffer:	PBS, pH 7.4
Elution buffer:	0.1 M acetic acid, pH 3.0
Flow rates:	4.65 to 0.46 ml/min (Residence times: 1 to 10 min)
System:	ÄKTAexplorer 10

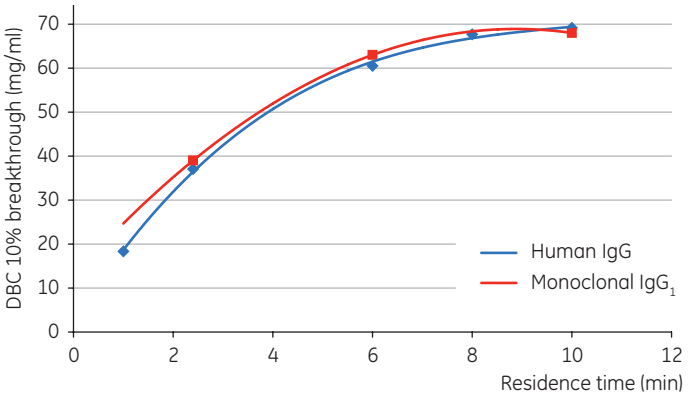
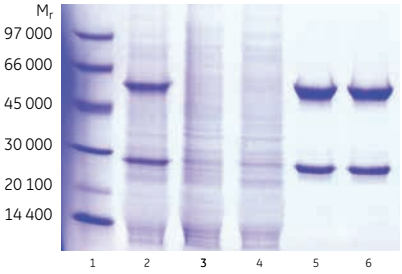


Fig 7. The dynamic binding capacity of MabSelect SuRe LX increases as a function of residence time in the column. Comparative data also show that MabSelect SuRe LX has a higher DBC than MabSelect SuRe at equivalent residence times.

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. The results for the two columns were compared (Figs 8 and 9).



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

Fig 8. 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.

Running conditions

Columns:	HiScreen MabSelect SuRe, (0.77 x 10 cm), 4.7 ml XK 16/20 packed with MabSelect SuRe, (1.6 x 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 rates:	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

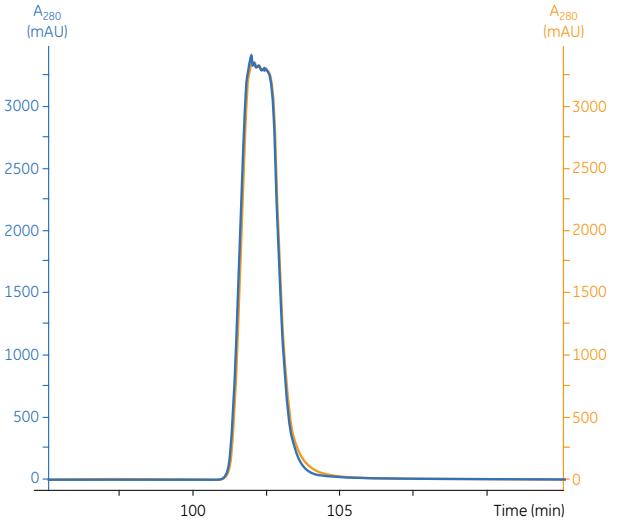


Fig 9. Overlay of UV absorbance curves (280 nm) for eluted peaks from HiScreen MabSelect SuRe (blue) and XK 16/20 packed with MabSelect SuRe (orange).

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

HiScreen Ni FF was used for purification of histidine-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.

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

Running conditions

Column:	HiScreen Ni FF
Sample:	40 ml 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:	300 cm/h (2.3 ml/min)
Linear gradient:	0% to 100% elution buffer in 10 CV
System:	ÄKTA avant 25

