HiPrep SP FF 16/10, HiPrep CM FF 16/10, HiPrep Q FF 16/10, HiPrep DEAE FF 16/10, HiPrep Q XL 16/10, and HiPrep SP XL 16/10

HiPrep™ 16/10 columns for ion exchange (IEX) chromatography are prepacked 20 ml cation and anion exchange columns for preparative separations of proteins and other biomolecules based on charge (Fig 1).

Key features include:

- Simple operation, high capacity, and good flow properties
- Ideal for rapid enrichment during initial capture of proteins from start material
- Excellent for scale-up from HiTrap™ IEX column family
- Reliable and reproducible separations
- Compatible with single pump-based configurations as well as ÄKTA™ design chromatography systems

Fig 1. Prepacked HiPrep 16/10 ion exchange columns allow for the purification of a wide range of biomolecules.

Ion exchange chromatography

IEX chromatography is based on the reversible interactions between charged molecules and immobilized ion exchange groups of opposite charges. The charged molecules bind reversibly to the matrix by displacing counter ions, and elution is promoted by changing the elution conditions to those unfavorable for electrostatic interaction. As biological molecules have various degrees of interaction to the ion exchanger (due to differences in charge, charge densities, and charge distribution), separation is achieved by decreasing the affinity of the biomolecule for the charged IEX groups. This is usually done by continuously or stepwise increasing the ionic strength of the elution buffer.

Sepharose™ Fast Flow ion exchangers include media that are called weak (DEAE and CM) or strong (Q and SP). The binding capacity of weak ion exchangers varies with pH considerably more than that of strong ion exchangers, which might affect selectivity. In contrast, the ligands of strong ion exchangers remain charged and consistently maintain high capacity over broad working pH ranges. Sepharose XL media includes strong anion (Q) and cation (SP) ion exchange ligands.

Chromatography media characteristics

HiPrep 16/10 IEX columns are prepacked with four different Sepharose Fast Flow ion exchange media: SP Sepharose Fast Flow, CM Sepharose Fast Flow, Q Sepharose Fast Flow, and DEAE Sepharose Fast Flow. HiPrep 16/10 columns are also available prepacked with two different Sepharose XL media. SP Sepharose XL and Q Sepharose XL are based on a robust, 6% highly cross-linked beaded agarose matrix with good flow properties and high loading capacity.

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

Experimental conditions are not generally transferable from Fast Flow media to XL media. Re-optimization of pH and other conditions may be necessary to achieve optimal results.

Sepharose Fast Flow and Sepharose XL ion exchangers are a preferred choice for separation early in purification schemes. Full technical and regulatory support for production-scale applications is available. The main characteristics of HiPrep 16/10 ion exchangers are shown in Table 1.

Table 1. Characteristics of HiPrep 16/10 ion exchangers

Cation exchangers

	HiPrep SP FF 16/10	HiPrep CM FF 16/10	HiPrep SP XL 16/10
Matrix	6% highly cross-linked agarose	6% highly cross-linked agarose	Dextran attached to 6% highly cross-linked, spherical agarose
Average particle size	90 µm	90 µm	90 µm
Type of medium	Strong cation	Weak cation	Strong cation
Charged group	$-CH_2-CH_2-CH_2SO_3^-$	$-O-CH_2COO^-$	$-SO_3^-$
Total ionic capacity	0.18–0.25 mmol H^+/ml medium	0.09–0.13 mmol H^+/ml medium	0.18–0.25 mmol H^+/ml medium
Dynamic binding capacity[1]	70 mg ribonuclease A/ml medium	50 mg ribonuclease A/ml medium	> 160 mg lysozyme/ml medium
pH stability			
Short term[2]	3–14	2–14	4–13
Long term[3]	4–13	4–13	3–14
Storage	4°C to 30°C in 20% ethanol, 0.2 M sodium acetate	4°C to 30°C in 20% ethanol	4°C to 30°C in 20 % ethanol, 0.2 M sodium acetate
Chemical stability	All commonly used buffers, 1 M NaOH, 8 M urea, 6 M guanidine hydrochloride, and 70% ethanol		All commonly used buffers, non-ionic detergents, 1 M NaOH, 6 M guanidine hydrochloride
Avoid	Oxidizing agents, cationic detergents, and buffers		

