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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Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

1 Product description

HiScreen column characteristics

HiScreen columns are made of biocompatible polypropylene that does not interact with the biomolecules. The arrow on the column label shows the recommended flow direction.

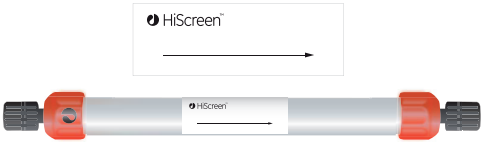

Fig 1. HiScreen column

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

Note: *Do not open or refill HiScreen columns.*

Note: *Check that the connector is tightened 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	0.8 MPa (8 bar, 116 psi)

Note: *The pressure over the packed bed varies depending on parameters such as the resin characteristics, sample/liquid viscosity, and the column tubing used.*

Properties of HIC resins

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

The resins have excellent flow properties with high physical and chemical stabilities. All Sepharose matrices show virtually no nonspecific 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 resins 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 ligand concentrations 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 particle size and is ideal for laboratory and intermediate process scale separations and for final step purifications where high resolution is needed. The ligand concentration 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 resins, 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 resin 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
Matrix	Cross-linked agarose, 6%, spherical	Cross-linked agarose, spherical	Cross-linked agarose, 4%, spherical
Ligand	Phenyl	Phenyl	Octyl
Average particle size, d_{50v} [1]	~ 90 µm	~ 34 µm	~ 90 µm
Ligand concentration	~ 45 µmol/mL resin (high sub) ~ 25 µmol/mL resin (low sub)	~ 25 µmol/mL resin	~ 5 µmol/mL resin
Recommended operating flow rate [2]	2.3 mL/min	0.6 mL/min	1.2 mL/min
Maximum operating flow rate	3.5 mL/min	1.2 mL/min	1.8 mL/min
pH stability, operational [3]	3 to 13	3 to 12	3 to 13
pH Stability, CIP [4]	2 to 14	3 to 12	2 to 14
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M NaOH [5], 8 M Urea, 6 M guanidine hydrochloride, 30% isopropanol, and 70% ethanol	Stable to commonly used aqueous buffers, 0.01 M NaOH, 8 M Urea, 6 M guanidine hydrochloride, 30% isopropanol, and 70% ethanol	Stable to commonly used aqueous buffers, 1.0 M NaOH[5], 8 M Urea, 6 M guanidine hydrochloride, 30% isopropanol, and 70% ethanol
Avoid	Solutions with pH <2, and phenol		
Storage	20% ethanol (or 0.01 M NaOH [6]), 4°C to 30°C	20% ethanol, 4°C to 30°C	20% ethanol (or 0.01 M NaOH[6]), 4°C to 30°C

1 Median particle size of the cumulative volume distribution.

2 Flow rates for the resins in HiScreen format at room temperature using buffers with the same viscosity as water. See also table 5.

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

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

[5] 1.0 M NaOH must only be used for cleaning purposes.

[6] In most cases, no long-term stability data has been generated by GE Healthcare in 0.01 M NaOH. In some cases, accelerated studies at elevated temperature indicate that storage in 0.01 M NaOH can be a viable option but no guarantees can be made regarding retained function of the product.

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
Matrix	Cross-linked agarose, 4%, spherical	Cross-linked agarose, spherical	Cross-linked agarose, 6%, spherical
Ligand	Butyl	Butyl	Butyl-S
Particle size (d_{50v}) [1]	~ 90 µm	~ 34 µm	~ 90 µm
Ligand concentration	~ 40 µmol/mL resin	~ 50 µmol/mL resin	~ 10 µmol/mL resin
Recommended operating flow rate [2]	1.2 mL/min	0.6 mL/min	2.3 mL/min
Maximum operating flow rate[2]	1.8 mL/min	1.2 mL/min	3.5 mL/min
pH stability, operational [3]	3 to 13	3 to 13	3 to 13
pH stability, CIP [4]	2 to 14	2 to 14	2 to 14
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M NaOH [5], 6 M guanidine hydrochloride, 30% isopropanol, and 70% ethanol		
Storage	20% ethanol (or 0.01 M NaOH [6]), 4°C to 30°C		

1 Median particle size of the cumulative volume distribution.

2 Flow rates for the resins in HiScreen format at room temperature using buffers with the same viscosity as water. See also table 5.