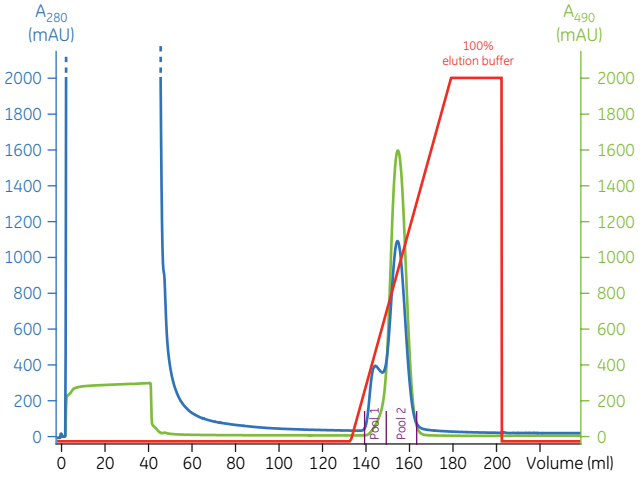
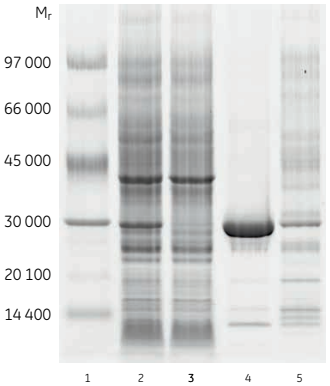


Fig 10. Purification of GFP-(His)$_6$ expressed in *E. coli* BL21 on HiScreen Ni FF. Indicated pools are analyzed by SDS-PAGE (Fig 11).



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.

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

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 ten 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

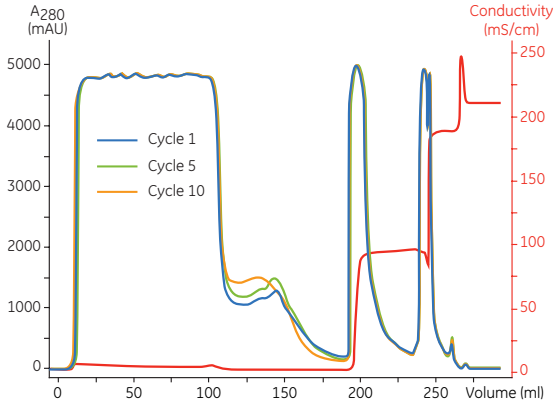


Fig 12. Two HiScreen Capto Q columns connected in series give a 20 cm bed height. Overlay of chromatograms for cycles 1, 5, and 10.

Table 11. DBC before and after cycles 5 and 10

2 x HiScreen Capto Q	DBC for BSA [mg/ml medium]	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.

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.

Running conditions

Columns:	2 × HiScreen Capto SP ImpRes (0.77 x 20 cm), 9.3 ml total, HiScale 16/20 packed with Capto SP ImpRes (1.6 x 20 cm) 40 ml, AxiChrom 50 packed with Capto SP ImpRes (5 x 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 rates:	150 cm/h 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

A) Two HiScreen columns in series (9.3 ml)

B) HiScale column (40 ml)

C) AxiChrom 50 column (398 ml)