Anion exchangers

	HiPrep Q FF 16/10	HiPrep DEAE FF 16/10	HiPrep Q XL 16/10
Matrix	6% highly cross-linked agarose	6% highly cross-linked agarose	Dextran attached to 6% highly cross-linked, spherical agarose
Average particle size	90 µm	90 µm	90 µm
Type of medium	Strong anion	Weak anion	Strong anion
Charged group	$-N^+(CH_3)_3$	$-N^+(C_2H_5)_2H$	$-N^+(CH_3)_3$
Total ionic capacity	0.18–0.25 mmol Cl^-/ml medium	0.11–0.16 mmol Cl^-/ml medium	0.18–0.26 mmol Cl^-/ml medium
Dynamic binding capacity[1]	120 mg HSA/ml medium	110 mg HSA/ml medium	> 130 mg BSA/ml medium
pH stability			
Short term[2]	1–14	1–14	3–13
Long term[3]	2–12	2–12	2–14
Storage	4°C to 30°C in 20% ethanol	4°C to 30°C in 20% ethanol	4°C to 30°C in 20% ethanol
Chemical stability	All commonly used buffers, 1 M NaOH, 8 M urea, 6 M guanidine hydrochloride, and 70% ethanol		All commonly used buffers, non-ionic detergents, 1 M NaOH, 6 M guanidine hydrochloride
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) or 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. Column: 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).

[2] Refers to the pH interval for regeneration and cleaning.

[3] Refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance.

Column characteristics

HiPrep 16/10 columns are made of polypropylene, which does not interact with biomolecules. HiPrep 16/10 column characteristics are shown in Table 2. Note, HiPrep columns are not designed to be opened or repacked.

Table 2. Characteristics of HiPrep 16/10 column

Column volume	20 ml
Column dimensions	1.6 × 10 cm
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	1.5 bar (0.15 MPa, 22 psi)
Column hardware pressure limit	5 bar (0.5 MPa, 73 psi)

[1] Water at room temperature.

Applications

1. High reproducibility

Figure 2 shows two purifications of GFP-(histidine)$_6$ using HiPrep Q FF 16/10. As shown in the figure, the replicates gave excellent reproducibility. Absorbance at 490 nm is the specific wavelength for the target protein, GFP.

Column: HiPrep Q FF 16/10
Sample: *E. coli* lysate with expressed GFP-(histidine)$_6$
Sample volume: 60 ml
Start buffer: 50 mM Tris, pH 8.0
Elution buffer: 50 mM Tris, 1 M NaCl, pH 8.0
Gradient: 0% to 100% elution buffer in 10 CV
Flow rate: 5 ml/min (150 cm/h)
System: ÄKTAexplorer™ 100

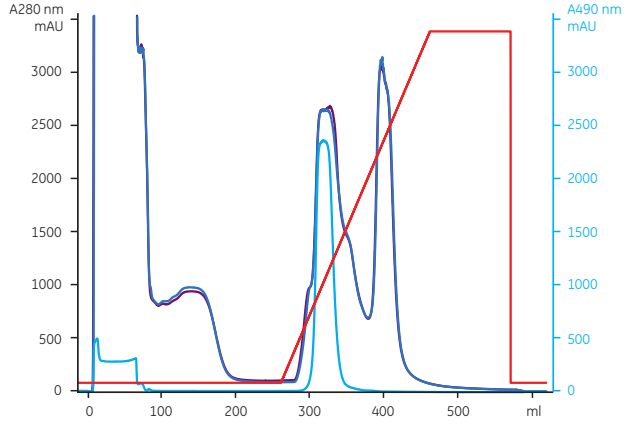

Fig 2. Repetitive purifications of GFP-(histidine)$_6$ using HiPrep Q FF 16/10 show high reproducibility (A280nm = dark blue and purple curves; A490nm = turquoise curve).

2. Scaling up using different prepacked Q Sepharose Fast Flow columns

Ease of scale-up is a key benefit of working with any Sepharose Fast Flow ion exchanger. In Figure 3, a purification is scaled up first five-fold and then twenty-fold on prepacked HiTrap Q FF and HiPrep Q FF 16/10 columns, respectively.

Columns: A) HiTrap Q FF 1 ml, B) HiTrap Q FF 5 ml, C) HiPrep Q FF 16/10 (20 ml)
Sample: 1. Conalbumin, 2 mg/ml
2. α-lactalbumin, 4 mg/ml
3. Soy trypsin inhibitor, 6 mg/ml
Sample volume: 1 CV (column volume) A) 1 ml, B) 5 ml, and C) 20 ml
Start buffer: 50 mM Tris-HCl, pH 7.3
Elution buffer: 50 mM Tris-HCl, 0.5 M NaCl, pH 7.3
Gradient: 0% to 100% elution buffer in 20 CV
A) 20 ml, B) 100 ml, and C) 400 ml
Flow rate: A) 1 ml/min (150 cm/h) and B-C) 5 ml/min (150 cm/h)
Detection: 280 nm
System: ÄKTAexplorer

