

HiScreen™ Phenyl FF (high sub)

HiScreen Phenyl FF (low sub)

HiScreen Phenyl HP

HiScreen Butyl FF

HiScreen Butyl HP

HiScreen Butyl-S FF

HiScreen Octyl FF

HiScreen Phenyl FF (high sub), HiScreen Phenyl FF (low sub), HiScreen Phenyl HP, HiScreen Butyl FF, HiScreen Butyl HP, HiScreen Butyl-S FF and HiScreen Octyl FF are prepacked, ready to use hydrophobic interaction chromatography (HIC) columns. The columns are ideal for screening of selectivity, binding and elution conditions, as well as small scale purifications.

The HiScreen HIC columns provide fast, reproducible and easy separations in a convenient format. The columns are used in an optimal way with liquid chromatography systems such as ÄKTA™.



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1 Product description

HiScreen column characteristics

HiScreen columns are made of biocompatible polypropylene that does not interact with biomolecules. The columns are delivered with stoppers at the inlet and outlet. The arrow on the column label indicates the column orientation and the recommended flow direction, see Figure 1.

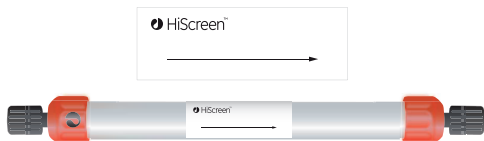


Fig 1. HiScreen column

For easy scaling-up and when a higher bed height is required, two columns can easily be connected in series using a union to give 20 cm bed height (see Section *Scaling up*).

Note: *HiScreen columns cannot be opened or refilled*

Note: *Make sure that the connector is tight to prevent leakage.*

Table 1. Characteristics of HiScreen column

Column volume (CV)	4.7 ml
Column dimensions	0.77 × 10 cm
Column hardware pressure limit ¹	8 bar (0.8 MPa)

¹ 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.

Properties of HIC media

The HIC media are based on the highly cross-linked beaded agarose matrices, Sepharose™ Fast Flow and Sepharose High Performance.

The media have excellent flow properties with high physical and chemical stabilities. All Sepharose matrices show virtually no non-specific adsorption and are resistant to microbial degradation due to the presence of the unusual sugar, 3,6-anhydro-L-galactose. The hydrophobic ligands are coupled to the monosaccharide units via glycidylethers. The resulting ether bonds are both stable and uncharged. Characteristics of the different HIC media are listed in Table 2 and 3.

Phenyl Sepharose 6 Fast Flow

Phenyl Sepharose 6 Fast Flow is ideal for initial and intermediate step purifications requiring a matrix with medium to high hydrophobicity. Two degrees of substitution grades are available (high sub and low sub), which increases the possibility of finding the best selectivity and capacity for a given application.

Phenyl Sepharose High Performance

Phenyl Sepharose High Performance is based on a 34 µm bead size and is ideal 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).

Butyl Sepharose 4 Fast Flow

Butyl Sepharose 4 Fast Flow is intended for initial and intermediate step purifications requiring a matrix with low to medium hydrophobicity. Butyl Sepharose 4 Fast Flow often works efficiently with rather 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.

Butyl Sepharose High Performance

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

Butyl-S Sepharose 6 Fast Flow

Butyl-S Sepharose 6 Fast Flow is the least hydrophobic medium and is intended for purification or removal of strongly hydrophobic biomolecules at low salt concentrations, with high recovery and low risk of denaturation.

The main differences between Butyl-S Sepharose 6 Fast Flow and Butyl Sepharose 4 Fast Flow are the length of their spacer arms, the concentration of the immobilized ligands, and the type of connector atom (O-ether or S-ether) linking each ligand to the Sepharose base matrix. Butyl-S Sepharose 6 Fast Flow contains a sulfur atom as a linker between the spacer arm and the butyl ligand.

Octyl Sepharose 4 Fast Flow

Octyl Sepharose 4 Fast Flow differs in hydrophobic characteristics from the phenyl and butyl ligands and is an important complement to the other hydrophobic matrices.