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

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

5 1.0 M NaOH must only be used for cleaning purposes.

6 In most cases, no long-term stability data has been generated by GE Healthcare Life Sciences in 0.01 M NaOH. In some cases, accelerated studies at elevated temperature indicate that storage in 0.01 M NaOH can be a viable option but no guarantees can be made regarding retained function of the product.

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 resin, and environmental constraints.

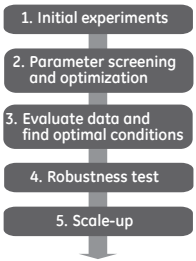

Fig 2. Typical steps during process development.

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. See handbook *Design of Experiments in Protein Production and Purification*. 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 can 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 resins depends on the hydrophobicity of the resin, 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 proteins to HIC resins is promoted, or otherwise modulated, by the presence of relatively high concentrations of anti-chaotropic salts such as ammonium sulfate and sodium sulfate (Fig 2). Elution of bound proteins is achieved simply by stepwise or gradient elution with buffers of low salt content.

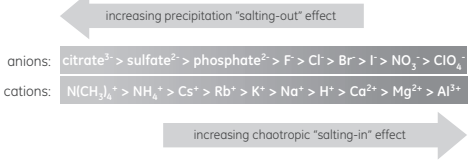

Fig 3. The Hofmeister series of some anions and cations arranged according to their effects on the solubility of protein in aqueous solutions. Increasing the salting-out effect promotes hydrophobic interactions and increases the binding capacity of the HIC resin for proteins. The opposite situation dominates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

HIC resins available from GE are produced as a graded series of hydrophobic resins 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 resins 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 a HIC resin 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 a HIC purification. 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 resins 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 a 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 a HIC resin 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 might be worthwhile to test several salts.

The most commonly used salts are $(NH_4)_2SO_4$, Na_2SO_4, NaCl, KCl and CH_3COONH_4. At a given concentration, ammonium sulfate often gives the best resolution of a mixture of standard proteins compared to other salts. Due to instability, ammonium sulphate is not suitable when working at pH values above 8.0. 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 might 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 resin should bind the protein of interest at a reasonably low concentration of salt. Binding conditions are dependent on the salt chosen. The salt concentration must be below that which causes precipitation of proteins in the sample.

- If the substance does not bind, a more hydrophobic resin should be chosen.
- If the substance binds so strongly that nonpolar additives are required for elution, a column with a less hydrophobic resin 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 resins. In the range of pH 5.0 to 8.5, the effect seems to be minimal or insignificant.

Temperature

It is generally accepted that the binding of proteins to HIC resins is entropy driven, which implies that the solute-resin interaction increases with increased temperature. In some instances, the reverse effect has been observed. In practical work, you need to 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 chaotrophic 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 resins 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 must 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	Adjust the sample to the composition of the start buffer, using one of these methods: • Dilute the sample with start buffer. ***Note:*** *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.* • Exchange buffer using a HiPrep™ 26/10 Desalting, HiTrap™ Desalting or PD-10 Desalting column (see table below). ***Note:*** *Use buffer exchange if chaotropic agents, such as guanidine hydrochloride or urea have been used for initial solubilization as they will inhibit hydrophobic interaction.*
2	Filter the sample through a 0.45 µm filter or centrifuge immediately before loading it to the column. This prevents clogging and increases the life time of the column when loading large sample volumes.

Table 4. Prepacked columns.

