

HiTrap™ DEAE FF, 1 mL and 5 mL

HiTrap CM FF, 1 mL and 5 mL

HiTrap Q FF, 1 mL and 5 mL

HiTrap SP FF, 1 mL and 5 mL

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

HiTrap SP FF, HiTrap CM FF, HiTrap Q FF, HiTrap DEAE FF, and HiTrap ANX FF (high sub) are prepacked 1 mL and 5 mL, ready to use cation and anion exchange HiTrap columns for method scouting, group separations, sample concentration and sample clean-up of charged biomolecules. The special design of the column, together with a well-established chromatography resin, provides fast, reproducible, and easy separations in a convenient format.

The columns can be operated with a syringe, peristaltic pump or liquid 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 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 DEAE FF, CM FF, Q FF, SP FF and ANX FF columns.

Column volume (CV)	1 mL	5 mL
Column dimensions	0.7 × 2.5 cm	1.6 × 2.5 cm
Column hardware pressure limit	5 bar (0.5 MPa)	5 bar (0.5 MPa)
Recommended maximum flow rate <sup>1</sup>	4 mL/min,	20 mL/min
Recommended operating flow rate	1mL/min	5 mL/min

<sup>1</sup> At room temperature in water.

**Note:** *The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the 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
Stop plug female, 1/16"	For sealing bottom of HiTrap column	2, 5 or 7

## Resin Properties

Q Sepharose™ Fast Flow, DEAE Sepharose Fast Flow, SP Sepharose Fast Flow, and CM Sepharose Fast Flow are based on a robust, 6% cross-linked beaded agarose matrix with excellent flow properties and high loading capacities.

ANX Sepharose 4 Fast Flow (high sub) is based on 4% cross-linked beaded agarose. This results in a resin with higher porosity, which is particularly useful for the purification of high molecular mass proteins.

Characteristics of the ion exchangers are listed in Table 2.

**Table 2.** Characteristics of Sepharose Fast Flow cation exchangers

Cation exchangers	SP Sepharose Fast Flow	CM Sepharose Fast Flow
Matrix	Cross-linked agarose, 6%, spherical	Cross-linked agarose, 6%, spherical
Bead size	45–165 µm	45–165 µm
Ion exchange type	Strong cation	Weak cation
Charged group	-CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> SO <sub>3</sub> <sup>-</sup>	-O-CH <sub>2</sub> COO <sup>-</sup>
Ionic capacity	0.18–0.25 mmol H <sup>+</sup> /mL resin	0.09–0.13 mmol H <sup>+</sup> /mL resin
Particle size, d <sub>50V</sub> <sup>1</sup>	~ 90 µm	~ 90 µm
Dynamic binding capacity <sup>2</sup>	~ 70 mg Ribonuclease A/ mL resin	~ 50 mg Ribonuclease A/ mL resin
pH stability, operational <sup>3</sup>	4 to 13	4 to 13
pH stability, CIP <sup>4</sup>	3 to 14	2 to 14
pH stability, ligand fully charged	Entire operational pH range	Above 6 <sup>5</sup>
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M NaOH <sup>6</sup> , 8 M urea, 6 M guanidine hydrochloride, 70% ethanol	
Avoid	Oxidizing agents, cationic detergents and buffers. See Table 4 and Figure 4.	
Storage	20% ethanol, 0.2 M sodium acetate, 4°C to 30°C	20% ethanol, 4°C to 30°C

<sup>1</sup> Median particle size of the cumulative volume distribution.

<sup>2</sup> Determination of dynamic binding capacity: SP Sepharose Fast Flow and CM Sepharose Fast Flow: Samples were applied at 75 cm/h until 50% breakthrough. Columns: 0.5 x 5 cm. Buffer: 0.1 M acetate, (+ 2 M NaCl in the elution buffer), pH 5.0 ANX Sepharose 4 Fast Flow (high sub): Sample was applied at 300 cm/h until 10% breakthrough. Column: 1.6 x 13 cm. Buffer: 0.05 M Tris, (+ 1 M NaCl in the elution buffer), pH 7.5.

