Instructions 11003452 AH **Prepacked columns**

HiTrap™ HIC columns

HiTrap Phenyl FF (high sub), 1 mL and 5 mL

HiTrap Phenyl FF (low sub), 1 mL and 5 mL

HiTrap Phenyl HP, 1 mL and 5 mL

HiTrap Butyl FF, 1 mL and 5 mL

HiTrap Butyl-S FF, 1 mL and 5 mL

HiTrap Butyl HP, 1 mL and 5 mL

HiTrap Octyl FF, 1 mL and 5 mL

HiTrap HIC Selection Kit

HiTrap Phenyl FF (high sub), HiTrap Phenyl FF (low sub), HiTrap Phenyl HP, HiTrap Butyl FF, HiTrap Butyl-S FF, HiTrap Butyl HP, and HiTrap Octyl FF are prepacked 1 mL and 5 mL, ready-to-use hydrophobic interaction chromatography (HIC) columns. The column together with modern chromatography resins, provides fast, reproducible and easy separation in a convenient format.

HiTrap HIC Selection Kit consists of seven Hydrophobic Interaction Chromatography (HIC) resins with different hydrophobic characteristics. The kit provides the possibility to screen for the most appropriate HIC resin for specific applications. The recommended test procedures help to optimize salt concentration, sample loading, resolution and other chromatographic parameters. The seven different resins are prepacked in ready to use 1 mL HiTrap columns.

Separations are easily performed with a syringe, a pump or a chromatography system such as ÄKTA™.

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Read these instructions carefully before using HiTrap columns.

Intended use

HiTrap columns 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 product in a safe way, refer to the Safety Data Sheet.

1 Product description

HiTrap column characteristics

The columns are made of biocompatible polypropylene that does not interact with biomolecules.

The columns are delivered with a stopper at the inlet and a snap-off end at the outlet. Table 1 lists the characteristics of HiTrap HIC columns.

Fig 1. HiTrap, 1 mL column.

Fig 2. HiTrap, 5 mL column.

Note: *HiTrap columns cannot be opened or refilled.*

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

Table 1. Characteristics of HiTrap HIC columns.

Column volume (CV)	1 mL	5 mL
Column dimensions	0.7 × 2.5 cm	1.6 x 2.5 cm
Column hardware pressure limit	5 bar (0.5 MPa)	5 bar (0.5 MPa)
Recommended flow rate	1 mL/min	5 mL/min
Maximum flow rate[1]	4 mL/min	20 mL/min

[1] Room temperature, aqueous buffers.

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

Supplied Connector kit with HiTrap column

Connectors supplied	Usage	No. supplied
Union 1/16" male/ luer female	For connection of syringe to HiTrap column	1 or 2
Stop plug female, 1/16"	For sealing bottom of HiTrap column	2, 5 or 7

Resin properties

The HIC resins are based on the crosslinked 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 HiTrap HIC resins are listed in Table 2 and their chemical stability is described on page 6.

Phenyl Sepharose High Performance is based on a ~ 34 µm matrix and is well suited 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 High Performance is based on a ~ 34 µm matrix. The small beads with high rigidity gives high resolution at high flow rate, 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 as for those products for the test proteins used in the functional test.

Phenyl Sepharose 6 Fast Flow (low sub) and Phenyl Sepharose 6 Fast Flow (high sub) are based on a ~ 90 µm matrix. They are ideal for initial and intermediate step purifications requiring a matrix with medium to high hydrophobicity. The availability of two ligand concentration grades increases the possibility of finding the best selectivity and capacity for a given application.

Butyl Sepharose 4 Fast Flow is based on a ~ 90 µm matrix. It 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.

Butyl-S Sepharose 6 Fast Flow is based on a ~ 90 µm matrix. The main differences between Butyl-S Sepharose 6 Fast Flow and Butyl Sepharose 4 Fast Flow lie in 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. It is the least hydrophobic resin in the GE portfolio and is intended for purification or removal of strongly hydrophobic biomolecules at low salt concentrations, with high recovery and low risk of denaturation.

Octyl Sepharose 4 Fast Flow is based on a ~ 90 µm matrix. It has a different hydrophobic character from the phenyl and butyl ligands and is an important complement to the other hydrophobic matrices.