Column	Loading volume	Elution volume
HiPrep 26/10 Desalting [1]	2.5 to 15 mL	7.5 to 20 mL
HiTrap Desalting [2]	0.25 to 1.5 mL	1.0 to 2.0 mL
PD-10 Desalting [3]	1.0 to 2.5 mL [4] 1.75 to 2.5 mL [5]	3.5 mL Up to 2.5 mL
PD MiniTrap™ G-25	0.1 to 2.5 mL [4] 0.2 to 0.5 mL [5]	1.0 mL Up to 0.5 mL
PD MidiTrap™ G-25	0.5 to 1 mL [4] 0.75 to 1 mL [5]	1.5 mL Up to 1 mL

1 Prepacked with Sephadex™ G-25 Fine and requires a pump or a chromatography system to run.

2 Prepacked with Sephadex G-25 Superfine and requires a syringe or pump to run.

3 Prepacked with Sephadex G-25 and can be run by the gravity flow or centrifugation.

4 Volumes with gravity elution.

5 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)	Maximum operating flow velocity (cm/h)	Maximum operating flow rate (mL/min)
HiScreen Phenyl FF (high sub)	300	2.3	450	3.5
HiScreen Phenyl FF (high sub)	300	2.3	450	3.5
HiScreen Phenyl HP	75	0.6	150	1.2
HiScreen Butyl FF	150	1.2	230	1.8
HiScreen Butyl HP	75	0.6	150	1.2
HiScreen Butyl-S FF	300	2.3	450	3.5
HiScreen Octyl FF	150	1.2	230	1.8

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, make sure that the connectors are tight. Use fingertight 1/16" connector (28401081).*
2	Wash out the ethanol with, at least 5 column volumes (CV) of distilled water or 3 CV elution buffer at 0.6 to 1.2 mL/min (75 to 150 cm/h).
3	Equilibrate the column with 10 CV 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 start buffer or until the UV trace of the effluent returns to near baseline.
6	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. • *Linear gradient elution* Elute with 0% to 100% elution buffer in 10 to 20 CV. • *Step elution* Elute with 2 to 5 CV 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 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 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 maintains columns in good condition. However, reduced performance, increased back pressure or blockage indicates that the column needs cleaning.

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

CIP must be performed regularly to prevent the build-up of contaminants and to maintain the capacity, flow properties, and general performance of prepacked columns.

It is recommended to perform a CIP:

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

CIP protocol

The HiScreen HIC resins can normally be regenerated by washing with distilled water, but are also alkali-tolerant resins. The nature of the sample will ultimately determine the final CIP protocol so the CIP procedure below might 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 (≤ 0.78 mL/min (≤ 100 cm/h)).
3	Wash with at least 3 CV water.

CAUTION

70% ethanol can require the use of explosion-proof areas and equipment.

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 (18112093) to give 20 cm bed height.

Note: *The back pressure is increased with longer bed height. This is easily addressed by lowering the flow rate.*

Other factors, such as the clearance of critical impurities can change, when column bed height is modified. The factors must be validated using the final bed height.

Scale-up to a larger column 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).

7 Adjusting pressure limits

The pressure in chromatography system software is generated by the flow through a column. The pressure affects the packed bed and the column hardware, see the figure below. The pressure is increased during 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 recommended flow rates in Table 2 and 3) can damage the column.*

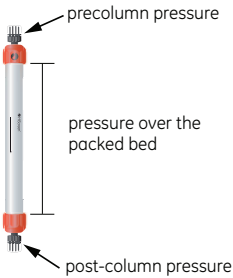

Fig 4. Precolumn and post-column measurements.

ÄKTA avant and ÄKTA pure

The system will automatically monitor the pressures (precolumn pressure and pressure over the packed bed, Δp). The precolumn pressure limit is the column hardware pressure limit (see Table 1 and Table 2).

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

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

To obtain the optimal functionality in ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC, and other systems with pressure sensor in the pump, the pressure limit in the software can be adjusted as follows:

Step	Action
1	• Replace the column with a piece of tubing. • Run the pump at the maximum intended flow rate. • Record the pressure as total system pressure, P1.

Step	Action
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. • Record the 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. *Result:* The actual pressure over the packed bed (Δp) during the run is equal to the actual measured pressure which is the total system pressure (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 [1] at 4°C to 30°C. Do not freeze.

