Instructions 71-5017-52 AK

HiTrap™ IEX Selection Kit

HiTrap SP FF, 1 ml
HiTrap CM FF, 1 ml
HiTrap Q FF, 1 ml
HiTrap DEAE FF, 1 ml
HiTrap ANX FF (high sub), 1 ml
HiTrap Q XL, 1 ml
HiTrap SP XL, 1 ml

HiTrap IEX Selection Kit consists of seven HiTrap 1 ml columns, prepacked with different ion exchangers.

HiTrap IEX Selection Kit is a tool which offers a fast, easy and convenient way to decide which ion exchange ligand is best for a given application. After choosing the optimal medium, prepacked columns and bulk media are available for larger scale preparative work.

The columns can be operated with a syringe, peristaltic pump or liquid chromatography system such as ÄKTA™.

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Please 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, please 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.

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

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

Table 1. Characteristics of HiTrap columns.

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

[1] Water at room temperature

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

Media Properties

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

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

Q Sepharose XL and SP Sepharose XL media have long chains of dextran coupled to a robust, 6% highly cross-linked agarose matrix. The dextran chains increase the exposure of the Q or SP charged groups which in certain applications results in higher loading capacity.

The functional groups are coupled to the matrices via chemically stable ether linkages.

Characteristics of the ion exchangers are listed in Table 2.

Table 2. Characteristic of Sepharose Fast Flow and Sepharose XL ion exchangers.

Cation exchangers	**SP Sepharose Fast Flow**	**SP Sepharose XL**	**CM Sepharose Fast Flow**
Bead structure	6% highly cross-linked agarose	6% highly cross-linked agarose with bound dextran	6% highly cross-linked agarose
Bead size	45 to 165 µm	45 to 165 µm	45 to 165 µm
Type of medium	Strong cation	Strong cation	Weak cation
Charged group	$-CH_2CH_2CH_2SO_3^-$	$-CH_2CH_2CH_2SO_3^-$	$-O-CH_2COO^-$
Total ionic capacity	0.18 to 0.25 mmol H^+/ ml medium	0.18 to 0.25 mmol H^+/ml medium	0.09 to 0.13 mmol H^+/ml medium
Dynamic binding capacity[1]	70 mg Ribonuclease A/ ml medium	>160 mg Lysozyme/ ml medium	50 mg Ribonuclease A/ ml medium
pH stability[2]			
Short term	3 to 14	3 to 14	2 to 14
Working	4 to 13	4 to 13	6 to 10
Long term	4 to 13	4 to 13	4 to 13
Storage temperature	4°C to 30°C	4°C to 30°C	4°C to 30°C
Storage buffer	20% ethanol, 0.2 M sodium acetate	20% ethanol, 0.2 M sodium acetate	20% ethanol
Chemical stability	All commonly used buffers, 1 M NaOH, 8 M urea, 6 M guanidine hydrochloride, 70% ethanol		
Avoid	Oxidizing agents, cationic detergents and buffers		

Anion exchangers	**Q Sepharose Fast Flow**	**Q Sepharose XL**	**DEAE Sepharose Fast Flow**	**ANX Sepharose 4 Fast Flow (high sub)**
Bead structure	6% highly cross-linked agarose	6% highly cross-linked agarose with bound dextran	6% highly cross-linked agarose	4% highly cross-linked agarose
Bead size	45 to 165 µm	45 to 165 µm	45 to 165 µm	45 to 165
Type of medium	Strong anion	Strong anion	Weak anion	Weak anion
Charged group	$-N^+(CH_3)_3$	$-N^+(CH_3)_3$	$-N^+H_2(C_2H_5)_2$	$-N^+H(C_2H_5)_2$
Total ionic capacity	0.18 to 0.25 mmol Cl^-/ ml medium	0.18 to 0.26 mmol Cl^-/ ml medium	0.11 to 0.16 mmol Cl^-/ ml medium	0.13 to 0.17 mmol Cl^-/ ml medium
Dynamic binding capacity[1]	120 mg HSA/ ml medium	>130 mg BSA/ ml medium	110 mg HSA/ ml medium	43 mg BSA/ ml medium
pH stability[2]				
Short term	1 to 14	2 to 14	1 to 14	2 to 14
Working	2 to 12	2 to 12	2 to 9	3 to 13
Long term	2 to 12	3 to 13	2 to 12	3 to 13
Storage temperature	4°C to 30°C	4°C to 30°C	4°C to 30°C	4°C to 30°C
Storage	20% ethanol	20% ethanol	20% ethanol	20% ethanol
Chemical	All commonly used buffers, 1 M NaOH, 8 M urea, 6 M guanidine stability hydrochloride, 70% ethanol			
Avoid	Oxidizing agents, anionic detergents and buffers			