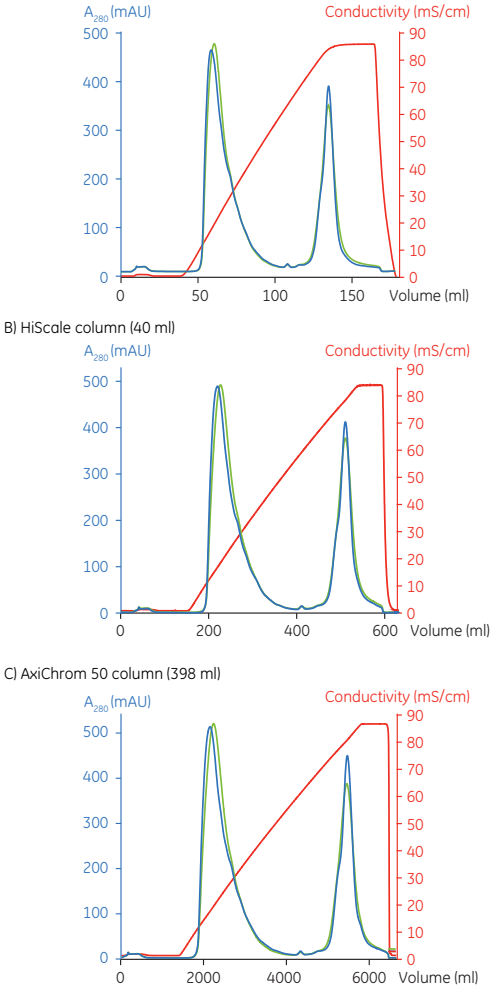


Fig 13. Separation of BSA and lactoferrin at three increasing scales (9.3, 40 and 398 ml columns) and two flow rates (150 cm/h [green curves] and 300 cm/h [blue curves]). Chromatographic performance was maintained during scale-up and at increased flow rate.

7. High resolution for late-stage purification and polishing

GE Healthcare Life Sciences offers several "S-type" cation exchange media. Even though the charged groups of the S-ligand are similar for these media, 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 peaks, Capto S ImpAct, Capto SP ImpRes, and SP Sepharose™ High Performance deliver improved resolution compared to Capto S. This is due to the three former having smaller bead sizes, 50 µm, 40 µm, and 34 µm, respectively, compared to Capto S (90 µm). Capto S is also designed for the capture step. The chromatograms in Figure 14 also indicate that the four media have a high separation potential, as the selectivity of the two test proteins clearly differs.

Running conditions

Column:	(A) HiScreen Capto S; (B) HiScreen Capto S ImpAct; (C) HiScreen SP Sepharose HP; (D) 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 (5.4 min residence time)
Gradient:	Linear, 0 to 100% elution buffer in 20 CV
System:	ÄKTA™ avant 25

