GE Healthcare

Instructions 28-4026-57 AB

HiPrep 16/10 Q FF, HiPrep 16/10 SP FF and HiPrep 16/10 ANX FF (high sub)

Introduction

HiPrep™ 16/10 Q FF, HiPrep 16/10 SP FF and HiPrep 16/10 ANX FF (high sub) are prepacked, ready to use columns for ion exchange chromatography. They provide fast, preparative separations of proteins and other biomolecules. The columns are prepacked with Q Sepharose™ Fast Flow, SP Sepharose Fast Flow and ANX Sepharose 4 Fast Flow respectively. See table below for column characteristics.

Column data

Matrix	6% highly cross-linked spherical agarose (Q, SP) 4% highly cross-linked spherical agarose (ANX)
Mean particle size	90 µm
Bed volume	20 ml
Bed height	100 mm
i.d.	16 mm
Column composition	Polypropylene
Recommended flow rate[1]	2–10 ml/min (60–300 cm/h)
Maximum flow rate[1]	10 ml/min (300 cm/h)
Maximum pressure over the packed bed during operation, Δp[3]	0.15 MPa, 1.5 bar, 22 psi
HiPrep column hardware pressure limit[3]	0.5 MPa, 5 bar, 73 psi
Storage	+4° to +30 °C in 20% ethanol (Q, ANX) and 20% ethanol, 0,2 M sodium acetate (SP).

	Q	SP	ANX (high sub)
Type of exchanger	Strong anion	Strong cation	Weak anion
Charged group	$-N^+(CH_3)_3$	$-CH_2CH_2CH_2SO_3^-$	$-N^+(C_2H_5)_2H$
pH stability			
short term	1–14	3–14	2–14
long term and working range	2–12	4–13	3–13
Total ionic capacity (mmol/ml medium)	0.18–0.25 (Cl^-)	0.18–0.25 (H^+)	0.13–0.17 (Cl^-)
Dynamic binding capacity[2]	120 mg HSA/ml medium	70 mg Ribonuclease A/ml medium	43 mg BSA/ml medium

[1] Water at room temperature. Flow rate is determined by $v \bullet \eta \leq 10$ ml/min where v=flow rate and η=viscosity.

[2] Determination of dynamic binding capacity: Q Sepharose Fast Flow and SP 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 0.1 M acetate, (+2 M NaCl in the elution buffer), pH 5.0 (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.

[3] Many chromatography systems are equipped with pressure gauges to measure the pressure at a particular point in the system, usually just after the pumps. The pressure measured here is the sum of the pre-column pressure, the pressure drop over the medium bed, and the post-column pressure. It is always higher than the pressure drop over the bed alone. We recommend keeping the pressure drop over the bed below 1.5 bar. Setting the upper limit of your pressure gauge to 1.5 bar will ensure the pump shuts down before the medium is overpressured. If necessary, post-column pressure of up to 3.5 bar can be added to the limit without exceeding the column hardware limit. To determine postcolumn pressure, proceed as follows:

To avoid breaking the column, the post-column pressure must never exceed 3.5 bar.

1. Connect a piece of tubing in place of the column.
2. Run the pump at the maximum flow you intend to use for chromatography. Use a buffer with the same viscosity as you intend to use for chromatography. Note the backpressure as total pressure.
3. Disconnect the tubing and run at the same flow rate used in step 2. Note this backpressure as pre-column pressure.
4. Calculate the post-column pressure as total pressure minus pre-column pressure. If the post-column pressure is higher than 3.5 bar, take steps to reduce it (shorten tubing, clear clogged tubing, or change flow restrictors) and perform steps 1–4 again until the post-column pressure is below 3.5 bar. When the post-column pressure is satisfactory, add the post-column pressure to 1.5 bar and set this as the upper pressure limit on the chromatography system.

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 the ANX Sepharose 4 Fast Flow has a diethylaminopropyl-group attached.

First time use

Ensure an appropriate pressure limit has been set. Equilibrate the column for first time use or after long-term storage by running:

a. 100 ml of start buffer at 5 ml/min at room temperature (see section "Choice of buffer" for buffer recommendations).
b. 100 ml of elution buffer at 5 ml/min at room temperature.
c. 100 ml of start buffer at 5 ml/min at room temperature.