Chemical stability

Good chemical stability allows the use of the prepacked columns with commonly used buffers and organic solvents, like for example 1.0 M NaOH[1] (0.01 M NaOH[1] for Phenyl Sepharose High Performance), 70% ethanol, 30% isopropanol, and 6 M GuHCl. The compatibility with other solvents have been verified by conducting stability test for some of the resins with for example, 3 M (NH_4) SO_4[2].

CAUTION

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

[1]Sodium hydroxide should only be used for cleaning purposes

[2]Due to instability, ammonium sulphate is not suitable when working at pH values above 8.0

Table 2. HiTrap HIC resin characteristics

Resin	Hydrophobic ligand	Ligand concentration	Particle size, d_{50V}[1]	pH stability, CIP	pH stability, operational
Phenyl Sepharose High Performance	Phenyl	~ 25 µmol/mL resin	~ 34 µm	3 to 12	3 to 13
Phenyl Sepharose 6 Fast Flow (low sub)	Phenyl	~ 25 µmol/mL resin	~ 90 µm	2 to 14	3 to 13
Phenyl Sepharose 6 Fast Flow (high sub)	Phenyl	~ 45 µmol/mL resin	~ 90 µm	2 to 14	3 to 13
Butyl Sepharose High Performance	Butyl	~ 50 µmol/mL resin	~ 34 µm	2 to 14	3 to 13
Butyl Sepharose 4 Fast Flow	Butyl	~ 40 µmol/mL resin	~ 90 µm	2 to 14	3 to 13
Butyl-S Sepharose 6 Fast Flow	Butyl-S	~ 10 µmol/mL resin	~ 90 µm	2 to 14	3 to 13
Octyl Sepharose 4 Fast Flow	Octyl	~ 5 µmol/mL resin	~ 90 µm	2 to 14	3 to 13

[1] Median particle size of the cumulative volume distribution

Note the following:

pH stability, operational: pH range where the resin can be operated without significant change in function.

pH stability, CIP: pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

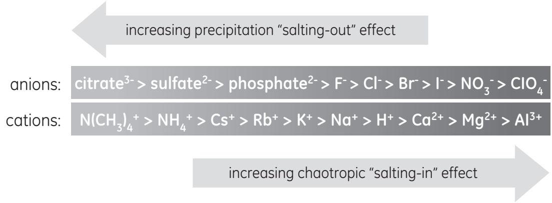
Matrices: All resins are based on 6% cross-linked agarose with spherical beads except for the two Sepharose 4 Fast Flow with the Butyl and Octyl ligands, which are based on 4% cross-linked agarose, spherical beads.

Storage: 20% ethanol. 4°C to 30°C.

2 Hydrophobic Interaction Chromatography

Overview

Separation of biomolecules on HIC resins is based on an interplay between 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 adsorption of biological solutes to HIC adsorbents is promoted, or otherwise modulated, by the presence of relatively high concentrations of anti-chaotropic salts such as ammonium sulfate, sodium sulfate, etc (Figure 3). Desorption of bound solutes is achieved simply by stepwise or gradient elution with buffers of low salt content.



Increasing the salting-out effect promotes hydrophobic interactions and increases the adsorption capacity of the HIC resin for proteins. The opposite situation dominates when the

Fig 3. The Hofmeister series of some anions and cations arranged according to their effects on the solubility of protein in aqueous solutions.

HIC resins available from GE are produced as a graded series of hydrophobic adsorbents based on alkyl or aryl ligands attached to a hydrophilic base matrix, e.g. 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 adsorbents 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 are:

- 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 type and concentration of ligand as well as the type and concentration of salt added during the adsorption step** are of paramount importance in determining the outcome of a HIC event.

In general, the type of immobilized ligand determines its adsorption selectivity toward the proteins in a sample while its concentration determines its adsorption capacity.

In general, HIC resins fall into two groups, depending on their interactions with sample components. Straight alkyl chains (butyl, octyl) show a ‘‘pure” hydrophobic character, while 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.

The characteristics of the target protein (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 adsorption behavior of a protein exposed to a HIC resin has to be determined on a case-by-case basis.