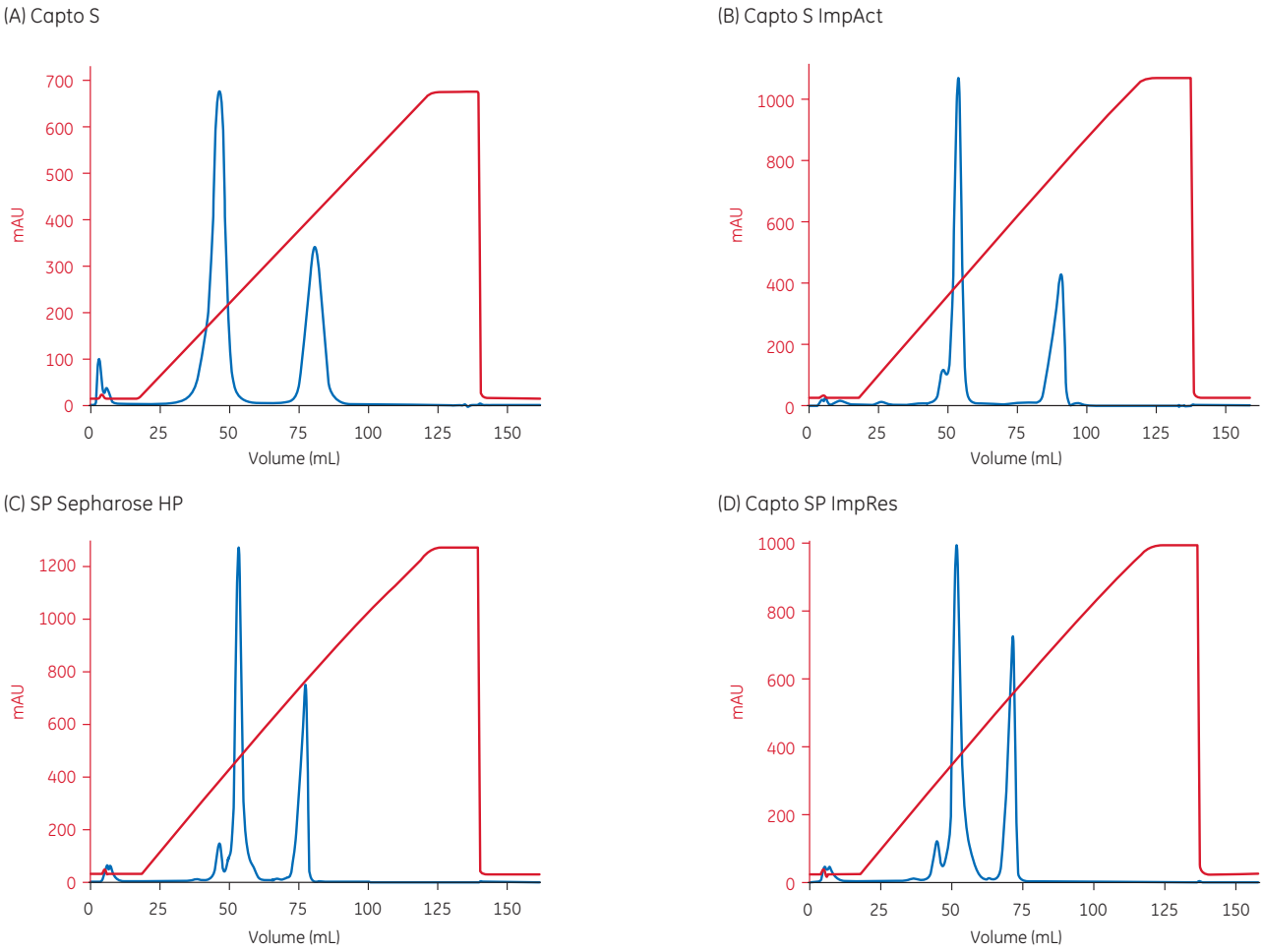


Fig 14. Chromatograms showing different selectivity and resolution, comparing four cation ion exchange media. Peaks (left to right) are α-chymotrypsinogen A and lysozyme. Compared to (A) Capto S (90 µm), the smaller bead size gives (B) Capto S ImpAct (50 µm), (C) SP Sepharose HP (34 µm), and (D) Capto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

8. Screening on different HIC media

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 contributes to the final hydrophobicity of the medium and hence also the selectivity. The binding capacity of HIC media 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 media.

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 volumes:	4.7 ml
Sample:	Mixture of cytochrome C, ribonuclease A, β-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 rates:	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