Table 2. Characteristics of Phenyl Sepharose 6 FF (high and low sub), Phenyl Sepharose HP and Octyl Sepharose 4 FF

	Phenyl Sepharose 6 FF (high and low sub)	Phenyl Sepharose HP	Octyl Sepharose 4 FF
Ligand	Phenyl	Phenyl	Octyl
Matrix	6% cross-linked agarose	6% cross-linked agarose	4% cross-linked agarose
Average particle size (d_{50v})¹	90 μm	34 μm	90 μm
Ligand density	40 $\mu\text{mol/ml}$ medium (high sub) 25 $\mu\text{mol/ml}$ medium (low sub)	25 $\mu\text{mol/ml}$ medium	5 $\mu\text{mol/ml}$ medium
Recommended flow velocity²	300 cm/h	30 to 150 cm/h	150 cm/h
Maximum flow velocity²	450 cm/h	150 cm/h	240 cm/h
pH stability³			
Working range	3 to 13	3 to 13	3 to 13
Cleaning-in-place	2 to 14	2 to 14	2 to 14
Chemical stability	All commonly used aqueous buffers, 1 M acetic acid, 1 M NaOH, 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol and 70% ethanol		
Storage	4°C to 30°C in 20% ethanol or 0.01 M NaOH		

¹ d_{50v} is the average particle size of the cumulative volume distribution.

² Water at room temperature.

For viscous buffers and samples the flow velocity must be optimized. Starting with a low flow velocity is recommended.

³ Working range: pH interval where the medium can be handled without significant change in function.

Cleaning-in-place: pH interval where the medium can be subjected to cleaning-in-place without significant change in function.

Table 3. Characteristics of Butyl Sepharose 4 FF, Butyl Sepharose HP and Butyl-S Sepharose 6 FF

	Butyl Sepharose 4 FF	Butyl Sepharose HP	Butyl-S Sepharose 6 FF
Ligand	Butyl	Butyl	Butyl-S
Matrix	4% cross-linked agarose	6% cross-linked agarose	6% cross-linked agarose
Average particle size (d_{50v})¹	90 µm	34 µm	90 µm
Ligand density	40 µmol/ml medium	50 µmol/ml medium	10 µmol/ml medium
Recommended flow velocity²	150 cm/h	30 to 150 cm/h	300 cm/h
Maximum flow velocity²	240 cm/h	150 cm/h	450 cm/h
pH stability³			
Working range	3 to 13	3 to 13	3 to 13
Cleaning-in-place	2 to 14	2 to 14	2 to 14
Chemical stability	All commonly used aqueous buffers, 1 M acetic acid, 1 M NaOH, 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol and 70% ethanol		
Storage	4°C to 30°C in 20% ethanol or 0.01 M NaOH		

¹ d_{50v} is the average particle size of the cumulative volume distribution.

² Water at room temperature.

For viscous buffers and samples the flow velocity must be optimized. Starting with a low flow velocity is recommended.

³ Working range: pH interval where the medium can be handled without significant change in function.

Cleaning-in-place: pH interval where the medium can be subjected to cleaning-in-place without significant change in function.

2 General process development

HiScreen column format is ideal to use for parameter and method optimization and for robustness testing when developing a new purification process. The small column volume, 4.7 ml, and the 10 cm bed height makes it possible to perform scalable experiments at relevant process flow rates. If necessary, two columns can easily be connected in series with a union to give 20 cm bed height (see Section *Scaling up*).

The figure below outlines typical steps during general process development.

Already from start in process development it is necessary to consider process cost, cleaning of the medium and environmental constraints.

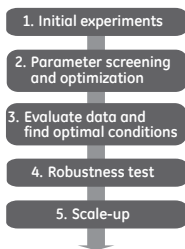


Fig 2. Typical steps during process development.

Design of Experiments (DoE) is an effective tool for investigating the effect of several parameters on protein recovery in order to establish the optimal purification protocol.

A common approach in DoE is to define a reference experiment (center point) and perform representative experiments around that point. Some initial experiments are required in order to define the center point and the variable ranges. DoE may be used for parameter screening and optimization as well as robustness testing.