<sup>3</sup> pH range where resin can be operated without significant change in function.

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

<sup>5</sup> pH range where ligand is fully charged. Although the ligand is fully charged throughout the entire pH range, only use the resin within the stated stability ranges.

<sup>6</sup> 1.0 M NaOH should only be used for cleaning purposes.

**Table 3.** Characteristics of Sepharose Fast Flow anion exchangers.

	<b>Q Sepharose Fast Flow</b>	<b>DEAE Sepharose Fast Flow</b>	<b>ANX Sepharose 4 Fast Flow (high sub)</b>
Matrix	Cross-linked agarose, 6%, spherical	Cross-linked agarose, 6%, spherical	Cross-linked agarose, 4%, spherical
Ion exchange type	Strong anion	Weak anion	Weak anion
Charged group	-N <sup>+</sup> (CH <sub>3</sub> ) <sub>3</sub>	-N <sup>+</sup> (C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> H	-N <sup>+</sup> (C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> H
Ionic capacity	0.18–0.24 mmol Cl <sup>-</sup> /mL resin	0.11–0.16 mmol Cl <sup>-</sup> /mL resin	0.13–0.18 mmol Cl <sup>-</sup> /mL resin
particle size, d <sub>50v</sub> <sup>1</sup>	~ 90 μm	~ 90 μm	~ 90 μm
Dynamic binding capacity <sup>2</sup>	~ 120 mg HSA/ mL resin	~ 110 mg HSA/ mL resin	~ 43 mg BSA/ mL resin
pH stability operational <sup>3</sup>	2 to 12	2 to 12	3 to 13
pH stability, CIP <sup>4</sup>	2 to 14	2 to 14	2 to 14
pH ligand fully charged	Entire operational pH range	Below 9 <sup>5</sup>	Below 9 <sup>5</sup>
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M NaOH <sup>6</sup> , 8 M urea, 6 M guanidine hydrochloride, 70% ethanol		
Avoid	Oxidizing agents, anionic detergents and buffers. See Table 5 and Figure 5		
Storage	20% ethanol, 4°C to 30°C	20% ethanol, 4°C to 30°C	20% ethanol, 4°C to 30°C

<sup>1</sup> Median particle size of the cumulative volume distribution.

<sup>2</sup> Determination of dynamic binding capacity: DEAE Sepharose Fast Flow, Q Sepharose Fast Flow: Samples were applied at 75 cm/h until 50% BREAKTHROUGH. COLUMNS: 0.5 X 5 cm. Buffer: 0.05 M Tris (+2 M NaCl in the elution buffer), pH 7.5. ANX Sepharose 4 Fast Flow (high sub): Sample was applied as 300 cm/h until 10% breakthrough. Column: 1.6 x 13 cm. Buffer: 0.05 M Tris, (+1 M NaCl in the elution buffer), pH 7.5.

<sup>3</sup> pH range where resin can be operated without significant change in function.

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

<sup>5</sup> pH range where ligand is fully charged. Although the ligand is fully charged throughout the entire pH range, only use the resin within the stated stability ranges.

<sup>6</sup> 1.0 M NaOH should only be used for cleaning purposes.

**Note:** *The active end of the charged group is the same for DEAE Sepharose Fast Flow and ANX Sepharose Fast Flow (high sub), the difference is the length of the carbon chain of the charged group. DEAE Sepharose Fast Flow has a diethylaminoethyl-group bound to the agarose whilst ANX Sepharose 4 Fast Flow has a diethylaminopropyl-group attached.*

## 2 General considerations

### Selection of ion exchanger

Ion exchange chromatography is based on the binding of charged sample molecules to oppositely charged groups attached to an insoluble matrix.