[1] Determination of dynamic binding capacity: **DEAE Sepharose Fast Flow, Q Sepharose Fast Flow, SP Sepharose Fast Flow and CM Sepharose Fast Flow:** Samples were applied at 75 cm/h until 50% breakthrough. Columns: 0.5 × 5 cm. Buffers: 0.05 M Tris, (+ 2 M NaCl in the elution buffer), pH 7.5 (Q and DEAE), 0.1 M acetate, (+ 2 M NaCl in the elution buffer), pH 5.0 (SP and CM).
Q Sepharose XL and SP Sepharose XL: Samples were applied at 300 cm/h until 10% breakthrough. Columns: 0.75 × 10 cm. Buffers: 0.05 M Tris, (+ 0.5 M NaCl in the elution buffer), pH 7.5 (Q), 0.05 M glycine, (+ 0.5 M NaCl in the elution buffer), pH 9.0 (SP).
ANX Sepharose 4 Fast Flow (high sub): Sample was applied at 300 cm/h until 10% breakthrough. Column: 1.6 × 13 cm. Buffer: 0.05 M Tris, (+1 M NaCl in the elution buffer), pH 7.5

[2] The ranges given are estimates based on our knowledge and experience. Please note the following:
pH stability, long term refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance.
pH stability, short term refers to the pH interval for regeneration, cleaning-in-place and sanitization procedures.

Note: *The active end of the charged group is the same for DEAE Sepharose Fast Flow and ANX Sepharose 4 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 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 2). If the sample components are most stable below their pI's, a cation exchanger should 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 3).

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.

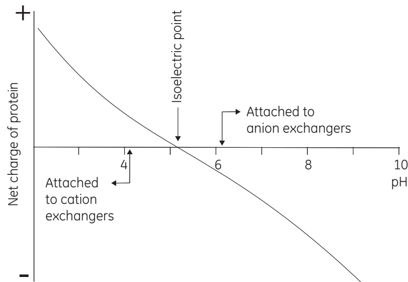

Fig 2. The net charge of a protein as a function of pH.

3 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, SP Sepharose XL and CM Sepharose Fast Flow, or high pH for Q Sepharose Fast Flow, Q Sepharose XL, DEAE Sepharose Fast Flow and ANX Sepharose 4 Fast Flow (high sub). The ionic strength should 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 3 and 4 and Figures 3 and 4. In the majority of cases a concentration of at least 10 mM is required to ensure adequate buffering capacity. The ionic strength of the buffer should 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 should not be so low that protein denaturation or precipitation occurs.

The buffering ion should 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 may take part in the ion exchange process and cause local disturbances in pH.

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.

Table 3. Buffers for anion exchange chromatography.

pH interval	Substance	Conc. (mM)	Counter-ion	pKa (25°C)[1]
4.3–5.3	N-Methyl-piperazine	20	Cl^-	4.75
4.8–5.8	Piperazine	20	Cl^- or $HCOO^-$	5.33
5.5–6.5	L-Histidine	20	Cl^-	6.04
6.0–7.0	bis-Tris	20	Cl^-	6.48
6.2–7.2; 8.6–9.6	bis-Tris propane	20	Cl^-	6.65; 9.10
7.3–8.3	Tri-ethanolamine	20	Cl^- or CH_3COO^-	7.76
7.6–8.6	Tris	20	Cl^-	8.07
8.0–9.0	N-Methyl-diethanolamine	20	SO_4^{2-}	8.52
8.0–9.0	N-Methyl-diethanolamine	50	Cl^- or CH_3COO^-	8.52
8.4–9.4	Diethanolamine	20 at pH 8.4 50 at pH 8.8	Cl^-	8.88
8.4–9.4	Propane 1,3-Diamino	20	Cl^-	8.88
9.0–10.0	Ethanolamine	20	Cl^-	9.50
9.2–10.2	Piperazine	20	Cl^-	9.73
10.0–11.0	Propane 1,3-Diamino	20	Cl^-	10.55
10.6–11.6	Piperidine	20	Cl^-	11.12

[1] Ref: Handbook of chemistry and physics, 83rd edition, CRC, 2002–2003.

Table 4. Buffers for cation exchange chromatography.

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

[1] Ref: Handbook of chemistry and physics, 83rd edition, CRC, 2002– 2003.

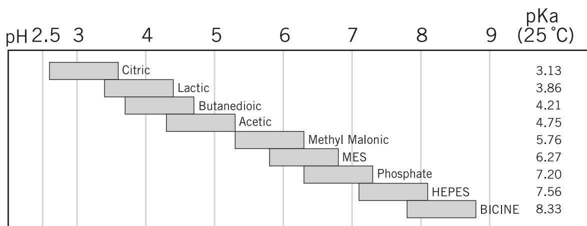

Fig 3. Recommended buffer substances for cation exchange chromatography.

