GE Healthcare
Life Sciences

Instructions 28-9413-23 AC **Prepacked columns**

HiPrep™ CM FF 16/10
HiPrep DEAE FF 16/10
HiPrep Q FF 16/10
HiPrep SP FF 16/10
HiPrep Q HP 16/10
HiPrep SP HP 16/10

HiPrep CM FF 16/10, HiPrep DEAE FF 16/10, HiPrep Q FF 16/10, HiPrep SP FF 16/10, HiPrep Q HP 16/10, and HiPrep SP HP 16/10 are prepacked, ready to use columns for ion exchange chromatography. They provide fast preparative separations of proteins and other biomolecules. The columns are used in an optimal way with liquid chromatography systems such as ÄKTA™.

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Please read these instructions carefully before using the HiPrep columns.

Intended use
HiPrep 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

HiPrep column characteristics

HiPrep columns are made of biocompatible polypropylene that does not interact with biomolecules. The columns are delivered with stoppers at the inlet and outlet. The arrow on the column label indicates the column orientation and the recommended flow direction, see Figure 1.

Fig 1. HiPrep 16/10 column

Note: *HiPrep columns cannot be opened or refilled*

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

Table 1. Characteristics of HiPrep 16/10 column

Column volume (CV)	20 ml
Column dimensions	16 × 100 mm
Column hardware pressure limit [1]	5 bar (0.5 MPa)

[1] The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography medium and the column tubing used.

Properties of IEX chromatography media

Q Sepharose Fast Flow, SP Sepharose Fast Flow, DEAE Sepharose Fast Flow, and CM Sepharose Fast Flow are based on 6% highly cross-linked agarose with an average bead size of 90 µm. The media have good flow properties and high loading capacities.

Q Sepharose High Performance, and SP Sepharose High Performance are based on rigid, highly cross-linked, 6% agarose with an average bead size of 34 µm. The smaller bead size will result in higher resolution and sharper peaks.

The functional groups are coupled to the matrix via chemically stable ether linkages and remain charged over the entire pH working range, as well as maintain high capacity.

Type of media	Ion exchanger type	Functional group
Q	Strong anion exchanger	Quaternary amine group
SP	Strong cation exchanger	Sulfoethyl group
DEAE	Weak anion exchanger	Diethylaminoethyl group
CM	Weak cation exchanger	Carboxymethyl group

The characteristics of the different media are listed in Tables 2, 3 and 4.

Table 2. Characteristics of Q Sepharose FF and SP Sepharose FF

	Q Sepharose FF	SP Sepharose FF
Matrix	Highly cross-linked 6% agarose	
Average particle size (d_{50v}) [1]	90 µm	90 µm
Ion exchange type	Strong anion	Strong cation
Charged group	$-N^+(CH_3)_3$	$-CH_2CH_2CH_2SO_3^-$
Total ionic capacity	0.18 to 0.25 mmol Cl^-/ml medium	0.18 to 0.25 mmol H^+/ml medium
Dynamic binding capacity [2]	120 mg HSA/ml medium	70 mg Ribonuclease A/ml medium
Recommended flow velocity [3]	150 cm/h	150 cm/h
Maximum flow velocity [3]	300 cm/h	300 cm/h
pH stability [4]		
Working range	2 to 12	4 to 13
Cleaning-in-place	1 to 14	3 to 14
Chemical stability	All commonly used aqueous buffers, 1 M NaOH, 8 M urea, 6 M guanidine hydrochloride, 70% ethanol	
Avoid	Oxidizing agents, and anionic detergents/ buffers	Oxidizing agents, and cationic detergents/ buffers
Storage	4°C to 30°C in 20% ethanol	4°C to 30°C in 0.2 M sodium acetate in 20% ethanol

1 d_{50v} is the average particle size of the cumulative volume distribution.

2 Running conditions:
Q Sepharose FF and DEAE Sepharose FF: 0.05 M Tris-HCl, pH 7.5 at 75 cm/h.
SP Sepharose FF: 0.1 M sodium acetate, pH 5.0 at 75 cm/h.

3 Water at room temperature.
For viscous buffers and samples the flow velocity must be optimized. Starting with a low flow velocity is recommended.

4 Working range: pH interval where the medium can be handled without significant change in function.
Cleaning-in-place: pH interval where the medium can be subjected to cleaning-in-place without significant change in function.