The robustness of a process is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters, and provides an indication of its reliability during normal usage. An objective of a robustness test is the evaluation of factors potentially causing variability in the responses of the method, for example, purity and yield. For this purpose, small variations in method parameters are introduced.

For scale-up, see Section *Scaling up*.

3 Optimization

General

Separation of biomolecules on HIC media is based on interplay between the hydrophobicity of the medium, the nature and composition of the sample, the prevalence and distribution of surface-exposed hydrophobic amino acid residues, and the type and concentration of salt in the binding buffer. Unlike reversed phase chromatography (RPC), which is a separation method closely related to HIC, the binding of biological solutes to HIC media is promoted, or otherwise modulated, by the presence of relatively high concentrations of anti-chaotropic salts such as ammonium sulfate and sodium sulfate (Figure 3). Elution of bound solutes is achieved simply by stepwise or gradient elution with buffers of low salt content.

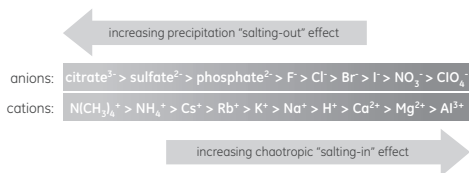


Fig 3. The Hofmeister series of some anions and cations arranged according to their effects on the solubility of protein in aqueous solutions. Increasing the salting-out effect promotes hydrophobic interactions and increases the binding capacity of the HIC medium for proteins. The opposite situation dominates when the chaotropic effect of the salts is increased.

HIC media available from GE Healthcare are produced as a graded series of hydrophobic media based on alkyl or aryl ligands attached to a hydrophilic base matrix, for example Capto™ and Sepharose. In each instance, the type and concentration of ligand has been optimized to cover the range of hydrophobicities of the proteins in a biological extract, varying from weak to moderate to strong hydrophobic proteins. This strategy results in HIC media for "all occasions" where the emphasis is on high recovery, purity, and reduced risk for denaturation of the target proteins in a biological extract.

Factors affecting HIC

The main parameters to consider when selecting an HIC medium and optimizing its chromatographic performance is:

- The nature of the base matrix (e.g., agarose, organic co-polymers, etc.)
- Structure of the ligand
- Concentration of the ligand
- Characteristics of the target protein and other sample components
- Type of salt
- Concentration of salt
- Temperature
- pH

Of these parameters, the structure and concentration of ligand as well as the type and concentration of salt added during the binding step are of highest importance in determining the outcome of an HIC event. In general, the type of immobilized ligand determines its binding selectivity toward the proteins in a sample while its concentration determines its binding capacity.

HIC media fall into two groups, depending on their interactions with sample components:

- Straight alkyl chains (butyl, octyl) show a “pure” hydrophobic character.
- Aryl ligands (phenyl) show a mixed mode behavior, where both aromatic and hydrophobic interactions as well as lack of charge play simultaneous roles.

The choice of ligand must be determined empirically through screening experiments for each individual separation problem.

Target protein

The target protein characteristics (in an HIC context) are usually not known since minimal data are available in this respect. There are some published data regarding the hydrophobicity indices for a number of purified proteins based on amino acid composition, the number and distribution of surface-exposed hydrophobic amino acids, and the order of their elution from RPC columns but few, if any, have proved to be useful when purifying a protein in a real biological sample. For this and other reasons, the binding behavior of a protein exposed to an HIC medium has to be determined on a case-by-case basis.

Solvent

The solvent is one of the most important parameters, which influence the capacity and selectivity in HIC. In general, the binding process is more selective than the elution process. It is therefore important to optimize the start buffer with respect to pH, type of solvent, type of salt and concentration of salt.

Salts

The addition of various "salting-out" salts to the sample promotes ligand-protein interactions in HIC. As the concentration of salt is increased, the amount of bound protein increases up to the precipitation point for the protein. Each type of salt differs in its ability to promote hydrophobic interactions and it may be worthwhile testing several salts.