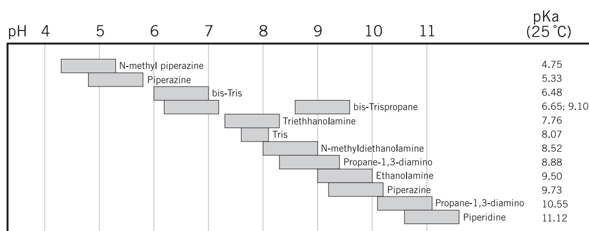

Fig 4. Recommended buffer substances for anion exchange chromatography.

4 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 should be of high purity. It is recommended to filter the buffers by passing them through a 0.45 µm filter before use. See Tables 3 and 4 and Figures 3 and 4 for recommended buffers.

Sample preparation

The sample should 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 5. The sample should be filtered through a 0.45 µm filter or centrifuged immediately before it is applied to the column.

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 luer 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 ml of start buffer, at 1 ml/min.
4. Wash with 5 ml of elution buffer (start buffer with 1 M NaCl).
5. Finally equilibrate with 5 to 10 ml of start buffer.
6. Apply the sample at 1 ml/min using a syringe fitted to the luer connector or by pumping it onto the column.
7. Wash with 5 ml of start buffer or until no material appears in the effluent.
8. Elute with 5 to 10 ml of elution buffer (see page 16 for "Choice of gradient type").
9. The purified fractions can be desalted using a HiTrap Desalting, HiPrep 26/10 Desalting or a PD-10 column if necessary.
10. After the completed elution, regenerate the column by washing with 5 ml of regeneration buffer (start buffer with 1 M NaCl) followed by 5 to 10 ml of start buffer. The column is now ready for a new sample.

For a first experiment the following conditions are recommended:

Flow rate:	1 ml/min
Start buffer:	See Tables 3 and 4, Figures 3 and 4.
Elution buffer:	Start buffer + 1 M NaCl
Gradient volume:	20 ml

5 Optimizing

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 4–8 (SP, CM) or 5–9 (Q, DEAE, ANX), with 0.5–1 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. Collect the eluate.

5 Wash with at least 5 ml of start buffer or until no material appears in effluent. Collect the eluate.

6 Elute bound material with elution buffer. 3 to 5 ml is usually sufficient but other volumes may 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 2–7 for the next buffer pH.

9 Decide which pH should 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 0–0.5 M, with intervals of 0.05–0.1 M salt between each buffer.

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 may be optimized to meet the special needs can be found in the Handbook, *Ion Exchange Chromatography & Chromatofocusing, Principles and Methods*, see "Ordering information".

A wide range of ion exchange chromatography media for purification of biomolecules at all scales is available, see "Ordering information".

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 may 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".
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. Collect eluate.
5. Wash with 5 to 10 ml of start buffer or until no material appears in effluent. 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 ml is usually sufficient. Collect eluate.
7. Elute with next ionic strength buffer. Collect eluate.
8. After completed elution, regenerate the column by washing with 5 ml of regeneration buffer (start buffer with 1 M NaCl) followed by 5 to 10 ml 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. 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., an ÄKTA system with two pumps.

1. Choose starting conditions as outlined under “Optimizing”.
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. Collect eluate.
5. Wash with 5 to 10 ml of start buffer or until no material appears in the effluent.
6. Start the gradient elution. A gradient volume of 10 to 20 ml and an increase in ionic strength to 0.5 M NaCl is usually sufficient.
7. Regenerate the column by washing with 5 ml of start buffer with 1 M NaCl followed by 5 to 10 ml of start buffer. The column is now ready for a new sample.

6 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 5.

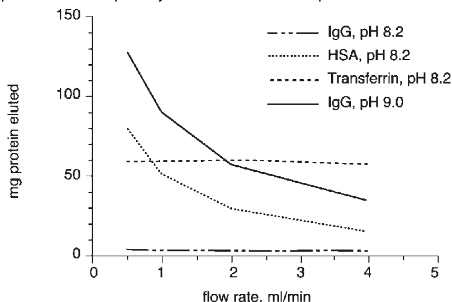

Fig 5. Binding capacity of human IgG, HSA and human transferrin at different pH´s on HiTrap Q HP, 1 ml.

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.

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 ml of start buffer or until no material appears in effluent.
6 Elute bound proteins with 3 to 5 ml 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.

7 Scaling up

For quick scale-up of purification, two or three HiTrap ion exchange columns can be connected in series (back pressure will increase). For further scale-up Q Sepharose Fast Flow, SP Sepharose Fast Flow, CM Sepharose Fast Flow, DEAE Sepharose Fast Flow, ANX Sepharose Fast Flow (high sub), Q Sepharose XL and SP Sepharose XL are available as prepacked HiPrep 16/10 columns or as bulk media packs, see “Ordering information”.