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

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 adsorbents. In the range of pH 5 to 8.5, the effect seems to be minimal or insignificant.

In regard to **temperature**, it is generally accepted that the binding of proteins to HIC adsorbents is entropy driven, which implies that the soluteadsorbent interaction increases with increase in temperature. In some instances, the reverse effect has been observed, In practical work, one must be aware that a downstream purification process that is 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.

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).

Further information about hydrophobic interaction chromatography can be found in the Handbook "HIC & RPC, Principles and Methods", *see* "Ordering information". Check also *www.gelifesciences.com/hitrap* and *www.gelifesciences.com/protein-purification* for further information.

Screening experiments

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. As mentioned before, binding conditions are dependent on the salt chosen. The salt concentration must be below that which causes precipitation of proteins in the sample. We recommend 1 M ammonium sulphate as a good starting buffer for screening experiments. If the substance does not bind, a more hydrophobic resin needs to be chosen. If the substance binds so strongly that nonpolar additives are required for elution, a less hydrophobic resin should be tried. The bound protein should be eluted from the column with high recovery.

Figures 4 to 9 show some typical elution profiles from screening experiments and recommendations for further experimental work. The shaded area shows the elution position of the protein of interest.

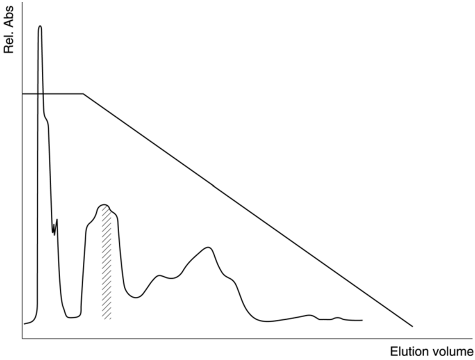


Fig 4.

Result: The protein is eluted early in the gradient. Resolution is not satisfactory.

Discussion: Not much can be gained by changing the salt concentration. A decrease in the salt concentration of the start buffer will decrease binding capacity and might even lead to co-elution with the unbound fraction. An increase in the salt concentration might lead to the co-adsorption of unwanted impurities leading to a decrease in selectivity. Change of pH in the start buffer might result in stronger binding and higher selectivity. Trying another salt might also improve performance.

Next step: Repeat the experiment at a lower or higher pH or with a salt of higher salting-out strength. If no improvement in selectivity is obtained, try a resin with a different ligand or with a higher degree of ligand substitution.

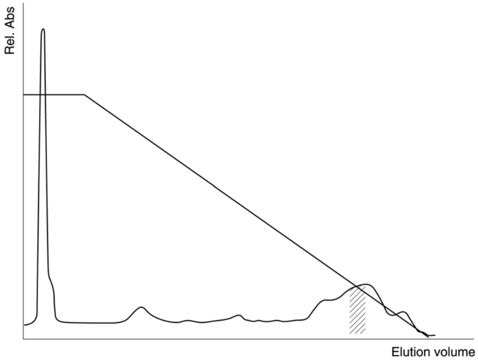


Fig 5.

Result: The protein is eluted near the end of the gradient. Resolution is not satisfactory.

Discussion: A decrease in the initial salt concentration will weaken the binding, resulting in earlier elution of the protein. It might, however, not have a positive effect on selectivity, since the contaminants are eluted very close to, both before and after, the protein of interest. Changing the pH of the start buffer or changing to another salt might have more impact on resolution and should be tried.

Next step: Repeat the experiment at a lower or higher pH value or with a salt with lower salting-out strength. If no improvement in selectivity is obtained, try a resin with a different ligand or with a lower degree of ligand substitution.

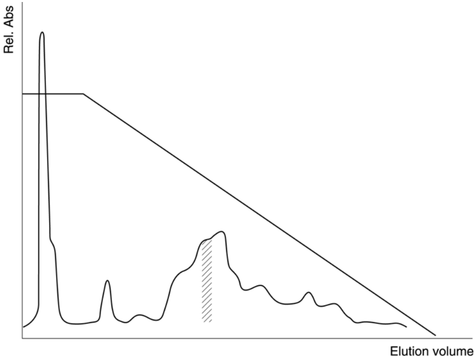


Fig 6.

