cytiva

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

Prepacked columns

Instructions for Use

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 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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Read these instructions carefully before using the products.

Intended use

The products 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 products in a safe way, refer to the Safety Data Sheets.

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 below.

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]	0.5 MPa (5 bar, 72.5 psi)

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

Properties of IEX chromatography resins

Q Sepharose Fast Flow, SP Sepharose Fast Flow, DEAE Sepharose Fast Flow, and CM Sepharose Fast Flow are based on 6% cross-linked agarose with a particle size of ~ 90 µm. The resins have good flow properties and high loading capacities.

Q Sepharose High Performance, and SP Sepharose High Performance are based on rigid cross-linked, agarose with a particle size of ~ 34 µm. The smaller particle 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 resins	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 resins are listed in *Table 2, on page 5*, *Table 3, on page 6* and *Table 4, on page 7*

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

	Q Sepharose FF	SP Sepharose FF
Matrix	Cross-linked 6% agarose, spherical	
Particle size d_{50v} [1]	~90 µm	~90 µm
Ion exchange type	Strong anion	Strong cation
Charged group	$-N^+(CH_3)_3$	$-CH_2CH_2CH_2SO_3^-$
Ionic capacity	0.18 to 0.25 mmol Cl^-/mL resin	0.18 to 0.25 mmol H^+/mL resin
Dynamic binding capacity [2]	~42 mg BSA/mL resin	~70 mg Ribonuclease A/mL resin
Recommended operating flow rate (HiPrep) [3]	150 cm/h (5 mL/min)	150 cm/h (5 mL/min)
Maximum operating flow rate (HiPrep)	300 cm/h (10 mL/min)	300 cm/h (10 mL/min)
pH stability, operational [4]	2 to 12	4 to 13
pH stability, CIP [5]	2 to 14	3 to 14
pH ligand fully charged	Entire operational pH range	Entire operational pH range
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M NaOH[6], 8 M urea, 6 M guanidine hydrochloride, 70% ethanol	
Avoid	Oxidizing agents, and anionic detergents/ buffers	Oxidizing agents, and cationic detergents/ buffers
Storage	20% ethanol, 4°C to 30°C	0.2 M sodium acetate in 20% ethanol, 4°C to 30°C

1 Median 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 (DEAE) and 300 cm/h (Q).SP Sepharose FF: 0.1 M sodium acetate, pH 5.0 at 75 cm/h.

3 At room temperature using buffers with the same viscosity as water.

4 pH range where resin can be operated without significant change in function.

5 pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

6 1.0 M NaOH should only be used for cleaning purposes.

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

	DEAE Sepharose FF	CM Sepharose FF
Matrix	Cross-linked 6% agarose, spherical	
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 resin	0.09 to 0.13 mmol H^+/mL resin
Dynamic binding capacity [2]	~110 mg HSA/mL resin	~50 mg Ribonuclease A/mL resin
Recommended operating flow rate (HiPrep) [3]	150 cm/h (5 mL/min)	150 cm/h (5 mL/min)
Maximum operating flow rate (HiPrep)	300 cm/h (10 mL/min)	300 cm/h (10 mL/min)
pH stability, operational [4]	2 to 12	4 to 13
pH range, CIP [5]	2 to 14	2 to 14
pH ligand fully charged	Below 9[6]	Above 6[6]
Chemical stability	Stable to commonly used aqueous buffers, 1 M NaOH[7], 8 M urea, 6 M guanidine hydrochloride, 70% ethanol	
Avoid	Oxidizing agents, and anionic detergents/ buffers	Oxidizing agents, and cationic detergents/ buffers
Storage	20% ethanol, 4°C to 30°C	20% ethanol, 4°C to 30°C

1 Median 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 (DEAE) and 300 cm/h (Q). SP Sepharose FF: 0.1 M sodium acetate, pH 5.0 at 75 cm/h.

3 At room temperature using buffers with the same viscosity as water.

4 pH range where resin can be operated without significant change in function.

5 pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

6 pH range where ligand is fully charged; although the ligand is fully charged throughout the range stated, only use the resin within the stated stability ranges.

7 1.0 M NaOH should only be used for cleaning purposes.