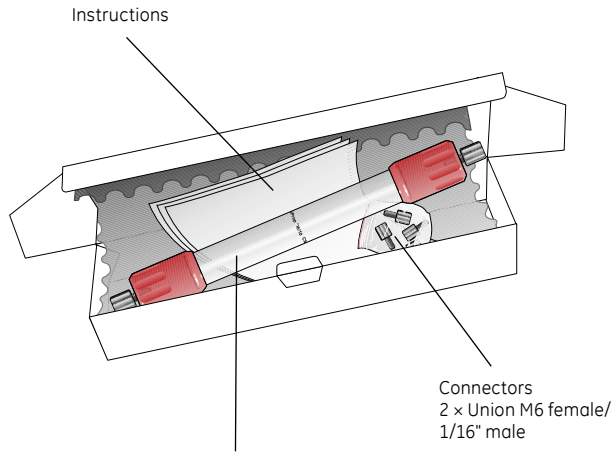
All these HiPrep columns can be used directly on ÄKTAdesign™ systems without the need for any extra connectors.

Try these conditions first

Flow rate:	5 ml/min at room temperature
Gradient:	0–100% elution buffer in 200 ml (10 CV)
Start buffer:	See section "Choice of buffer"
Elution buffer:	Start buffer + 1 M NaCl

Equilibration before a new run

Proceed according to steps b and c in the section "First time use". Extended equilibration may be needed if detergents were included in the eluent.



HiPrep 16/10 Q FF, HiPrep 16/10 SP FF
or HiPrep 16/10 ANX FF (high sub)

Buffers and solvent resistance

De-gas and filter all solutions through a 0.45 µm filter to increase column life-time.

Daily use

All commonly used aqueous buffers (see "Column data" for recommended pH)
Urea, up to 8 M

Cleaning

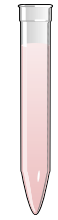
Sodium hydroxide, up to 1 M
Ethanol, up to 70%
Acetic acid, up to 1 M
Isopropanol, up to 30%

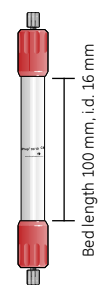
Avoid

Oxidizing agents
Cationic detergents and buffers (SP)
Anionic detergents and buffers (Q, ANX)

Sample preparation

Net charge of protein:	Positive (SP), negative (Q, ANX)
Recommended sample load:	Not more than 10–20% of the dynamic capacity (see section "Column data").
Preparation:	Dissolve the sample in start buffer, filter through 0.45 µm or centrifuge at 10 000 × g for 10 min





Delivery/storage

The column is supplied in 20% ethanol (Q, ANX) or 20% ethanol, 0.2 M sodium acetate (SP). If the column is to be stored for more than two days after use, clean the column according to the procedure described under "Cleaning-in-place (CIP)". Then equilibrate with at least 100 ml of 20% ethanol (Q, ANX) or 20% ethanol, 0.2 M sodium acetate (SP) at a flow rate of 5 ml/min at room temperature.

DO NOT OPEN THE COLUMN!

Choice of buffer

To avoid local disturbances in pH caused by buffering ions participating in the ion exchange process, select an eluent with buffering ions of the same charge as the substituent groups on the ion exchanger.

The start buffer pH should be chosen so that substances to be bound to the ion exchanger are charged, e.g. at least 1 pH unit above the isoelectric point for anion exchangers or at least 1 pH unit below the isoelectric point for cation exchangers.

HiPrep

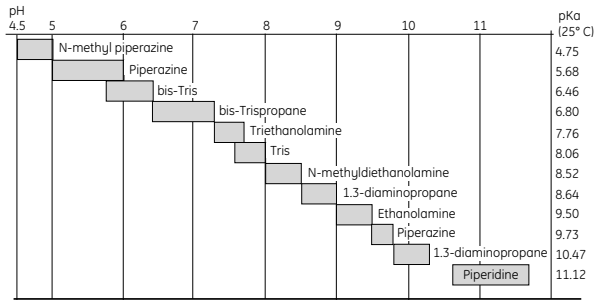


Fig 1. Recommended buffer substances for anion exchange chromatography.