Result: The protein is eluted in the middle of the gradient. Resolution is not satisfactory.

Discussion: Changing the concentration of salt in the start buffer will have a limited effect since the contaminants are eluted very close to, both before and after, the protein of interest. Changing the pH of the start buffer or changing to a different salt might have an impact on resolution and should be tried. Trying a different gradient slope might also be an effective way to increase the resolution.

Next step: Repeat the experiment at a lower or higher pH or with a salt with higher salting-out effect. If no improvement in selectivity is obtained, try a resin with a different ligand or with a higher degree of ligand substitution.

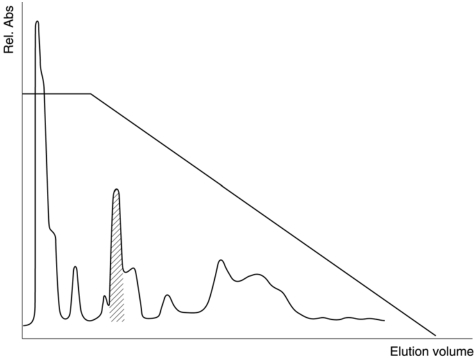


Fig 7.

Result: The protein is eluted early in the gradient. Resolution is satisfactory.

Discussion: This can be a good choice of resin. The fact that the protein is eluted early in the gradient indicates that the binding capacity might be low. An increase in salt concentration to compensate for this might lead to a decrease in selectivity since some of the unbound proteins might be adsorbed together with the protein of interest. Not much can be gained by changing the pH of the start buffer since the resolution was considered satisfactory.

Next step: If low capacity is a problem and a moderate increase in salt concentration leads to loss in resolution, try a resin with a different ligand or a resin with a higher degree of ligand substitution.

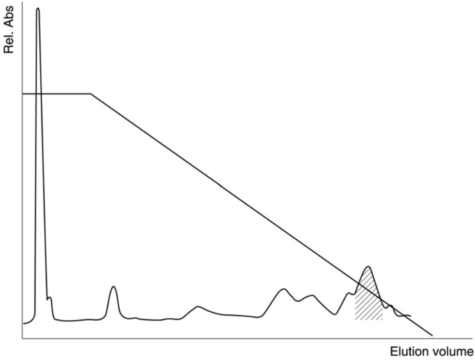


Fig 8.

Result: The product is eluted near the end of the gradient. Resolution is satisfactory.

Discussion: This can be a good choice of resin. A disadvantage might be that some of the most hydrophobic substances bind so strongly that they are difficult to remove from the resin. Decreasing the concentration of salt in the start buffer will give earlier elution of the protein and reduce the risk that strongly bound proteins are difficult to elute. It also leads to a reduction in cycle time.

Next step: If problems with strong binding of contaminants are discovered, try a resin with a different ligand or with a lower degree of ligand substitution.

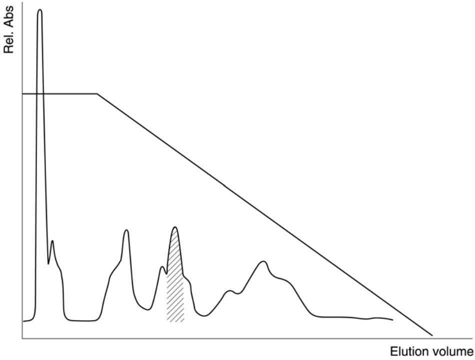


Fig 9.

Result: The protein is eluted in the middle of the gradient. Resolution is satisfactory.

Discussion: The choice of ligand is very good and there is little risk for strong binding of the most hydrophobic contaminants.

3 Operation

Buffer preparation

Water and chemicals must be of high purity. When using high salt concentration buffers, especially ammonium sulphate, use a salt of high quality to prevent baseline drift.

Recommended buffers

Start buffer: 50 mM sodium phosphate, 1.5 M ammonium sulphate, pH 7.0.

Elution buffer: 50 mM sodium phosphate, pH 7.0.