Substances are bound to ion exchangers when they carry a net charge opposite to that of the ion exchanger. This binding is electrostatic and reversible.

The pH value at which a biomolecule carries no net charge is called the isoelectric point (pI). When exposed to a pH below its pI, the biomolecule will carry a positive net charge and will bind to a cation exchanger (SP and CM). At pH's above its pI the biomolecule will carry a negative net charge and will bind to an anion exchanger (Q, DEAE and ANX) (Fig 3).

If the sample components are most stable below their pI's, a cation exchanger must be used. If they are most stable above their pI's, an anion exchanger is used. If stability is high over a wide pH range on both side of the pI, either type of ion exchanger can be used.

Weak ion exchangers have a limited pH working range (Table 4).

Information on the pI and how the net charge on the molecule varies with pH gives valuable information regarding the choice of starting conditions. Electrophoretic titration curves enable the determination of the charge/pH relationship for the molecules present across the pH range of interest.

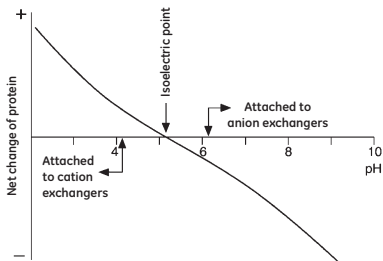


Fig 3. The net charge of a protein as a function of pH.

## Selection of buffer pH and ionic strength

Buffer pH and ionic strength are critical for the binding and elution of material (both target substances and contaminants) in ion exchange chromatography. Selection of appropriate pH and ionic strength for the start and elution buffers allows the use of three possible separation strategies.

### Strategy 1. Binding and elution of all sample components

Binding is achieved by choosing a start buffer with a low pH for SP Sepharose Fast Flow, and CM Sepharose Fast Flow, or high pH for Q Sepharose Fast Flow, DEAE Sepharose Fast Flow and ANX Sepharose 4 Fast Flow (high sub). The ionic strength must be kept as low as possible to allow all components to bind to the ion exchanger (< 5 mS/cm).

This results in a concentration of the target substance and a complete picture of the total sample. The drawback of this strategy is that the binding capacity of the ion exchanger for the target substance depends on the amount of contaminants in the sample. Strongly binding contaminants can also displace bound target protein if a large volume of sample is loaded.

**Note:** *Starting conditions are subject to the stability of the sample components.*



## Strategy 2. Enrichment of target protein

This is achieved by choosing a start buffer with a pH optimized to allow maximal binding of target protein, and as high as possible an ionic strength to suppress binding of sample contaminants.

This strategy results in a concentration of the target substances.

## Strategy 3. Binding of sample contaminants

This is achieved by choosing a start buffer with a pH and an ionic strength that promotes the binding of some or all contaminants but allows the target substance to pass through the column.

The drawback of this approach is that the target substance is not concentrated and the amount of sample that can be applied to the ion exchanger depends on the amount of contaminants in the sample.

## Start buffer

The concentration of buffer required to give effective pH control varies with the buffer system. A list of suitable buffers and suggested starting concentrations is shown in Tables 4 and 5, and Figures 4 and 5. In the majority of cases a concentration of at least 10 mM is required to make sure that there is adequate buffering capacity. The ionic strength of the buffer must be kept low ( $< 5$  mS/cm) so as not to interfere with sample binding. Salts also play a role in stabilizing protein structures in solution and it is important the ionic strength are not so low that protein denaturation or precipitation occurs.

The buffering ion must carry the same charge as the ion exchange group and should have a pKa within 0.5 pH units of the pH used in the separation. Buffering ions of opposite charge might take part in the ion exchange process and cause local disturbances in pH.