Make sure 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 backpressure during the run	Solutions with high viscosity are used.	Use a lower flow rate.
	The column is clogged	Clean the column, see section Cleaning-in-place (CIP)
Gradual broadening of the eluate peak	Insufficient elution and CIP, caused by contaminants accumulating in the column.	Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.
Gradual decrease in yield	Insufficient elution and CIP.	Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.
Precipitation during sample loading/ elution	High salt concentrations.	Optimize the start buffer/elution conditions. Perform a CIP.
Gradual increase in CIP peaks	Suboptimal elution conditions and CIP.	Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.
High back pressure during CIP	Proteins are precipitated in the column.	Check that all the components are eluted from the column Optimize elution conditions and/or perform a CIP at a lower flow rate.
Reduced column performance despite optimized elution and CIP	Column longevity, which depends mainly on the sample type and sample preparation.	Change to a new column.

[1] In most cases, no long term stability data has been generated by GE Healthcare Life Sciences in 0.01 M NaOH. In some cases, accelerated studies at elevated temperature indicate that storage in 0.01 M NaOH can be a viable option but no guarantees can be made regarding retained function of the product.

10 Ordering information

Product	Quantity	Product code
HiScreen Phenyl FF (high sub)	1 × 4.7 mL	28926988
HiScreen Phenyl FF (low sub)	1 × 4.7 mL	28926989
HiScreen Phenyl HP	1 × 4.7 mL	28950516
HiScreen Butyl FF	1 × 4.7 mL	28926984
HiScreen Butyl HP	1 × 4.7 mL	28978242
HiScreen Butyl-S FF	1 × 4.7 mL	28926985
HiScreen Octyl FF	1 × 4.7 mL	28926986

Related products	Quantity	Product code
HiTrap HIC Selection Kit, 7 different HIC resins	7 × 1 mL	28411007
HiTrap Phenyl FF (high sub)	5 × 1 mL	17135501
	5 × 5 mL	17519301
HiTrap Phenyl FF (low sub)	5 × 1 mL	17135301
	5 × 5 mL	17519401
HiTrap Phenyl HP	5 × 1 mL	17135101
	5 × 5 mL	17519501
HiTrap Butyl FF	5 × 1 mL	17135701
	5 × 5 mL	17519701
HiTrap Butyl HP	5 × 1 mL	28411001
	5 × 5 mL	28411005
HiTrap Butyl-S FF	5 × 1 mL	17097813
	5 × 5 mL	17097814
HiTrap Octyl FF	5 × 1 mL	17135901
	5 × 5 mL	17519601

Related products	Quantity	Product code
HiPrep Phenyl FF (high sub) 16/10	1 x 20 mL	28936545
HiPrep Phenyl FF (low sub) 16/10	1 x 20 mL	28936546
HiPrep Butyl FF 16/10	1 x 20 mL	28936547
HiPrep Octyl FF 16/10	1 x 20 mL	28936548
Phenyl Sepharose 6 Fast Flow (low sub)	25 mL	17096510
	200 mL [1]	17096505
Phenyl Sepharose 6 Fast Flow (high sub)	25 mL	17097310
	200 mL [1]	17097305
Phenyl Sepharose High Performance	75 mL [1]	17108201
Butyl Sepharose 4 Fast Flow	25 mL	17098010
	200 mL	17098001
	500 mL[1]	17098002
Butyl High Performance	25 mL	17543201
	200 mL [1]	17543202
Butyl-S Sepharose 6 Fast Flow	25 mL	17097810
	200 mL [1]	17097802
Octyl Sepharose 4 Fast Flow	25 mL	17094610
	200 mL [1]	17094602

[1] Process-scale quantities are available. Contact your local representative

Accessories HiScreen	Quantity	Product code
HiTrap/HiPrep, 1/16" male connector for ÄKTA *(For connection of columns with 1/16" fittings to ÄKTA)*	8	28401081
Union 1/16" male/1/16" male with 0.5 mm i.d. *(For connecting two columns with 1/16" fittings in series)*	2	18112093
Fingertight stop plug, 1/16" [1] *(For sealing a HiScreen column)*	5	11000355

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

Related literature	Product code
Hydrophobic Interaction Chromatography and Reversed Phase Chromatography, Principles and Methods	11001269
Prepacked chromatography columns for ÄKTA systems, Selection Guide	28931778

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