The most commonly used salts are $(\text{NH}_4)_2\text{SO}_4$, Na_2SO_4 , NaCl , KCl and $\text{CH}_3\text{COONH}_4$. At a given concentration, ammonium sulfate often gives the best resolution of a mixture of standard proteins compared to other salts. If sodium chloride is used, a concentration of up to 3 to 4 M is usually needed. Due to instability, ammonium sulfate is not suitable when working at pH values above 8.0. Sodium sulphate is also a very good salting-out agent but protein solubility problems may exclude its use at high concentrations.

Protein binding to HIC adsorbents is promoted by moderate to high concentrations of "salting-out" salts, which also have a stabilizing influence on protein structure. Elution is achieved by a linear or step-wise decrease in concentration of the salt.

The HIC medium should bind the protein of interest at a reasonably low concentration of salt. Binding conditions are dependent on the salt chosen. The salt concentration should be below that which causes precipitation of proteins in the sample.

- If the substance does not bind, a more hydrophobic medium should be chosen.
- If the substance binds so strongly that non-polar additives are required for elution, a HiScreen column with a less hydrophobic medium should be tried.

The bound protein should be eluted from the column with high recovery.

pH

The effect of pH is not well established. In general, an increase in pH above 8.5 weakens hydrophobic interactions whereas a decrease in pH below 5.0 results in an apparent increase in the retention of proteins on HIC media. In the range of pH 5 to 8.5, the effect seems to be minimal or insignificant.

Temperature

It is generally accepted that the binding of proteins to HIC media is entropy driven, which implies that the solute-media interaction increases with increased temperature. In some instances, the reverse effect has been observed. In practical work, you should be aware that a purification process developed at room temperature might not be reproduced in the cold room, or vice versa. In other instances, temperature control is mandatory in order to obtain reproducible results from run to run.

Additives

Sometimes it is necessary to weaken the protein-ligand interactions by including different additives. Commonly used are water-miscible alcohols (propanol, ethylene glycol), detergents (SDS) and solutions of chaotropic salts (lithium perchlorate, urea, guanidine hydrochloride).

Automated buffer preparation

Users of ÄKTA chromatography systems with BufferPrep or BufferPro functionality can select from a range of buffer recipes to conveniently screen media over a range of pH values and elution conditions

4 Operation

Prepare buffers

When using high salt concentration buffers, especially ammonium sulfate, use a salt of high quality to prevent baseline drift. Commonly used salts are ammonium sulfate, sodium chloride and sodium sulfate.

Selection of buffering ions is not critical for hydrophobic interaction. Phosphate buffers are often used.

The following buffers are recommended:

Start buffer

1.7 M ammonium sulfate, 50 mM sodium phosphate, pH 7.0

Elution buffer

50 mM sodium phosphate, pH 7.0

Note: *Water and chemicals used for buffers should be of high purity. It is highly recommended to filter buffers through a 0.22 μm or a 0.45 μm filter before use.*

Prepare the sample

Step	Action
1	<p>Adjust the sample to the composition of the start buffer, using one of these methods:</p> <ul style="list-style-type: none">• Dilute the sample with start buffer. Note: <i>The sample should be fully solubilized. If the sample starts to precipitate, reduce the ionic strength of the start buffer, or change to a different salt.</i>• Exchange buffer using a HiPrep™ 26/10 Desalting, HiTrap™ Desalting or PD-10 Desalting column (see Table below). Note: <i>Use buffer exchange if chaotropic agents, such as guanidine hydrochloride or urea have been used for initial solubilization as they will inhibit hydrophobic interaction.</i>
2	<p>Filter the sample through a 0.45 µm filter or centrifuge immediately before loading it to the column. This prevents clogging and increases the longevity of the column when loading large sample volumes.</p>