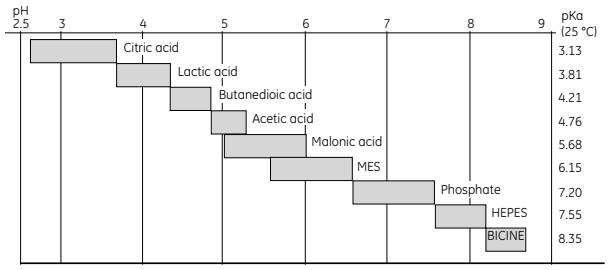


Fig 2. Recommended buffer substances for cation exchange chromatography.

Figure 1 and Figure 2 list a selection of standard aqueous buffers.

Table 1 lists suggested volatile buffers that can be used in cases where the purified substance has to be freeze-dried.

Table 1. Volatile buffer systems.

pH	Substances
2.3–3.5	Pyridine/formic acid
3.0–5.0	Trimethylamine/formic acid
4.0–6.0	Trimethylamine/acetic acid
6.8–8.8	Trimethylamine/HCl
7.0–8.5	Ammonia/formic acid
8.5–10.0	Ammonia/acetic acid
7.0–12.0	Trimethylamine/CO2
8.0–9.5	Ammonium carbonate/ammonia
8.5–10.5	Ethanolamine/HCl

Optimization

Perform your first run according to "Try these conditions first". If the obtained results are unsatisfactory, consider the following:

Action	Effect
Change pH/buffer salt (see Figs. 1 and 2 for buffers)	Selectivity change, weaker/stronger binding
Change salt, counter ions and/or co-ions	Selectivity change
Smaller sample loading	Improved resolution
Lower flow rate	Improved resolution, when running isocratically
Shallower gradient	Improved resolution, but broader peaks

and decreased concentration in fractions

For more information, please refer to the handbook "Ion Exchange Chromatography and Chromatofocusing, Principles and Methods", see ordering information.

Cleaning-in-place (CIP)

Regular cleaning

Wash the column with 40 ml of 2 M NaCl at a flow rate of 5 ml/min at room temperature after each run to elute material still bound to the column.

If detergents have been used, wash the column with 100 ml distilled water followed by 40 ml of 2 M NaCl at a flow rate of 5 ml/min at room temperature.

Re-equilibrate the column with at least 100 ml start buffer at a flow rate of 5 ml/min at room temperature or until the UV base-line and pH/conductivity values are stable.

More rigorous cleaning

Reverse the flow direction and run at a flow rate of 5 ml/min at room temperature the following sequence of solutions.

1. 80 ml of a 2 M NaCl solution (removes ionically bound proteins from the column) followed by 50 ml distilled water.
2. 80 ml of a 1 M NaOH solution (removes precipitated proteins, hydrophobically bound proteins and lipoproteins from the column) followed by 50 ml distilled water.
3. 80 ml of 70% ethanol or 30% isopropanol (removes proteins, lipoproteins and lipids that are strongly hydrophobically bound to the column) followed by 60 ml distilled water.

   or

   30 ml 0.5% non-ionic detergent in acidic solution (e.g. 0.1 M acetic acid) followed by 100 ml 70% ethanol (to remove the detergent) and 60 ml distilled water.

After cleaning, equilibrate the column before use with approximately 100 ml start buffer at a flow rate of 5 ml/min at room temperature in the normal flow direction.

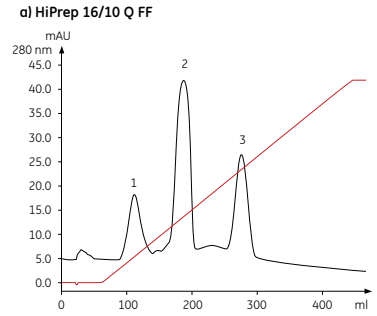
DO NOT OPEN THE COLUMN!