**Table 4.** Buffers for cation exchange chromatography

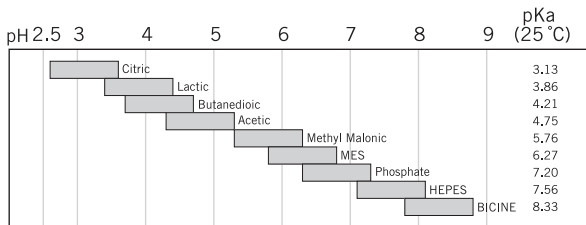
pH interval	Substance	Conc. (mM)	Counter-ion	pKa (25°C) <sup>1</sup>
1.4–2.4	Maleic acid	20	Na <sup>+</sup>	1.92
2.6–3.6	Methyl malonic acid	20	Na <sup>+</sup> or Li <sup>+</sup>	3.07
2.6–3.6	Citric acid	20	Na <sup>+</sup>	3.13
3.3–4.3	Lactic acid	50	Na <sup>+</sup>	3.86
3.3–4.3	Formic acid	50	Na <sup>+</sup> or Li <sup>+</sup>	3.75
3.7–4.7; 5.1–6.1	Succinic acid	50	Na <sup>+</sup>	4.21; 5.64
4.3–5.3	Acetic acid	50	Na <sup>+</sup> or Li <sup>+</sup>	4.75
5.2–6.2	Methyl malonic acid	50	Na <sup>+</sup> or Li <sup>+</sup>	5.76
5.6–6.6	MES	50	Na <sup>+</sup> or Li <sup>+</sup>	6.27
6.7–7.7	Phosphate	50	Na <sup>+</sup>	7.20
7.0–8.0	HEPES	50	Na <sup>+</sup> or Li <sup>+</sup>	7.56
7.8–8.8	BICINE	50	Na <sup>+</sup>	8.33

<sup>1</sup> Ref: Handbook of chemistry and physics, 83rd edition, CRC, 2002-2003.

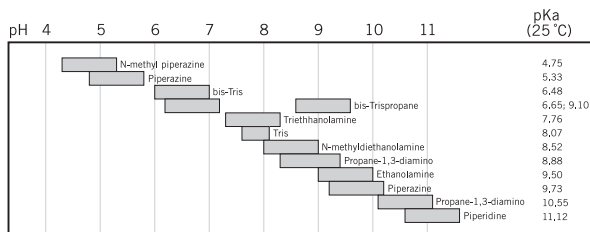
**Table 5.** Buffers for anion exchange chromatography

pH interval	Substance	Conc. (mM)	Counter-ion	pKa (25°C) <sup>1</sup>
4.3–5.3	N-Methylpiperazine	20	Cl <sup>-</sup>	4.75
4.8–5.8	Piperazine	20	Cl <sup>-</sup> or HCOO <sup>-</sup>	5.33
5.5–6.5	L-Histidine	20	Cl <sup>-</sup>	6.04
6.0–7.0	bis-Tris	20	Cl <sup>-</sup>	6.48
6.2–7.2; 8.6–9.6	bis-Tris propane	20	Cl <sup>-</sup>	6.65; 9.10
7.3–8.3	Triethanolamine	20	Cl <sup>-</sup> or CH <sub>3</sub> COO <sup>-</sup>	7.76
7.6–8.6	Tris	20	Cl <sup>-</sup>	8.07
8.0–9.0	N-Methyl- diethanolamine	20	SO <sub>4</sub> <sup>2-</sup>	8.52
8.0–9.0	N-Methyl- diethanolamine	50	Cl <sup>-</sup> or CH <sub>3</sub> COO <sup>-</sup>	8.52
8.4–9.4	Diethanolamine	20 at pH 8.4 50 at pH 8.8	Cl <sup>-</sup>	8.88
8.4–9.4	Propane 1,3-Diamino	20	Cl <sup>-</sup>	8.88
9.0–10.0	Ethanolamine	20	Cl <sup>-</sup>	9.50
9.2–10.2	Piperazine	20	Cl <sup>-</sup>	9.73
10.0–11.0	Propane 1,3-Diamino	20	Cl <sup>-</sup>	10.55
10.6–11.6	Piperidine	20	Cl <sup>-</sup>	11.12

<sup>1</sup> Ref: Handbook of chemistry and physics, 83<sup>rd</sup> edition, CRC, 2002–2003.