Table 4. Prepacked columns for desalting

Column	Code No.	Loading volume	Elution volume	Comments	Application
HiPrep 26/10 Desalting	17-5087-01	2.5 to 15 ml	7.5 to 20 ml	Prepacked with Sephadex™ G-25 Fine. Requires a laboratory pump or a chromatography system to run.	For desalting and buffer exchange of protein extracts ($M_r > 5000$).
HiTrap Desalting	17-1408-01	0.25 to 1.5 ml	1.0 to 2.0 ml	Prepacked with Sephadex G-25 Superfine. Requires a syringe or pump to run.	
PD-10 Desalting	17-0851-01	1.0 to 2.5 ml ¹ 1.75 to 2.5 ml ²	3.5 ml ¹ up to 2.5 ml ²	Prepacked with Sephadex G-25 Medium. Runs by gravity flow or centrifugation	For desalting, buffer exchange, and cleanup of proteins and other large biomolecules ($M_r > 5000$).
PD MiniTrap™ G-25	28-9180-07	0.1 to 0.5 ml ¹ 0.2 to 0.5 ml ²	1.0 ml ¹ up to 0.5 ml ²		
PD MidiTrap™ G-25	28-9180-08	0.5 to 1.0 ml ¹ 0.75 to 1.0 ml ²	1.5 ml ¹ up to 1.0 ml ²		

¹ Volumes with gravity elution² Volumes with centrifugation

Recommended flow rates

Table 5. Recommended flow rates for HiScreen HIC columns.

Column type	Flow velocity (cm/h)	Flow rate (ml/min)
HiScreen Phenyl FF (high sub)	300	2.3
HiScreen Phenyl FF (high sub)	300	2.3
HiScreen Phenyl HP	75	0.6
HiScreen Butyl FF	150	1.2
HiScreen Butyl HP	75	0.6
HiScreen Butyl-S FF	300	2.3
HiScreen Octyl FF	150	1.2

Purification

Flow rate: See Table 5

Column tubing: Choose the optimal tubing kit for the column and the application you intend to run. (i.d.: 0.25, 0.50 or 0.75 [mm]). A tubing with wider inner diameter gives broader peaks whereas a tubing with a smaller inner diameter gives higher back pressure.

Step	Action
------	--------

- | | |
|---|--|
| 1 | Remove the stoppers and connect the column to the system. Avoid introducing air into the column. |
|---|--|

Note:

To prevent leakage, ensure that the connectors are tight. Use fingertight 1/16" connector (28-4010-81).

- | | |
|---|--|
| 2 | Wash out the ethanol with at least 5 column volumes (CV) of distilled water or elution buffer at 0.6 to 1.2 ml/min (75 to 150 cm/h). |
| 3 | Equilibrate the column with 10 CV of start buffer at recommended flow rate (see Table 5). |

Step	Action
4	Adjust the sample to the chosen starting pH and conductivity and load on the column. If the sample has a high viscosity, use a lower flow rate during sample loading (approximately 0.5 to 1 ml/min (65 to 130 cm/h)).
5	Wash with 5 to 10 CV of start buffer or until the UV trace of the effluent returns to near baseline.
6	<p>Elute, either by linear gradient elution or a step elution, see below. If required, the collected eluted fractions can be buffer exchanged or desalted using columns listed in Table 4.</p> <ul style="list-style-type: none"> • <i>Linear gradient elution</i> Elute with 0 to 100% elution buffer in 10 to 20 CV. • <i>Step elution</i> Elute with 2 to 5 CV of elution buffer at a salt concentration lower than in the start buffer. Repeat, lowering the salt content at each step until the target protein has been eluted.
7	Wash with 5 CV of salt-free elution buffer to elute any remaining bound material.
8	If required, perform a CIP to clean the column.
9	Re-equilibrate with 5 to 10 CV of start buffer or until the UV baseline, eluent pH, and conductivity reach the required values.

Note: *Do not exceed the maximum recommended flow and back pressure for the column.*

5 Cleaning-In-Place (CIP)

General description

Correct preparation of samples and buffers should maintain columns in good condition. However, reduced performance, increased back pressure or blockage indicates that the column medium needs cleaning.

CIP removes very tightly bound, precipitated or denatured substances from the medium. If such contaminants are allowed to accumulate, they may affect the chromatographic properties of the prepacked column, reduce the capacity of the medium and, potentially, come off in subsequent runs. If the fouling is severe, it may block the column, increase back pressure and reduce flow rate.

CIP should be performed regularly to prevent the build-up of contaminants and to maintain the capacity, flow properties and general performance of prepacked columns. CIP is usually performed after the elution.

It is recommended to perform a CIP:

- When an increase in back pressure is seen.
- If reduced column performance is observed.
- Before first time use or after long term storage.
- Between runs when the same column is used for purification of different proteins to prevent possible cross-contamination.
- After every run with real feed.