Trouble shooting

Symptom	Remedy
Increased backpressure over the column	Reverse the flow direction and pump 100 ml elution buffer at a flow rate of 5 ml/min through the column. Return to normal flow direction and run 100 ml start buffer at a flow rate of 5 ml/min through the column.
Loss of resolution and/or decreased sample recovery	Clean the column according to the procedure described in the section "More rigorous cleaning".
Air in the column	Reverse the flow direction and pump 100 ml of well de gassed start buffer through the column at a flow rate of 5 ml/min.

Column performance control

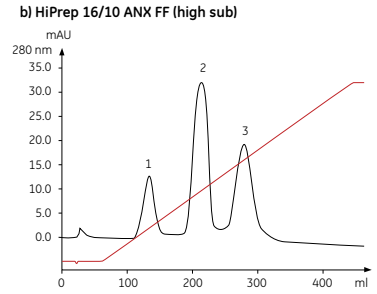
We recommend checking the column performance at regular intervals. Figure 3 describes how to check the function of the respective column.



Typical chromatogram from a function test of HiPrep 16/10 Q FF

Sample:
1. Conalbumin 2 mg/ml
2. α-lactalbumin 4 mg/ml
3. Soya Trypsin Inhibitor 6 mg/ml

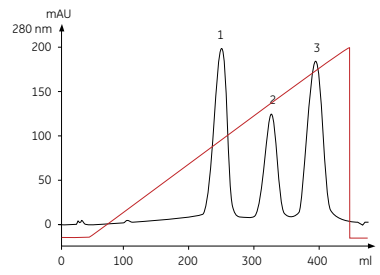
Sample volume: 1 ml
Gradient: 0-100% elution buffer in 20 CV (400 ml)
Start buffer: 50 mM Tris-HCl, pH 7.3
Elution buffer: 50 mM Tris-HCl, 0.5 M NaCl, pH 7.3
Flow rate: 5 ml/min (150 cm/h)
Instrumentation: ÄKTAexplorer™



Typical chromatogram from a function test of HiPrep 16/10 ANX FF

Sample:
1. Conalbumin 2 mg/ml
2. α-lactalbumin 4 mg/ml
3. Soya Trypsin Inhibitor 6 mg/ml

Sample volume: 1 ml
Gradient: 0-100% elution buffer in 20 CV (400 ml)
Start buffer: 50 mM Tris-HCl, pH 7.3
Elution buffer: 50 mM Tris-HCl, 0.5 M NaCl, pH 7.3
Flow rate: 5 ml/min (150 cm/h)
Instrumentation: ÄKTAexplorer



Typical chromatogram from a function test of HiPrep 16/10 SP FF

Sample:
1. Ribonuclease A 2 mg/ml
2. Cytochrome C 2 mg/ml
3. α-lactoglobulin 4 mg/ml

Sample volume: 1 ml
Gradient: 0-60% elution buffer in 20 CV (400 ml)
Start buffer: 50 mM sodium acetate, pH 4.5
Elution buffer: 50 mM sodium acetate, 1 M NaCl, pH 4.5
Flow rate: 5 ml/min (150 cm/h)
Instrumentation: ÄKTAexplorer

Fig 3. Typical chromatograms from function tests of (a) HiPrep 16/10 Q FF, (b) HiPrep 16/10 ANX FF (high sub) and (c) HiPrep 16/10 SP FF.

Ordering information

Products	No. per pack	Code No.
HiPrep 16/10 Q FF	1 (20 ml)	17-5190-01
HiPrep 16/10 SP FF	1 (20 ml)	17-5192-01
HiPrep 16/10 ANX FF (high sub)	1 (20 ml)	17-5191-01

Companion products

HiTrap™ IEX Selection Kit*	7 × 1 ml	17-6002-33
HiPrep 26/10 Desalting	1 (53 ml)	17-5087-01
HiPrep 26/10 Desalting	4 (53 ml)	17-5087-02

* contains 7 different ion exchange media

Accessories

HiTrap/HiPrep 1/16" male connector to ÄKTA design	8	24-4010-81
To connect columns with 1/16" connections to FPLC™ System:		
Union M6 female 1/16" male*	6	18-3858-01

*Two units (in red polypropylene) are included in HiPrep package

Related literature

Handbook, Ion Exchange Chromatography and Chromatofocusing, Principles & Methods	11-0004-21
Ion Exchange Column and Media, Selection Guide	18-1127-31

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