**Fig 4.** Recommended buffer substances for cation exchange chromatography.



**Fig 5.** Recommended buffer substances for anion exchange chromatography.

## Starting pH

Cation exchangers (SP, CM): At least 1 pH unit below the pI of substance to be bound.

Anion exchangers (Q, DEAE, ANX): At least 1 pH unit above the pI of substance to be bound.

## 3 Operation

The columns can be operated by a syringe, a peristaltic pump or a chromatography system.

### Buffer preparation

Water and chemicals used for buffer preparation must be of high purity. It is recommended to filter the buffers by passing them through a 0.45  $\mu\text{m}$  filter before use. See Tables 4 and 5, Figures 4 and 5 for recommended buffers.

### Sample preparation

The sample must be adjusted to the composition of the start buffer by buffer exchange using HiTrap Desalting, HiPrep™ 26/10 Desalting or PD-10 columns (See Table 6). The sample must be filtered through a 0.45  $\mu\text{m}$  filter or centrifuged immediately before it is applied to the column.

**Table 6.** Prepacked columns for desalting and buffer exchange

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

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

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

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

<sup>4</sup> Volumes with gravity elution.

<sup>5</sup> Volumes with centrifugation.

## 4 Purification

- 1 Fill the syringe or pump tubing with start buffer (low ionic strength). Remove the stopper and connect the column to the syringe (with the provided connector), or pump tubing, "drop to drop" to avoid introducing air into the column.
- 2 Remove the snap-off end at the column outlet.
- 3 Wash out the preservatives with 5 column volumes of start buffer, at 1 mL/min for HiTrap 1 mL and 5 mL/min for HiTrap 5 mL.
- 4 Wash with 5 column volumes of elution buffer (start buffer with 1 M NaCl).
- 5 Finally equilibrate with 5 to 10 column volumes of start buffer.
- 6 Apply the sample at 1 mL/min for HiTrap 1 mL and 5 mL/min for HiTrap 5 mL using a syringe fitted to the luer connector or by pumping it onto the column.
- 7 Wash with at least 5 column volumes of start buffer or until no material appears in the eluate.

- 8 Elute with 5 to 10 column volumes of elution buffer, see "Choice of gradient type".
- 9 The purified eluted fractions can be desalted using a HiTrap Desalting, HiPrep 26/10 Desalting or a PD-10 column if necessary.
- 10 After completed elution, regenerate the column by washing with 5 column volumes of regeneration buffer (start buffer with 1 M NaCl) followed by 5 to 10 columns volumes of start buffer. The column is now ready for a new sample.

**For a first experiment the following conditions are recommended:**

Flow rates:	1 mL/min using HiTrap 1 mL column 5 mL/min using HiTrap 5 mL column
Start buffer:	See Tables 4 and 5, Figures 4 and 5
Elution buffer:	Start buffer + 1 M NaCl
Gradient volume:	20 mL

## 5 Optimizing starting conditions

If the composition of the sample is unknown, a simple screening test using a syringe or pump can be performed to optimize starting pH and ionic strength.