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

	Q Sepharose HP	SP Sepharose HP
Matrix	Cross-linked agarose, spherical	
Particle size d_{50v} [1]	~ 34 µm	~ 34 µm
Ion exchange type	Strong anion	Strong cation
Charged group	$-N^+(CH_3)_3$	$-CH_2CH_2CH_2SO_3^-$
Ionic capacity	0.14 to 0.20 mmol Cl^-/mL resin	0.15 to 0.20 mmol H^+/mL resin
Dynamic binding capacity [2]	~ 70 mg BSA/mL resin	~ 55 mg Ribonuclease A/mL resin
Recommended operating flow rate (HiPrep) [3]	90 cm/h (3 mL/min)	90 cm/h (3 mL/min)
Maximum operating flow rate (HiPrep)	150 cm/h (5 mL/min)	150 cm/h (5 mL/min)
pH stability, operational [4]	2 to 12	4 to 13
pH range, CIP [5]	2 to 14	3 to 14
pH ligand fully charged	Entire operational pH range	Entire operational pH range
Chemical stability	Stable to commonly used aqueous buffers, 1 M NaOH[6], 8 M urea, 6 M guanidine hydrochloride, 70% ethanol	
Avoid	Oxidizing agents, and anionic detergents/ buffers	Oxidizing agents, and cationic detergents/ buffers
Storage	20% ethanol, 4°C to 30°C	0.2 M sodium acetate in 20% ethanol, 4°C to 30°C

1 Median 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.

3 SP Sepharose HP: 5 mg/mL Ribonuclease in 0.1 M sodium acetate, pH 6.0 at 150 cm/h. At room temperature using buffers with the same viscosity as water.

4 pH range where resin can be operated without significant change in function.

5 pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

6 1.0 M NaOH should only be used for cleaning purposes.

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 must 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 must 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 resins 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 must 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 must 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 must 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 must 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 resin 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.

Resin 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, on page 11*.

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, make sure that the connectors are tight. Use fingertight 1/16" connector (28401081).*
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.

Step	Action
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.
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, on page 11* 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 1.0 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.

CAUTION
Specific regulations may apply when using 70% ethanol or 30% isopropanol since it can require the use of explosion-proof areas and equipment.

5 Adjusting pressure limits

The pressure in chromatography system software is generated by the flow through a column. The pressure affects the packed bed and the column hardware, see the figure below. The pressure is increased during 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 recommended flow rates in Table 2, on page 5, Table 3, on page 6 and Table 4, on page 7) can damage the column.*

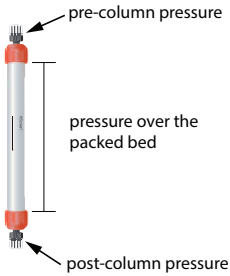

Fig 2. Precolumn and post-column measurements.

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The system will automatically monitor the pressures (precolumn pressure and pressure over the packed bed, Δp). The precolumn pressure limit is the column hardware pressure limit (see *Table 1, on page 3* and *Table 2, on page 5*).

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

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

To obtain the optimal functionality in ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC, and other systems with pressure sensor in the pump, the pressure limit in the software can be adjusted as follows:

Step	Action
1	• Replace the column with a piece of tubing. • Run the pump at the maximum intended flow rate. • Record 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. • Record the pressure as P2.
3	• Calculate the new pressure limit as a sum of P2 and the column hardware pressure limit (see *Table 1, on page 3*). • Replace the pressure limit in the software with the calculated value. *Result:* The actual pressure over the packed bed (Δp) during the run is equal to the actual measured pressure which is the 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 *Chapter 4 Cleaning-in-place, on page 14*. 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.*

Problem	Possible cause/*corrective action*
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 degassed start buffer through the column at room temperature.*

Note: *See Table 5, on page 11 for recommended flow rates.*

8 Ordering information

Product	Quantity	Product code
HiPrep CM FF 16/10	1 × 20 mL	28936542
HiPrep DEAE FF 16/10	1 × 20 mL	28936541
HiPrep Q FF 16/10	1 × 20 mL	28936543
HiPrep SP FF 16/10	1 × 20 mL	28936544
HiPrep Q HP 16/10	1 × 20 mL	29018182
HiPrep SP HP 16/10	1 × 20 mL	29018183

Related products	Quantity	Product code
HiTrap IEX Selection Kit, 7 different IEX resins	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

Related products	Quantity	Product code
HiTrap DEAE FF	5 × 1 mL	17505501
	5 × 5 mL	17515401
HiTrap CM FF	5 × 1 mL	17505601
	5 × 5 mL	17515501
HiTrap SP HP	5 × 1 mL	17115101
	5 × 5 mL	17115201
HiTrap Q HP	5 × 1 mL	17115301
	5 × 5 mL	17115401
HiPrep 26/10 Desalting	1 × 53 mL	17507801
	4 × 53 mL	17508702

Accessories	Quantity	Product code
HiTrap/HiPrep, 1/16" male connector for ÄKTA *(For connection of columns with 1/16" fittings to ÄKTA)*	8	28401081

Related literature	Product code
Ion Exchange Chromatography Handbook, Principles and Methods	11000421
Ion Exchange Columns and Resins, Selection Guide	18112731
Prepacked chromatography columns for ÄKTA systems,Selection Guide	28931778

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