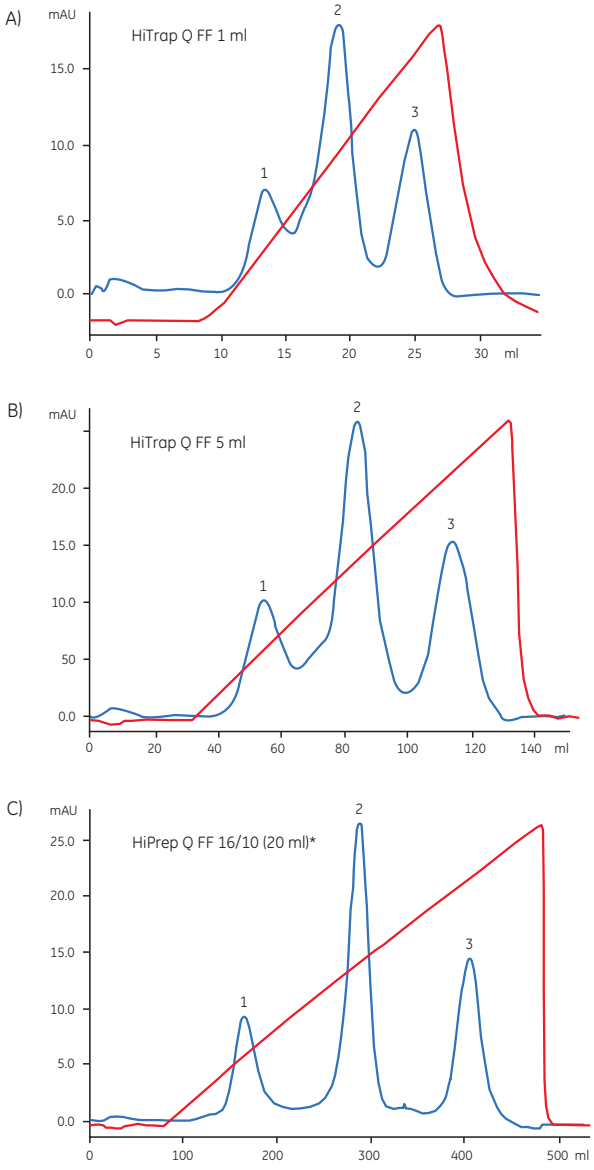

Fig 3. Scale-up from HiTrap columns to HiPrep Q FF 16/10.
A) HiTrap Q FF 1 ml (0.7 × 2.5 cm), B) HiTrap Q FF 5 ml (1.6 × 2.5 cm), and C) HiPrep Q FF 16/10 20 ml (1.6 × 10 cm).

* Note: Data was obtained using first-generation HiPrep 16/10 columns.

3. Effect of pH on the separation of standard proteins on HiPrep CM FF 16/10

Column:	HiPrep CM FF 16/10 (20 ml)*
Sample:	10 mg apotransferrin, ribonuclease A, and cytochrome C in 1 ml
Buffer:	CIEX pH 3–7.5 BufferPrep recipe in ÄKTAexplorer
Gradient:	0% to 50% elution buffer in 300 ml (15 CV), where 50% elution buffer = 0.5 M NaCl
Flow rate:	10 ml/min (300 cm/h)
Detection:	280 nm
System:	ÄKTAexplorer

* Note: Data was obtained using first-generation HiPrep 16/10 columns.