- 1 Set up a series of buffers with different pH's, in the range 4 to 8 (SP, CM) or 5 to 9 (Q, DEAE, ANX), with 0.5 to 1 pH unit intervals between each buffer. Make one series with 1 M NaCl included in the buffers (elution buffer) and the other without NaCl (start buffer).
- 2 Equilibrate the column with start buffer, see "Purification".
- 3 Adjust the sample to the chosen start buffer, see "Sample preparation".
- 4 Apply a constant known amount of the sample at 1 mL/min using HiTrap 1 mL column and at 5 mL/min using HiTrap 5 mL column. Collect the eluate.
- 5 Wash with at least 5 column volumes of start buffer or until no material appears in the eluate. Collect the eluate.
- 6 Elute bound material with elution buffer. 3 to 5 column volumes are usually sufficient but other volumes might be required dependent on the exact experimental conditions. Collect the eluate.
- 7 Analyze all eluates (by activity assay for example) and determine the purity and the amount bound to the column.
- 8 Perform steps 2 to 7 for the next buffer pH.
- 9 Decide which pH is to be used for the selected purification strategy.
- 10 To decide on starting ionic strength conditions, a similar screening is done, but the buffer pH is held constant and the salt concentration is varied in the interval 0 to 0.5 M, with intervals of 0.05 to 0.1 M salt between each buffer.



## 6 Further optimization

The recommendations given above will give a sound basis for developing an efficient purification step. Details of how flow rate, sample loading, particle size and elution scheme can be optimized to meet the special needs can be found in the handbook, *Ion Exchange Chromatography, Principles and Methods*, Product code 11000421.

A wide range of ion exchange resins for purification of biomolecules at all scales is available. See "Ordering information", [www.gelifesciences.com/protein-purification](http://www.gelifesciences.com/protein-purification) or contact your local representative.

### Choice of gradient type

- 1 Stepwise gradients are easy to produce and require minimal equipment. Eluted peaks are very sharp and elution volumes minimal. However, care must be exercised in the design of the steps and the interpretation of results for substances eluted by a sharp change in pH or small differences in ionic strength. Peaks tend to have sharp fronts and pronounced tailing since they frequently contain more than one component.
- 2 Continuous salt gradients are the most frequently used type of elution. Many types of gradient forming systems are available. Two buffers of differing ionic strength, the start and elution buffer (start buffer + 1 M NaCl or higher buffer salt concentration), are mixed together and if the volume ratio is changed linearly, the ionic strength changes linearly.

**Note:** *Another, but less common, method to desorb bound material is to increase (SP and CM) or decrease (Q, DEAE and ANX) the pH of the eluent. Continuous pH gradients are difficult to produce at constant ionic strength, since simultaneous changes in ionic strength, although small, also occur (buffering capacities are pH dependent). In the case of pH gradients using weak ion exchangers (CM, DEAE and ANX) the buffer might have to titrate the ion exchanger and there will be a short period of re-equilibration before the new pH is reached.*

## Elution with stepwise ionic strength gradients

Stepwise elution is the sequential use of the same buffer at different ionic strengths. It is technically simple and fast, and is 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.

- 1 Choose starting conditions as outlined under Optimizing starting conditions.
- 2 Equilibrate the column, see "Purification".
- 3 Adjust the sample to the chosen starting pH and ionic strength, see "Sample preparation".
- 4 Apply the sample at 1 mL/min using HiTrap 1 mL column and at 5 mL/min using HiTrap 5 mL column. Collect eluate.
- 5 Wash with at least 5 column volumes of start buffer or until no material appears in the eluate. Collect eluate.
- 6 Elute with the first step ionic strength buffer. The volumes required for stepwise elution depend on the operating conditions. However, 3 to 5 column volumes are usually sufficient. Collect eluate.
- 7 Elute with next ionic strength buffer. Collect eluate.
- 8 After completed elution, regenerate the column by washing with 5 column volumes of regeneration buffer (start buffer with 1 M NaCl) followed by 5 to 10 column volumes of start buffer. The column is now ready for a new sample.

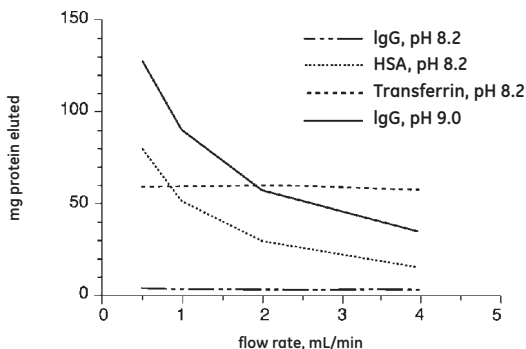
## Elution with continuous ionic strength gradients

Continuous salt gradient elution is the most frequently used type of elution in ion exchange chromatography. It is very reproducible and leads to improved resolution, since zone sharpening occurs during elution.