Table 3. Characteristics of DEAE Sepharose FF and CM Sepharose FF

	DEAE Sepharose FF	CM Sepharose FF
Matrix	Highly cross-linked 6% agarose	
Average particle size (d_{50v}) [1]	90 µm	90 µm
Ion exchange type	Weak anion	Weak cation
Charged group	$-N^+(C_2H_5)_2H$	$-O-CH_2COO^-$
Total ionic capacity	0.11 to 0.16 mmol Cl^-/ml medium	0.09 to 0.13 mmol H^+/ml medium
Dynamic binding capacity [2]	110 mg HSA/ml medium	50 mg Ribonuclease A/ml medium
Recommended flow velocity [3]	150 cm/h	150 cm/h
Maximum flow velocity [3]	300 cm/h	300 cm/h
pH stability [4]		
Working range	2 to 12	4 to 13
Cleaning-in-place	1 to 14	2 to 14
Chemical stability	All commonly used aqueous buffers, 1 M NaOH, 8 M urea, 6 M guanidine hydrochloride, 70% ethanol	
Avoid	Oxidizing agents, and anionic detergents/ buffers	Oxidizing agents, and cationic detergents/ buffers
Storage	4°C to 30°C in 20% ethanol	4°C to 30°C in 20% ethanol

1 d_{50v} is the average particle size of the cumulative volume distribution.

2 Running conditions:
Q Sepharose FF and DEAE Sepharose FF: 0.05 M Tris-HCl, pH 7.5 at 75 cm/h.
SP Sepharose FF: 0.1 M sodium acetate, pH 5.0 at 75 cm/h.

3 Water at room temperature.
For viscous buffers and samples the flow velocity must be optimized. Starting with a low flow velocity is recommended.

4 Working range: pH interval where the medium can be handled without significant change in function.
Cleaning-in-place: pH interval where the medium can be subjected to cleaning-in-place without significant change in function.

Table 4. Characteristics of Q Sepharose HP and SP Sepharose HP

	Q Sepharose HP	SP Sepharose HP
Matrix	Highly cross-linked 6% agarose	
Average particle size (d_{50v}) [1]	34 µm	34 µm
Ion exchange type	Strong anion	Strong cation
Charged group	$-N^+(CH_3)_3$	$-CH_2CH_2CH_2SO_3^-$
Total ionic capacity	0.14 to 0.20 mmol Cl^-/ml medium	0.15 to 0.20 mmol H^+/ml medium
Dynamic binding capacity [2]	70 mg BSA/ml medium	55 mg Ribonuclease A/ml medium
Recommended flow velocity [3]	90 cm/h	90 cm/h
Maximum flow velocity [3]	150 cm/h	150 cm/h
pH stability [4]		
Working range	2 to 12	4 to 13
Cleaning-in-place	1 to 14	3 to 14
Chemical stability	All commonly used aqueous buffers, 1 M NaOH, 8 M urea, 6 M guanidine hydrochloride, 70% ethanol	
Avoid	Oxidizing agents, and anionic detergents/ buffers	Oxidizing agents, and cationic detergents/ buffers
Storage	4°C to 30°C in 20% ethanol	4°C to 30°C in 0.2 M sodium acetate in 20% ethanol

1 d_{50v} is the average particle size of the cumulative volume distribution.

2 Running conditions:
Q Sepharose HP: 10.0 mg/ml BSA in 0.02 M Tris-HCl, pH 8.2 at 150 cm/h.
SP Sepharose HP: 5 mg/ml Ribonuclease in 0.1 M sodium acetate, pH 6.0 at 150 cm/h.

3 Water at room temperature.
For viscous buffers and samples the flow velocity must be optimized. Starting with a low flow velocity is recommended.

4 Working range: pH interval where the medium can be handled without significant change in function.
Cleaning-in-place: pH interval where the medium can be subjected to cleaning-in-place without significant change in function.

2 Optimization

Optimizing the process

The aim of designing and optimizing an ion exchange separation process is to identify conditions that promote binding of the highest amount of target molecule in the shortest possible time with highest possible product recovery. To reduce time, sample and buffer consumption during optimization the method should be designed in laboratory scale.

Optimizing binding conditions

Screen for optimal binding conditions by testing a range of pH values within which the target protein is known to be stable. If the isoelectric point (pI) of the target protein is known, then begin with a narrower pH range, for example, 0.5 to 1 pH unit away from pI. In some cases the sample conductivity is equally important as the pH when screening for optimal binding conditions.

Screening for buffer concentration at the temperature where the process is intended to be run will give the optimal dynamic binding capacity.