8 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 6. 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) may damage the column.*

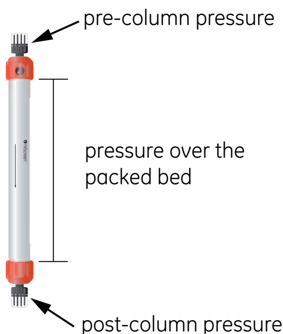

Fig 6. Pre-column and post-column measurements.

ÄKTA avant

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

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

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

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

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.*

9 Storage

HiTrap Q FF, HiTrap Q XL, HiTrap DEAE FF, HiTrap ANX FF (high sub) and HiTrap CM FF: Rinse with water and then wash with 5 ml 20% ethanol at 1 ml/min to prevent microbial growth.

HiTrap SP FF and HiTrap SP XL: Rinse with water then wash with 5 ml 20% ethanol containing 0.2 M sodium acetate at 1 ml/min.

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

Table 5. Prepacked columns for desalting and buffer exchange

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

[1] Volumes with gravity elution
[2] Volumes with centrifugation

10 Ordering information

Product	**No. Supplied**	**Code No.**
HiTrap IEX Selection Kit	7 × 1 ml	17-6002-33
HiTrap Q FF	5 × 1 ml	17-5053-01
	5 × 5 ml	17-5156-01
HiTrap SP FF	5 × 1 ml	17-5054-01
	5 × 5 ml	17-5157-01
HiTrap DEAE FF	5 × 1 ml	17-5055-01
	5 × 5 ml	17-5154-01
HiTrap CM FF	5 × 1 ml	17-5056-01
	5 × 5 ml	17-5155-01
HiTrap Q XL	5 × 1 ml	17-5158-01
	5 × 5 ml	17-5159-01
HiTrap SP XL	5 × 1 ml	17-5160-01
	5 × 5 ml	17-5161-01
HiTrap ANX FF (high sub)	5 × 1 ml	17-5162-01
	5 × 5 ml	17-5163-01

Related product	**No. Supplied**	**Code No.**
HiTrap SP HP	1 × 1 ml	29-0513-24
	5 × 1 ml	17-1151-01
	5 × 5 ml	17-1152-01
HiTrap Q HP	1 × 1 ml	29-0513-25
	5 × 1 ml	17-1153-01
	5 × 5 ml	17-1154-01
Q Sepharose Fast Flow	25 ml	17-0510-10
	300 ml[1]	17-1510-01
SP Sepharose Fast Flow	25 ml	17-0729-10
	300 ml[1]	17-0729-01
DEAE Sepharose Fast Flow	25 ml	17-0709-10
	500 ml[1]	17-0709-01
CM Sepharose Fast Flow	25 ml	17-0719-10
	500 ml[1]	17-0719-01
ANX Sepharose 4 Fast Flow (high sub)	25 ml	17-1287-10
	500 ml[1]	17-1287-01
Q Sepharose XL	300 ml[1]	17-5072-01
SP Sepharose XL	300 ml[1]	17-5073-01
HiPrep DEAE FF 16/10	1 × 20 ml	28-9365-41

Related product	No. Supplied	Code No.
HiPrep CM FF 16/10	1 × 20 ml	28-9365-42
HiPrep SP FF 16/10	1 × 20 ml	28-9365-44
HiPrep Q FF 16/10	1 × 20 ml	28-9365-43
HiPrep Q XL 16/10	1 × 20 ml	28-9365-38
HiPrep SP XL 16/10	1 × 20 ml	28-9365-40
HiTrap Desalting	1 × 5 ml	29-0486-84
	5 × 5 ml	17-1408-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02
PD-10 Desalting column	30	17-0851-01

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

Accessories	Quantity	Code No.
1/16" male/luer female *(For connection of syringe to top of HiTrap column*)	2	18-1112-51
Tubing connector flangeless/M6 female (*For connection of tubing to bottom of HiTrap column)*	2	18-1003-68
Tubing connector flangeless/M6 male *(For connection of tubing to top of HiTrap column)*	2	18-1017-98
Union 1/16" female/M6 male *(For connection to original FPLC System through bottom of HiTrap column)*	6	18-1112-57
Union M6 female /1/16" male *(For connection to original FPLC System through top of HiTrap column*)	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep, 1/16" male connector for ÄKTA design	8	28-4010-81
Stop plug female, 1/16" *(For sealing bottom of HiTrap column)*	5	11-0004-64
Fingertight stop plug, 1/16"	5	11-0003-55

Literature	Code No.
Ion Exchange Cromatography & Chromatofocusing Handbook, Principles and Methods	11-0004-21
Ion Exchange Columns and Media, Selection Guide	18-1127-31
Prepacked chromatography columns for ÄKTAdesign systems, Selection Guide	28-9317-78

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