- 1 Choose starting conditions as outlined under "Optimizing starting conditions".
- 2 Equilibrate the column, see "Purification".
- 3 Adjust the sample to the chosen starting pH and ionic strength, see "Sample preparation".
- 4 Apply the sample at 1 mL/min using HiTrap 1 mL column and at 5 mL/min using HiTrap 5 mL column. Collect eluate.
- 5 Wash with at least 5 column volumes of start buffer or until no material appears in the eluate.
- 6 Start the gradient elution. A gradient volume of 10 to 20 column volumes and an increase in ionic strength to 0.5 M NaCl is usually sufficient.
- 7 Regenerate the column by washing with 5 column volumes of start buffer with 1 M NaCl followed by 5 to 10 column volumes of start buffer. The column is now ready for a new sample.

## 7 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 of pH and ionic strength and the flow rate at which the separation is done. The influence of flow rate and pH on the capacity for some model proteins are shown in Figure 6. Samples were applied until 5% of the start material appeared in the eluent. The column was then washed with 10 mL 20 mM Tris-HCl, pH 8.2 or 9.0 before elution with elution buffer, 20 mM Tris-HCl, 1 M NaCl, pH 8.2 or 9.0.



**Fig 6.** Binding capacity of human IgG, HSA and human transferrin at different pH's on HiTrap Q HP, 1 mL.

- 1 Equilibrate the column, see "Purification".
- 2 Adjust the sample to the chosen starting pH and ionic strength, see "Sample preparation".
- 3 Determine the concentration of the specific proteins by UV, SDS-PAGE, ELISA or other appropriate techniques.

- 4 Apply the sample solution to the column with a pump or a syringe, at a flow rate equal to the flow rate to be used in the purification method. Collect fractions and continue sample application until the column is saturated.
- 5 Wash the column with 5 to 10 column volumes of start buffer or until no material appears in eluate.
- 6 Elute bound proteins with 3 to 5 column volumes of elution buffer (start buffer with 1 M NaCl) and collect eluate.
- 7 Analyze fractions and eluates from steps 4 and 6 for the specific protein and determine the breakthrough profile (sample concentration as a function of the amount of sample applied). The dynamic capacity is the amount that can be applied without any significant breakthrough. The total capacity for the specific protein is determined from step 6.

## 8 Scaling up

For quick scale-up of purification, two or three HiTrap ion exchange columns of the same type can be connected in series. For further scale-up Q Sepharose Fast Flow, SP Sepharose Fast Flow, CM Sepharose Fast Flow and DEAE Sepharose Fast Flow are available as prepacked HiPrep 16/10 columns or as lab packs. See "Ordering information".

The resins covered in this instruction are part of the GE range of BioProcess™ products and are also available in the process scale packs. 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.

## 9 Adjusting pressure limits in chromatography system software

Pressure generated by the flow through a column affects the packed bed and the column hardware, see Fig 7. 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) can damage the column.*

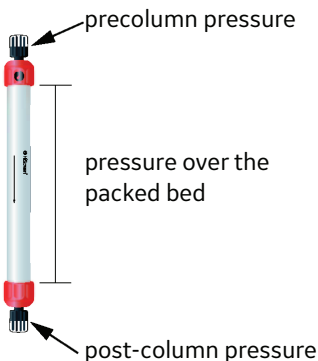


Fig 7. Precolumn and post-column measurements.