Optimizing elution conditions

Linear ionic strength gradients should always be used for method development or when starting with an unknown sample. Linear ionic strength gradients are easy to prepare and very reproducible when generated by a suitable chromatography system. The results obtained can then serve as a basis from which to optimize the separation.

Step-wise elution allows the target protein to be eluted in a more concentrated form, thus decreasing buffer consumption and shortening cycle times. Due to the high concentrations of protein in the eluted pool it might in rare cases be necessary to decrease the flow rate and thereby avoid exceeding the maximum back pressure for the column.

Automated buffer preparation

Users of ÄKTA chromatography systems with BufferPrep or BufferPro functionality can select from a range of buffer recipes to conveniently screen media over a range of pH values and elution conditions.

3 Operation

Prepare buffers

To avoid local disturbances in pH caused by buffering ions participating in the ion exchange process, select a buffer 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, that is, at least 1 pH unit above pI for anion exchangers or at least 1 pH unit below pI for cation exchangers.

The elution buffer is usually of the same composition and pH as the start buffer, but it contains additional salt, most often sodium chloride. The pH of the start buffer should be at least 0.5 to 1 pH unit above pI of the target molecule when using an anion exchanger and at least 0.5 to 1 pH unit below pI when using a cation exchanger.

The buffer species and buffer concentration are important for reproducible and robust methods. The buffer concentration depends partly on the buffer capacity at a given pH and should be at least 10 mM (only rarely above 100 mM). Where the conductivity of the buffers needs to be considered, it can be increased by increasing the buffer concentration or adding sodium chloride.

Try the following buffers for samples with unknown charge properties.

Anion exchange

Start buffer: 20 mM Tris-HCl, pH 8.0

Elution buffer: 20 mM Tris-HCl, 1 M NaCl, pH 8.0

Cation exchange

Start buffer: 50 mM sodium acetate, pH 5.0

Elution buffer: 50 mM sodium acetate, 1 M NaCl, pH 5.0

or

Start buffer: 50 mM MES, pH 6.0

Elution buffer: 50 mM MES, 1 M NaCl, pH 6.0

Note: *Water and chemicals used for buffer preparation should be of high purity. Filter buffers through a 0.22 µm or a 0.45 µm filter before use.*

Prepare the sample

Step	Action
1	Adjust the sample to the composition of the start buffer, using one of these methods: • Dilute the sample with start buffer. • Exchange buffer using a HiPrep 26/10 Desalting, HiTrap™ Desalting or PD-10 Desalting column.
2	Filter the sample through a 0.45 µm filter or centrifuge at 10 000 × g for 10 min immediately before loading it to the column. This prevents clogging and increases the life time of the column when loading large sample volumes.

Recommended flow rates

The table below outlines recommended flow rates for the different media types under different conditions. For viscous buffers and samples the flow rate must be optimized. Starting with a low flow rate is recommended.

Table 5. Recommended flow rates for HiPrep IEX columns.

Media type	First time use or after long time storage in 20% EtOH	Experimental condition	Cleaning-in-place (CIP)
High performance	0.8 ml/min	3 ml/min	3 ml/min
Fast flow	2.0 ml/min	5 ml/min	5 ml/min

Purification

Collect fractions throughout the separation.

Flow rate: See Table 5.

Column tubing: Choose the optimal tubing kit for the column and the application you intend to run. (i.d.: 0.25, 0.50 or 0.75 [mm]). A tubing with wider inner diameter gives broader peaks whereas a tubing with a smaller inner diameter gives higher back pressure.

Step	Action
1	Remove the stoppers and connect the column to the system. Avoid introducing air into the column. ***Note:*** *To prevent leakage, ensure that the connectors are tight. Use fingertight 1/16" connector (28-4010-81).*
2	Wash with 1 column volume (CV) distilled water. This step removes the ethanol and avoids the precipitation of buffer salts upon exposure to ethanol. The step can be omitted if precipitation is not likely to be a problem.
3	Equilibrate the column with at least 5 CV start buffer or until the UV baseline, eluent pH and conductivity are stable.
4	Adjust the sample to the chosen starting pH and conductivity and load on the column.
5	Wash with 5 to 10 CV start buffer or until the UV trace of the effluent returns to near baseline.
6	Elute, either by linear gradient elution or a step elution, see below. If required, the collected eluted fractions can be buffer exchanged or desalted. • *Linear gradient elution* Elute with 0% to 100% elution buffer (up to 1 M NaCl) in 10 to 20 CV. • *Step elution* Elute with 5 CV elution buffer including NaCl at chosen concentration. Repeat at higher NaCl concentrations until the target protein has been eluted.