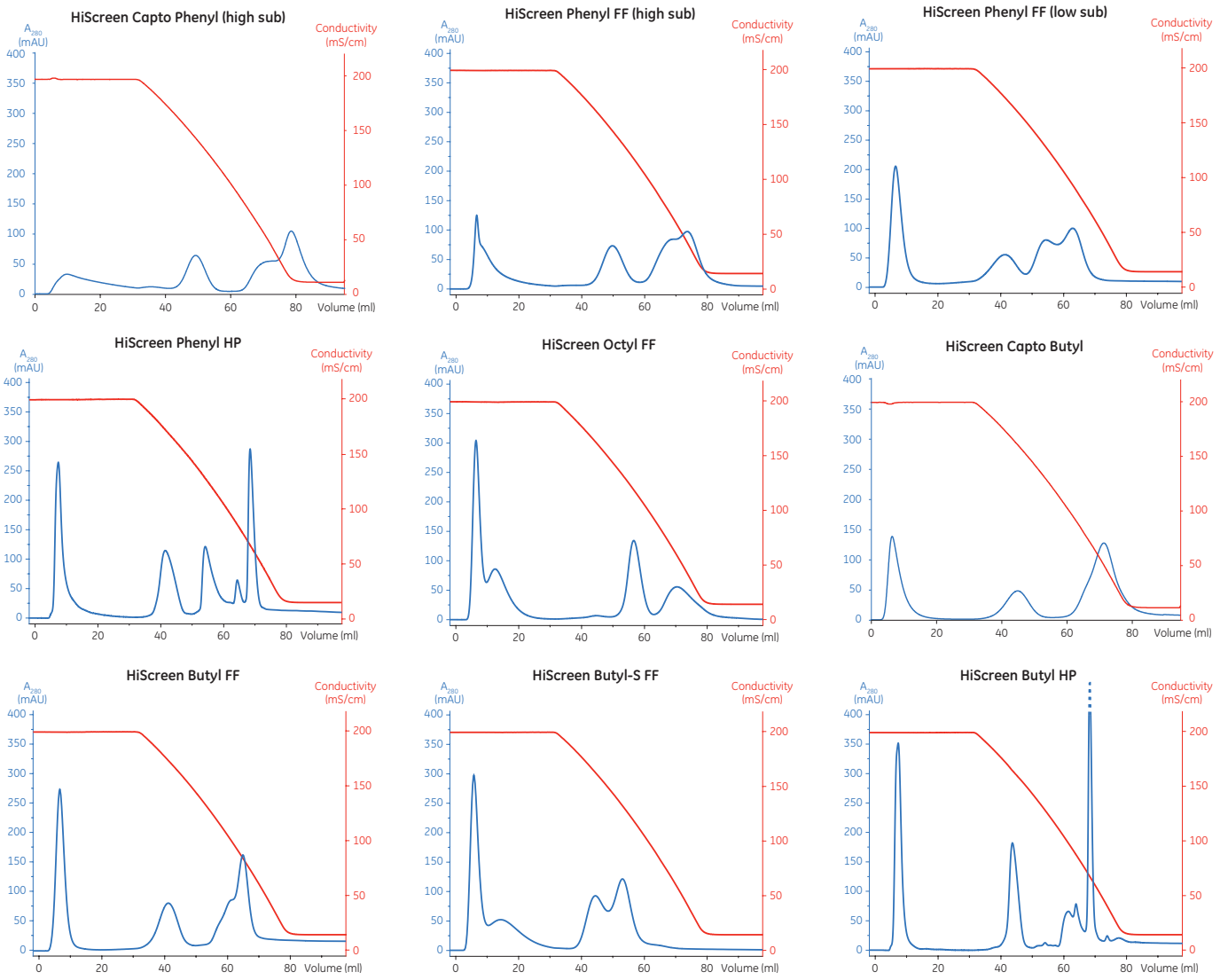


Fig 15. Comparison of the selectivity of nine different HiScreen HIC columns.

Comparison of HiScreen with other column formats

Chromatography media 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 media 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	Code number
HiScreen Capto L	1 × 4.7 ml	17-5478-14
HiScreen Capto Q ImpRes	1 × 4.7 ml	17-5470-15
HiScreen Capto SP ImpRes	1 × 4.7 ml	17-5468-15
HiScreen Capto Q	1 × 4.7 ml	28-9269-78
HiScreen Capto S	1 × 4.7 ml	28-9269-79
HiScreen Capto S ImpAct	1 × 4.7 ml	17-3717-47
HiScreen Capto Core 700	1 × 4.7 ml	17-5481-15
HiScreen Capto MMC	1 × 4.7 ml	28-9269-80
HiScreen Capto MMC ImpRes	1 × 4.7 ml	17-3716-20
HiScreen Capto adhere	1 × 4.7 ml	28-9269-81
HiScreen Capto adhere ImpRes	1 × 4.7 ml	17-3715-20
HiScreen Capto DEAE	1 × 4.7 ml	28-9269-82
HiScreen Capto Blue	1 × 4.7 ml	28-9924-74
HiScreen Capto Phenyl (high sub)	1 × 4.7 ml	28-9924-72
HiScreen Capto Phenyl ImpRes	1 × 4.7 ml	17-5484-10
HiScreen Capto Butyl	1 × 4.7 ml	28-9924-73
HiScreen MabSelect	1 × 4.7 ml	28-9269-73
HiScreen MabSelect Xtra	1 × 4.7 ml	28-9269-76
HiScreen MabSelect SuRe	1 × 4.7 ml	28-9269-77
HiScreen MabSelect SuRe LX	1 × 4.7 ml	17-5474-15
HiScreen Ni FF	1 × 4.7 ml	28-9782-44
HiScreen IMAC FF	1 × 4.7 ml	28-9505-17
HiScreen Q FF	1 × 4.7 ml	28-9505-10
HiScreen DEAE FF	1 × 4.7 ml	28-9782-45
HiScreen SP FF	1 × 4.7 ml	28-9505-13
HiScreen Q HP	1 × 4.7 ml	28-9505-11
HiScreen SP HP	1 × 4.7 ml	28-9505-15
HiScreen Blue FF	1 × 4.7 ml	28-9782-43
HiScreen Phenyl HP	1 × 4.7 ml	28-9505-16
HiScreen Phenyl FF (high sub)	1 × 4.7 ml	28-9269-88
HiScreen Phenyl FF (low sub)	1 × 4.7 ml	28-9269-89
HiScreen Butyl HP	1 × 4.7 ml	28-9782-42
HiScreen Butyl FF	1 × 4.7 ml	28-9269-84
HiScreen Butyl-S FF	1 × 4.7 ml	28-9269-85
HiScreen Octyl FF	1 × 4.7 ml	28-9269-86