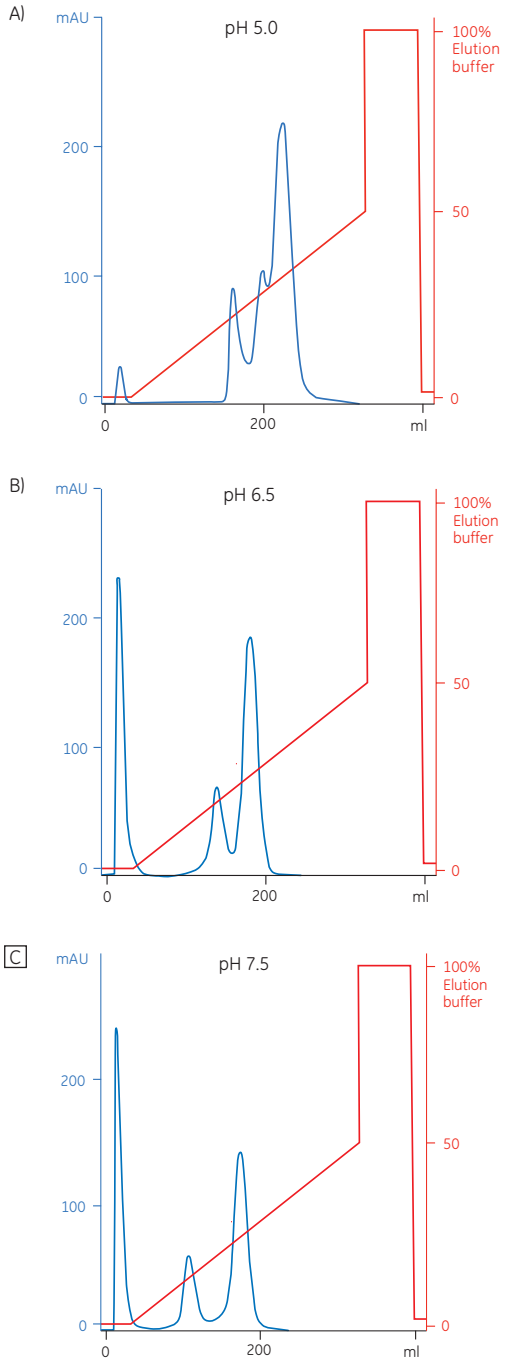

Fig 4. Separations of standard proteins on HiPrep CM FF 16/10 at A) pH 5.0, B) pH 6.5, and C) pH 7.5. In this case, pH 7.5 gave the most distinct separation.

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. Figures 5 and 6 show a selection of standard aqueous buffers, and Table 3 lists the pH ranges of some volatile buffer systems.

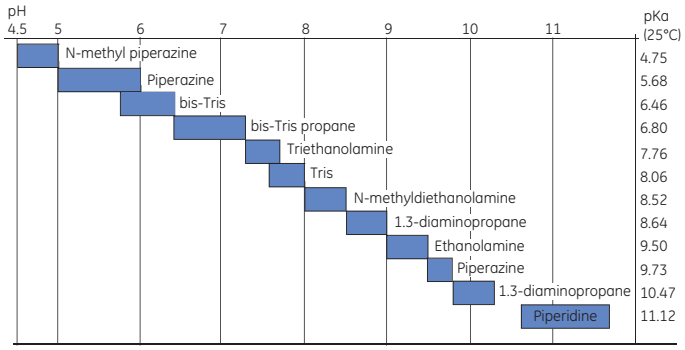

Fig 5. Recommended buffers for anion exchange chromatography.

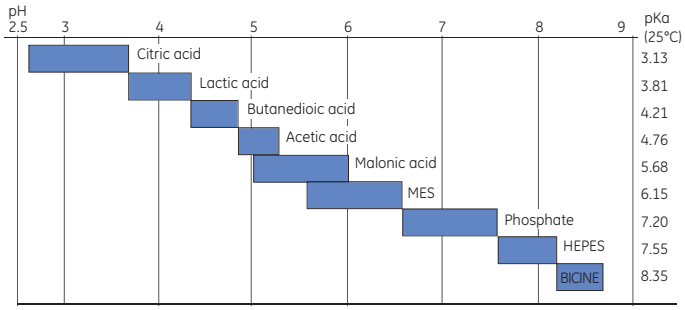

Fig 6. Recommended buffers for cation exchange chromatography.

Table 3. 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/CO_2
8.0–9.5	Ammonium carbonate/ammonia
8.5–10.5	Ethanolamine/HCl

Storage

HiPrep CM FF 16/10, HiPrep Q FF 16/10, HiPrep DEAE FF 16/10, and HiPrep Q XL 16/10 should be stored in 20% ethanol.

HiPrep SP FF 16/10 and HiPrep SP XL 16/10 should be stored in 20% ethanol with 0.2 M sodium acetate.

The recommended storage temperature is 4°C to 30°C.

Ordering information

Products	Quantity	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 DEAE FF 16/10	1 × 20 ml	28-9365-41
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

Related products	Quantity	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
Q Sepharose Fast Flow	25 ml	17-0510-10
	300 ml†	17-1510-01
SP Sepharose Fast Flow	25 ml	17-0729-10
	300 ml†	17-0729-01
DEAE Sepharose Fast Flow	25 ml	17-0709-10
	500 ml†	17-0709-01
CM Sepharose Fast Flow	25 ml	17-0719-10
	500 ml†	17-0719-01
Q Sepharose XL	300 ml	17-5072-01
SP Sepharose XL	300 ml	17-5073-01
Q Sepharose XL virus licensed	25 ml	17-5437-10
	300 ml	17-5437-01
HiTrap Desalting	5 × 5 ml	17-1408-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02

† Process-scale quantities are available. Please contact your local GE Healthcare representative.

Accessories	Quantity	Code No.
HiTrap/HiPrep 1/16" male connector for ÄKTA design	8	28-4010-81
Union M6 female 1/16" male	5	18-3858-01

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

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