Sample preparation

Since adsorption is carried out at high salt concentration, the composition of the sample needs to be adjusted to the pH and ionic strength of the start buffer (high salt buffer) for consistent and reproducible results. When possible, dissolve the sample in start buffer. Buffer exchange can be carried out using HiPrep™ 26/10 Desalting, HiTrap Desalting or PD-10 columns for ionic strengths up to ~ 1.5 M (at higher ionic strengths there is a risk that the resin will shrink).

Another way to increase the ionic strength is by addition of solid salt (note: precipitation might occur due to high local salt concentrations), by addition of the salt as a high concentration stock solution or by dilution of the sample with start buffer followed by pH adjustment.

The sample must be fully solubilized. We recommend centrifugation or filtration immediately before loading on the column to remove particulate material (0.45 μm filter). Never apply turbid solutions to the column. Turbidity indicates sample insolubility which might be due to incorrect ionic strength.

High sample viscosity causes high backpressure, instability of the sample zone and gives irregular flow pattern with decreased resolution. High backpressure can also damage the column packing. Recommended maximum sample viscosity corresponds to a protein concentration of ~ 50 mg/mL in aqueous solution. If lipids or other very hydrophobic substances are present in the sample, they might interact very strongly with the HIC column, diminishing capacity and being very difficult to remove. In such cases, using a slightly less hydrophobic column as a precolumn can be very efficient. The precolumn should be chosen to bind the most hydrophobic material and allow the substance of interest to pass through under starting conditions.

Column equilibration

1 Fill the syringe or pump tubing with elution buffer (low salt buffer). Remove the stopper. To avoid introducing air into the column, connect the column drop-to-drop to either the syringe (via the luer connector) or to the pump tubing.

2 Remove the snap-off end at the column outlet.

3 Wash the column with 5 column volumes (CV) of elution buffer at 1 mL/min.

4 Wash with 5 to 10 CV start buffer (high salt buffer). If air is trapped in the column, wash with buffer until the air disappears.

Note: *If a P1-pump is used a max flow rate of 1 to 3 mL/min can be run on a HiTrap 1 mL column packed with Sepharose High Performance resin.*

Elution with linear descending gradients

A linear decrease of the salt concentration is the most frequently used type of elution in hydrophobic interaction chromatography. Continuous gradients can be prepared in different ways depending on available equipment:

- A peristaltic pump and a gradient mixer e.g., pump P-1, gradient mixer GM-1
- A one pump system, e.g., ÄKTAprime plus
- A two pump system, e.g., ÄKTA design

1 Equilibrate the column (see "Column equilibration").

2 Adjust the sample to the chosen starting pH and ionic strength (see "Sample preparation").

3 Apply the sample.

4. Wash with 5 to 10 CV start buffer until the UV trace of the effluent returns to near baseline.
5. Start the gradient elution. A gradient volume of 10 to 20 CV is usually enough.
6. Regenerate the column by washing with 5 CV distilled water followed by 5 CV start buffer. The column is now ready for a new sample. Avoid storage of the column or the chromatography system in high salt buffer to prevent crystal build-up in the equipment.

Elution with stepwise descending gradients

Stepwise elution is the sequential use of the same buffer at different ionic strengths. It is technically simple and fast and suitable for syringe operation. It is often used for sample concentration and sample clean up. Stepwise elution gives small peak volumes and the resolution depends on the difference in elution power between each step. When stepwise elution is applied, one has to keep in mind the danger of artifactual peak when a subsequent step is executed too early after a tailing peak. For this reason it is recommended to start with a continuous gradient to characterize the sample and its chromatographic behavior.

4 Determination of binding capacity

The amount of sample which can be applied to a column depends on the capacity of the column and the degree of resolution required. The capacity is dependent on the sample composition, chosen starting conditions such as pH, ionic strength, buffer salts and the flow rate at which the adsorption is done. The dynamic capacity can be determined by frontal analysis using real sample:

1. Equilibrate the column (see ''Column equilibration'').
2. Adjust the sample to the chosen starting pH and ionic strength (see ''Sample preparation'').
3. Determine the concentration of the actual protein in the sample by UV, PAGE, ELISA or other appropriate techniques.
4. Apply the sample to the column with a pump or a syringe at the flow rate to be used in the purification method. Collect fractions and continue to apply sample until the column is saturated.
5. Wash the column with 5 to 10 CV start buffer until the baseline is stable.
6. Elute bound proteins with 2 to 5 CV elution buffer (low salt buffer) and collect the eluate.
7. Analyze fractions and eluates from steps 4 and 6 for the protein in question and determine the breakthrough profile. The practical capacity is the amount that can be applied without any breakthrough and the total capacity available is determined by analyzing eluate from step 6.