Related products

IEX*

Product	Quantity	Code number
HiTrap Capto Q ImpRes	5 × 1 ml	17-5470-51
HiTrap Capto SP ImpRes	5 × 1 ml	17-5468-51
HiTrap Capto Q	5 × 1 ml	11-0013-02
HiTrap Capto S	5 × 1 ml	17-5441-22
HiTrap Capto S ImpAct	5 × 1 ml	17-3717-51
HiTrap Capto DEAE	5 × 1 ml	28-9165-37
HiTrap Q FF	5 × 1 ml	17-5053-01
HiTrap DEAE FF	5 × 1 ml	17-5055-01
HiTrap SP FF	5 × 1 ml	17-5054-01
HiTrap Q HP	5 × 1 ml	17-1153-01
HiTrap SP HP	5 × 1 ml	17-1151-01
HiPrep™ Q FF 16/10	1 × 20 ml	28-9365-43
HiPrep DEAE FF 16/10	1 × 20 ml	28-9365-41
HiPrep SP FF 16/10	1 × 20 ml	28-9365-44
Capto Q ImpRes†	100 ml	17-5470-02
Capto SP ImpRes†	100 ml	17-5468-02
Capto Q†	100 ml	17-5316-02
Capto S†	100 ml	17-5441-01
Capto S ImpAct†	100 ml	17-3717-02
Capto DEAE†	100 ml	17-5443-01
Q Sepharose High Performance†	75 ml	17-1014-01
SP Sepharose High Performance†	75 ml	17-1087-01
Q Sepharose Fast Flow†	300 ml	17-0510-01
SP Sepharose Fast Flow†	300 ml	17-0729-01
DEAE Sepharose Fast Flow†	500 ml	17-0709-01

AC*

HiTrap Protein L	5 × 1 ml	17-5478-51
HiTrap MabSelect SuRe	5 × 1 ml	11-0034-93
HiTrap MabSelect	5 × 1 ml	28-4082-53
HiTrap MabSelect Xtra	5 × 1 ml	28-4082-58
HiTrap IMAC FF	5 × 1 ml	17-0921-02
HisTrap™ FF	5 × 1 ml	17-5319-01
HiTrap Blue HP	5 × 1 ml	17-0412-01
Capto L	200 ml	17-5478-02
Capto Blue†	25 ml	17-5448-01
	500 ml	17-5448-02
MabSelect SuRe†	200 ml	17-5438-02
MabSelect SuRe LX†	200 ml	17-5474-02
MabSelect†	200 ml	17-5199-02
MabSelect Xtra†	200 ml	17-5269-02
Ni Sepharose 6 Fast Flow†	500 ml	17-5318-03
IMAC Sepharose Fast Flow†	100 ml	17-0921-08
Blue Sepharose 6 Fast Flow†	500 ml	17-0948-02

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

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

Related products

Multimodal*	Quantity	Code number
HiTrap Capto Core 700	5 × 1 ml	17-5481-51
HiTrap Capto MMC	5 × 1 ml	11-0032-73
HiTrap Capto MMC ImpRes	5 × 1 ml	17-3716-10
HiTrap Capto adhere	5 × 1 ml	28-4058-44
HiTrap Capto adhere ImpRes	5 × 1 ml	17-3715-10
Capto Core 700†	100 ml	17-5481-02
Capto MMC†	100 ml	17-5317-02
Capto MMC ImpRes†	25 ml	17-3716-01
Capto adhere†	100 ml	17-5444-01
Capto adhere ImpRes†	25 ml	17-3715-01
Capto MMC ImpRes	100 ml	17-3716-02
Capto adhere ImpRes	100 ml	17-3715-02