Step	Action
7	Wash with 5 CV of 1 M NaCl (100% elution buffer) to elute any remaining ionically bound material.
8	If required, perform a CIP to clean the column.
9	Re-equilibrate with 5 to 10 CV start buffer or until the UV baseline, eluent pH, and conductivity reach the required values.

To save time, higher flow rates during regeneration and re-equilibration steps can be used.

Note: *Do not exceed the maximum recommended flow and back pressure for the column.*

4 Cleaning-in-place

Regular cleaning

Wash the column with 40 ml of 2 M NaCl at room temperature after each run to elute material still bound to the column. See Table 5 for recommended flow rates.

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

Re-equilibrate the column with at least 100 ml start buffer at room temperature, until the UV baseline and pH/conductivity values are stable.

Rigorous cleaning

Reverse the flow direction and run the following sequence of solutions at room temperature:

- 80 ml of a 2 M NaCl solution (removes ionically bound proteins from the column) followed by 50 ml distilled water.
- 80 ml of a 1 M NaOH solution (removes precipitated proteins, hydrophobically bound proteins, and lipoproteins from the column) followed by 80 ml distilled water.
- 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.

After cleaning, equilibrate the column with approximately 100 ml start buffer at room temperature before use.

5 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 below. 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 2, 3 and 4) may damage the column.*

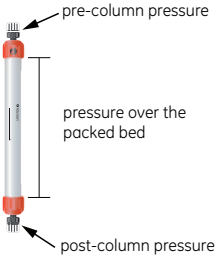

Fig 2. Pre-column and post-column measurements.

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:

Step	Action
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.

Step	Action
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.*

6 Storage

If the column is to be stored for more than two days after use, clean the column according to the procedure described in section *Cleaning-in-Place (CIP).* Then equilibrate as follows:

HiPrep CM FF 16/10, HiPrep DEAE FF 16/10, HiPrep Q FF 16/16 and HiPrep Q HP 16/10: equilibrate with at least 100 ml of 20% ethanol.

HiPrep SP FF 16/10 and HiPrep SP HP 16/10: equilibrate with at least 100 ml of 0.2 M sodium acetate in 20% ethanol.

Store at 4°C to 30°C. Do not freeze.

Make sure that the column is tightly sealed to avoid drying out.

7 Troubleshooting

Problem	Possible cause/*corrective action*
High back pressure during the run	The column is clogged. *Reverse the flow direction and try to pump 100 ml elution buffer through the column. Return to normal flow direction and run 100 ml buffer through the column at low flow rate. If back pressure is not decreased, reverse the flow direction again and follow the rigorous cleaning protocol in Section Cleaning-in-place (CIP).* High viscosity of solutions. *Use lower flow rate.*
Loss of resolution and/or decreased sample recovery	Insufficient elution and CIP. *Follow the rigorous cleaning protocol in Section Cleaning-in-place (CIP). Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.*
Unstable pressure curve	Air in the column. *Reverse the flow direction and pump 100 ml of well de-gassed start buffer through the column at room temperature.*

Note: *See Table 5 for recommended flow rates.*

8 Ordering information

Product	Quantity	Code No.
HiPrep CM FF 16/10	1 × 20 ml	28-9365-42
HiPrep DEAE FF 16/10	1 × 20 ml	28-9365-41
HiPrep Q FF 16/10	1 × 20 ml	28-9365-43
HiPrep SP FF 16/10	1 × 20 ml	28-9365-44
HiPrep Q HP 16/10	1 × 20 ml	29-0181-82
HiPrep SP HP 16/10	1 × 20 ml	29-0181-83

Related products	Quantity	Code No
HiTrap IEX Selection Kit, 7 different IEX media	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 SP HP	5 × 1 ml	17-1151-01
	5 × 5 ml	17-1152-01
HiTrap Q HP	5 × 1 ml	17-1153-01
	5 × 5 ml	17-1154-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5078-01
	4 × 53 ml	17-5087-02

Accessories	Quantity	Code No
HiTrap/HiPrep, 1/16" male connector for ÄKTA *(For connection of columns with 1/16" fittings to ÄKTA)*	8	28-4010-81

Related literature	Code No.
Ion Exchange Chromatography and Chromatofocusing Handbook, Principles and Methods	11-0004-21
Ion Exchange Columns and Media, Selection Guide	18-1127-31
Prepacked chromatography columns for ÄKTA systems, Selection Guide	28-9317-78

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