CIP protocol

The HiScreen HIC media can normally be regenerated by washing with distilled water, but are also alkali-tolerant media. The nature of the sample will ultimately determine the final CIP protocol so the CIP procedure below may require optimization. NaOH concentration, contact time and frequency are typically the main parameters to vary during the optimization of the CIP. The CIP procedure below removes common contaminants.

- Regular cleaning with 0.5 to 1.0 M NaOH. NaOH has the ability to dissolve proteins and saponify fats.
- Strongly bound substances can be removed by washing with 5 to 10 CV of up to 70% ethanol or 30% isopropanol.

Flow rate: It is recommended to use a lower flow rate than during the purification.

Step	Action
1	Wash with 3 column volumes (CV) of water or elution buffer.
2	Wash with 4 CV 0.5 to 1.0 M NaOH at a low flow rate (approximately 0.5 to 1 ml/min (65 to 130 cm/h)).
3	Wash with at least 3 CV of water.

6 Scaling up

After optimizing the method at laboratory scale, the process is ready for scaling up.

For quick scale-up of purification, two HiScreen columns can easily be connected in series with a union (18-1120-93) to give 20 cm bed height. Note that the back pressure will increase. This can easily be addressed by lowering the flow rate.

Factors, such as clearance of critical impurities, may change when column bed height is modified and should be validated using the final bed height.

Scale-up is typically performed by keeping bed height and flow velocity (cm/h) constant while increasing bed diameter and flow rate (ml/min or l/h).

Bulk media is available for further scale-up, see Section *Ordering information*.

7 Adjusting pressure limits in chromatography system software

Pressure generated by the flow through a column affects the packed bed and the column hardware, see Figure below. Increased pressure is generated when running/using one or a combination of the following conditions:

- High flow rates
- Buffers or sample with high viscosity
- Low temperature
- A flow restrictor

Note: Exceeding the flow limit (see Table 2 and 3) may damage the column.

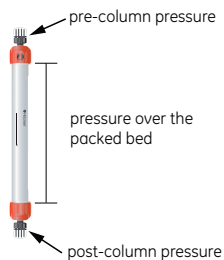


Fig 4. Pre-column and post-column measurements.

ÄKTA avant

The system will automatically monitor the pressures (pre-column pressure and pressure over the packed bed, Δp). The pre-column pressure limit is the column hardware pressure limit (see Table 1).

The maximum pressure the packed bed can withstand depends on media characteristics and sample/liquid viscosity. The measured value also depends on the tubing used to connect the column to the instrument.

ÄKTAexplorer™, ÄKTApurifier™, ÄKTAFFPLC™ and other systems with pressure sensor in the pump

To obtain optimal functionality, the pressure limit in the software may be adjusted according to the following procedure:

Step	Action
1	Replace the column with a piece of tubing. Run the pump at the maximum intended flow rate. Note the pressure as <i>total system pressure</i> , P1.
2	Disconnect the tubing and run the pump at the same flow rate used in step 1. Note that there will be a drip from the column valve. Note this pressure as P2.
3	Calculate the new pressure limit as a sum of P2 and the column hardware pressure limit (see Table 1). Replace the pressure limit in the software with the calculated value. The actual pressure over the packed bed (Δp) will during run be equal to actual measured pressure - <i>total system pressure</i> (P1).

Note: Repeat the procedure each time the parameters are changed.

8 Storage

Store the HiScreen HIC columns equilibrated with 5 to 10 CV 20% ethanol or 0.01 M NaOH at 4°C to 30°C. Do not freeze.

Ensure that the column is tightly sealed to avoid drying out.

Note: Never store the HiScreen HIC columns in a high salt concentration solution.