HIC*		
HiTrap HIC Selection Kit	7 × 1 ml	28-4110-07
HiTrap Capto Phenyl ImpRes	5 × 1 ml	17-5484-11
HiTrap Capto Phenyl ImpRes	5 × 5 ml	17-5484-12
HiTrap Phenyl HP	5 × 1 ml	17-1351-01
HiTrap Phenyl FF (high sub)	5 × 1 ml	17-1355-01
HiTrap Phenyl FF (low sub)	5 × 1 ml	17-1353-01
HiTrap Butyl HP	5 × 1 ml	28-4110-01
HiTrap Butyl FF	5 × 1 ml	17-1357-01
HiTrap Butyl-S FF	5 × 1 ml	17-0978-13
HiTrap Octyl FF	5 × 1 ml	17-1359-01
HiPrep Phenyl FF (high sub) 16/10	1 × 20 ml	28-9365-45
HiPrep Phenyl FF (low sub) 16/10	1 × 20 ml	28-9365-46
HiPrep Butyl FF 16/10	1 × 20 ml	28-9365-47
HiPrep Octyl FF 16/10	1 × 20 ml	28-9365-48
Capto Phenyl (high sub)†	25 ml	17-5451-01
Capto Phenyl ImpRes†	25 ml	17-5484-01
Capto Butyl†	25 ml	17-5459-01
Phenyl Sepharose High Performance†	75 ml	17-1082-01
Butyl Sepharose High Performance†	200 ml	17-5432-02
Phenyl Sepharose 6 Fast Flow (low sub)†	200 ml	17-0965-05
Phenyl Sepharose 6 Fast Flow (high sub)†	200 ml	17-0973-05
Butyl-S Sepharose 6 Fast Flow†	200 ml	17-0978-02
Butyl Sepharose 4 Fast Flow†	200 ml	17-0980-01
Octyl Sepharose 4 Fast Flow†	200 ml	17-0946-02

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

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

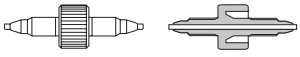
Desalting and buffer exchange

HiTrap Desalting	5 × 5 ml	17-1408-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02

Related literature

Antibody Purification Handbook	18-1037-46
Antibody Purification, Selection Guide	28-9351-97
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
Affinity Chromatography, Column and Media Selection Guide	18-1121-86
Recombinant Protein Purification Handbook, Principles and Methods	18-1142-75
Ni Sepharose and IMAC Sepharose, Selection Guide	28-4070-92
Ion Exchange Chromatography and Chromatofocusing Handbook, Principles and Methods	11-0004-21
Ion Exchange Columns and Media, Selection Guide	18-1127-31
Hydrophobic Interaction Chromatography and Reversed Phase Chromatography Handbook, Principles and Methods	11-0012-69
Gel Filtration Handbook, Principles and Methods	18-1022-18
Prepacked chromatography columns for ÄKTA systems, Selection Guide	28-9317-78

Related products

Accessories	Usage	Quantity	Code number
HiTrap/HiPrep, 1/16" male connector for ÄKTA design	Connection of columns with 1/16" fittings to ÄKTA design	8	28-4010-81
Union 1/16" male/1/16" male (0.5 mm i.d.)	Connecting two HiScreen columns in series 	2	18-1120-93
Fingertight stop plug, 1/16"‡	Sealing top and bottom of a HiScreen column 	5	11-0003-55

‡ One fingertight stop plug is connected to the inlet and the outlet of each HiScreen column at delivery.

For local office contact information, visit
www.gelifesciences.com/contact

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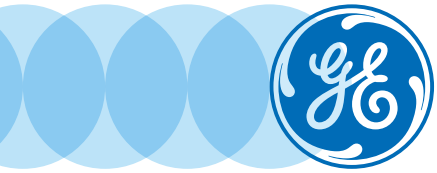

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