## ÄKTA avant and ÄKTA pure

The system will automatically monitor the pressures (precolumn pressure and pressure over the packed bed,  $\Delta p$ ). 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, ÄKTAFFPLC 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 ( $\Delta p$ ) will during run be equal to actual measured pressure - *total system pressure* (P1).

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

# 10 Storage

**HiTrap Q FF, HiTrap DEAE FF, HiTrap ANX FF (high sub) and HiTrap CM FF:** Rinse with water then wash with 5 column volumes of 20% ethanol at 1 mL/min (HiTrap 1 mL column) or at 5 mL/min (HiTrap 5 mL column) to prevent microbial growth.

**HiTrap SP FF:** Rinse with water then wash with 5 column volumes of 20% ethanol containing 0.2 M sodium acetate at 1 mL/min (HiTrap 1 mL column) or at 5 mL/min (HiTrap 5 mL column).

Seal the column with the supplied stoppers. The recommended storage temperature is 4°C to 30°C.



# 11 Ordering information

Product	No. supplied	Product code
HiTrap IEX Selection Kit	7 × 1 mL	17600233
HiTrap Q FF	5 × 1 mL	17505301
	5 × 5 mL	17515601
HiTrap SP FF	5 × 1 mL	17505401
	5 × 5 mL	17515701
HiTrap DEAE FF	5 × 1 mL	17505501
	5 × 5 mL	17515401
HiTrap CM FF	5 × 1 mL	17505601
	5 × 5 mL	17515501
HiTrap Q XL	5 × 1 mL	17515801
	5 × 5 mL	17515901
HiTrap SP XL	5 × 1 mL	17516001
	5 × 5 mL	17516101
HiTrap ANX FF (high sub)	5 × 1 mL	17516201
	5 × 5 mL	17516301
HiTrap Desalting	1 × 5 mL	29048684
	5 × 5 mL	17140801
	100 × 5 mL <sup>2</sup>	11000329
Q Sepharose Fast Flow <sup>1</sup>	25 mL	17051010
	300 mL	17151001
SP Sepharose Fast Flow <sup>1</sup>	25 mL	17072910
	300 mL	17072901
DEAE Sepharose Fast Flow <sup>1</sup>	25 mL	17070910
	500 mL	17070901
CM Sepharose Fast Flow <sup>1</sup>	25 mL	17071910
	500 mL	17071901
ANX Sepharose 4 Fast Flow (high sub) <sup>1</sup>	25 mL	17128710
	500 mL	17128701
HiPrep DEAE FF 16/10	1 × 20 mL	28936541
HiPrep CM FF 16/10	1 × 20 mL	28936542
HiPrep SP FF 16/10	1 × 20 mL	28936544
HiPrep Q FF 16/10	1 × 20 mL	28936543
HiPrep 26/10 Desalting	1 × 53 mL	17508701
	4 × 53 mL	17508702
PD-10 Desalting column	30	17085101

<sup>1</sup> Process scale quantities are available. Please contact your local representative.

<sup>2</sup> Special package. Delivered on specific customer order.

<b>Accessories</b>	<b>Quantity</b>	<b>Product code</b>
1/16" male/luer female <i>(For connection of syringe to top of HiTrap column)</i>	2	18111251
Tubing connector flangeless/M6 female <i>(For connection of tubing to bottom of HiTrap column)</i>	2	18100368
Tubing connector flangeless/M6 male <i>(For connection of tubing to top of HiTrap column)</i>	2	18101798
Union 1/16" female/M6 male <i>(For connection to original FPLC System through bottom of HiTrap column)</i>	6	18111257
Union M6 female /1/16" male <i>(For connection to original FPLC System through top of HiTrap column)</i>	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" <i>(For sealing bottom of HiTrap column)</i>	5	11000464
Fingertight stop plug, 1/16"	5	11000355

<b>Literature</b>	<b>Product code</b>
Ion Exchange Chromatography Handbook, Principles and Methods	11000421
Ion Exchange Chromatography Columns and Resins, Selection Guide	18112731

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