9 Troubleshooting

Problem	Possible cause/corrective action
High back pressure during the run	The column is clogged. <i>Clean the column, see Section Cleaning-in-place (CIP).</i> High viscosity of solutions. <i>Use lower flow rate.</i>
Gradual broadening of the eluate peak	Insufficient elution and CIP, caused by contaminants accumulating in the column. <i>Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.</i>
Gradual decrease in yield	Insufficient elution and CIP. <i>Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.</i>
Precipitation during sample loading/elution	Might be due due to high local salt concentrations. Optimize the start buffer/elution conditions. Perform a CIP.
Gradual increase in CIP peaks	Sub-optimal elution conditions and CIP. <i>Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.</i>
High back pressure during CIP	Proteins precipitated in column. <i>Make sure all components are eluted from column, optimize elution conditions and/or perform a CIP at lower flow rate.</i>
Reduced column performance despite optimized elution and CIP	<i>Change to a new column. The longevity of the column depends mainly on the sample and sample preparation.</i>

10 Ordering information

Product	Quantity	Code No.
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 Phenyl HP	1 × 4.7 ml	28-9505-16
HiScreen Butyl FF	1 × 4.7 ml	28-9269-84
HiScreen Butyl HP	1 × 4.7 ml	28-9782-42
HiScreen Butyl-S FF	1 × 4.7 ml	28-9269-85
HiScreen Octyl FF	1 × 4.7 ml	28-9269-86

Related products	Quantity	Code No
HiTrap HIC Selection Kit, 7 different HIC media	7 × 1 ml	28-4110-07
HiTrap Phenyl FF (high sub)	5 × 1 ml	17-1355-01
	5 × 5 ml	17-5193-01
HiTrap Phenyl FF (low sub)	5 × 1 ml	17-1353-01
	5 × 5 ml	17-5194-01
HiTrap Phenyl HP	5 × 1 ml	17-1351-01
	5 × 5 ml	17-5195-01
HiTrap Butyl FF	5 × 1 ml	17-1357-01
	5 × 5 ml	17-5197-01
HiTrap Butyl HP	5 × 1 ml	28-4110-01
	5 × 5 ml	28-4110-05
HiTrap Butyl-S FF	5 × 1 ml	17-0978-13
	5 × 5 ml	17-0978-14
HiTrap Octyl FF	5 × 1 ml	17-1359-01
	5 × 5 ml	17-5196-01

Related products	Quantity	Code No
HiPrep Phenyl FF (high sub) 16/10	1 x 20 ml	28-9365-45
HiPrep Phenyl FF (low sub) 16/10	1 x 20 ml	28-9365-46
HiPrep Butyl FF 16/10	1 x 20 ml	28-9365-47
HiPrep Octyl FF 16/10	1 x 20 ml	28-9365-48
Phenyl Sepharose 6 Fast Flow (low sub)	25 ml	17-0965-10
	200 ml ¹	17-0965-05
Phenyl Sepharose 6 Fast Flow (high sub)	25 ml	17-0973-10
	200 ml ¹	17-0973-05
Phenyl Sepharose High Performance	75 ml ¹	17-1082-01
Butyl Sepharose 4 Fast Flow	25 ml	17-0980-10
	200 ml	17-0980-01
	500 ml ¹	17-0980-02
Butyl High Performance	25 ml	17-5432-01
	200 ml ¹	17-5432-02
Butyl-S Sepharose 6 Fast Flow	25 ml	17-0978-10
	200 ml ¹	17-0978-02
Octyl Sepharose 4 Fast Flow	25 ml	17-0946-10
	200 ml ¹	17-0946-02

¹ Process-scale quantities are available. Please contact your local representative

Accessories HiScreen	Quantity	Code No
HiTrap/HiPrep, 1/16" male connector for ÄKTA <i>(For connection of columns with 1/16" fittings to ÄKTA)</i>	8	28-4010-81
Union 1/16" male/1/16" male with 0.5 mm i.d. <i>(For connecting two columns with 1/16" fittings in series)</i>	2	18-1120-93
Fingertight stop plug, 1/16" ¹ <i>(For sealing a HiScreen column)</i>	5	11-0003-55

¹ One fingertight stop plug is connected to the inlet and the outlet of each HiScreen column at delivery.

Related literature	Code No.
Hydrophobic Interaction Chromatography and Reversed Phase Chromatography, Principles and Methods	11-0012-69
Prepacked chromatography columns for ÄKTA systems, Selection Guide	28-9317-78

For local office contact information, visit
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