5 Cleaning and regeneration

HIC adsorbents can normally be regenerated by washing with distilled water. To prevent gradual build-up of contaminants on the column, regular cleaning is recommended. Precipitated proteins can be removed by washing with 5 CV 0.5 to 1.0 M NaOH followed by 5 to 10 CV water at a flow rate of 1 mL/min. Strongly bound substances can be removed by washing with 5 to 10 CV of up to 70% ethanol or 30% isopropanol.

6 Scaling up

Columns and resins for scale-up are available. For quick scale up of purifications, two or three HiTrap HIC columns of the same type can be connected in series (backpressure will increase). All HiTrap HIC columns included in HiTrap HIC Selection Kit are available as individual packages of 5 × 1 mL and 5 × 5 mL. Further scale up can be achieved using the prepacked columns HiPrep Phenyl FF (high sub) 16/10, HiPrep Phenyl FF (low sub) 16/10, HiPrep Butyl FF 16/10, HiPrep Octyl FF 16/10 or HiLoad™ Phenyl Sepharose High Performance or bulk resin packs, see "Ordering information".

BioProcess chromatography resins are developed and supported for production-scale chromatography. BioProcess resins are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production scale. Regulatory Support Files (RSF) are available to assist process validation and submissions to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing.

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 10. 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 1) might damage the column.*

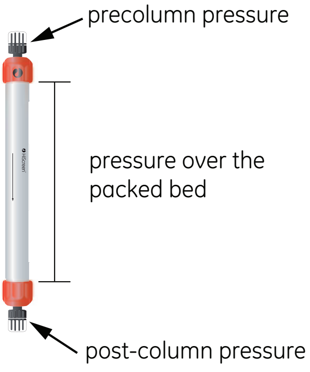


Fig 10. Precolumn and post-column measurements.

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).

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 optimal functionality, the pressure limit in the software can be adjusted according to the following procedure:

1 Replace the column with a piece of tubing. Run the pump at the maximum intended flow rate. Note the pressure as *total system pressure*, 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 - *total system pressure* (P1).

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

8 Storage

Store the HiTrap HIC columns equilibrated with 5 to 10 CV of 20% ethanol. The recommended storage temperature is 4°C to 30°C.

9 Ordering information

Product	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-S FF	5 × 1 mL	17097813
	5 × 5 mL	17097814
HiTrap Butyl HP	5 × 1 mL	28411001
	5 × 5 mL	28411005
HiTrap Octyl FF	5 × 1 mL	17135901
	5 × 5 mL	17519601

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

1 Larger quantities are available. Contact your local representative for further information

Accessories	Quantity	Product code
1/16" male/luer female *(For connection of syringe to top of HiTrap column*)	2	18111251
Tubing connector flangeless/M6 female (*For connection of tubing to bottom of HiTrap column)*	2	18100368
Tubing connector flangeless/M6 male *(For connection of tubing to top of HiTrap column)*	2	18101798
Union 1/16” female/M6 male *(For connection to original FPLC System through bottom of HiTrap column)*	6	18111257
Union M6 female /1/16” male *(For connection to original FPLC System through top of HiTrap column*)	5	18385801
Union luerlock female/M6 female	2	18102712
HiTrap/HiPrep, 1/16" male connector for ÄKTA design	8	28401081
Stop plug female, 1/16” *(For sealing bottom of HiTrap column)*	5	11000464
Fingertight stop plug, 1/16"	5	11000355

Literature	Product code
Hydrophobic Interaction Chromatography & Reversed Phase Chromatography, Principles and Methods, Handbook	11001269

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

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Björkgatan 30
751 84 Uppsala
Sweden

www.gelifesciences.com/hitrap
www.gelifesciences.com/protein-purification

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