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

ION EXCHANGE CHROMATOGRAPHY

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™ chromatography systems

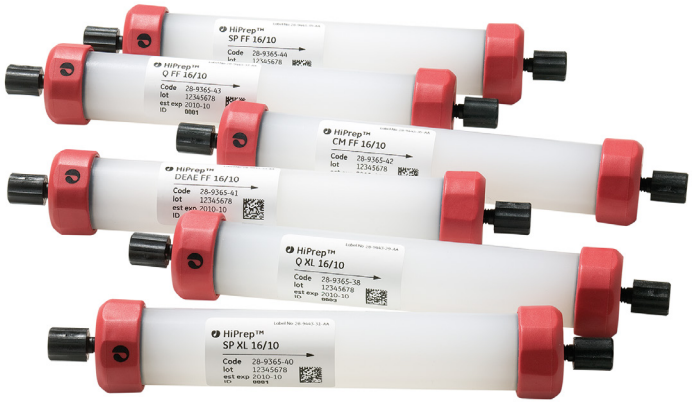

Fig 1. HiPrep ion exchange chromatography columns.

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 resins 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 resins includes strong anion (Q) and cation (SP) ion exchange ligands.

Chromatography resin characteristics

HiPrep 16/10 IEX columns are prepacked with four different Sepharose Fast Flow ion exchange resins: 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 resins. 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 resins to XL resins. Re-optimization of pH and other conditions might 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	Cross-linked agarose, 6%, spherical	Cross-linked agarose, 6%, spherical	Cross-linked agarose, with dextran surface extender, spherical
Particle size, d_{50v} [1]	~ 90 µm	~ 90 µm	~ 90 µm
Type of resin	Strong cation	Weak cation	Strong cation
Charged group	$-CH_2-CH_2-CH_2SO_3^-$	$-O-CH_2COO^-$	$-SO_3^-$
Ionic capacity	0.18 to 0.25 mmol H^+/mL resin	0.09 to 0.13 mmol H^+/mL resin	0.18 to 0.25 mmol H^+/mL resin
Dynamic binding capacity	~ 70 mg ribonuclease A/mL resin[2]	~ 50 mg ribonuclease A/mL resin[2]	≥ 160 mg Lysozyme/mL resin[3]
pH stability, operational[4]	4 to 13	4 to 13	4 to 13
pH stability, CIP[5]	3 to 14	2 to 14	3 to 14
pH ligand fully charged[6]	Entire pH range	Above 6	Entire pH range
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M NaOH[7], 8 M urea, 6 M guanidine hydrochloride, and 70% ethanol		Stable to commonly used aqueous buffers, non-ionic detergents, 1.0 M NaOH[7], 6 M guanidine hydrochloride
Avoid	Oxidizing agents, cationic detergents and buffers		
Storage	20% ethanol in 0.2 M sodium acetate, 4˚C to 30˚C	20% ethanol, 4˚C to 30˚C	20% ethanol in 0.2 M sodium acetate, 4˚C to 30˚C

Anion exchangers

	HiPrep Q FF 16/10	**HiPrep DEAE FF 16/10**	**HiPrep Q XL 16/10**
Matrix	Cross-linked agarose, 6%, spherical	Cross-linked agarose, 6%, spherical	Cross-linked agarose, with dextran surface extender, spherical
Particle size, d_{50v} [1]	~ 90 µm	~ 90 µm	~ 90 µm
Type of resin	Strong anion	Weak anion	Strong anion
Charged group	$-N^+(CH_3)_3$	$-N^+(C_2H_5)_2H$	$-N^+(CH_3)_3$
Ionic capacity	0.18 to 0.24 mmol Cl^-/mL resin	0.11 to 0.16 mmol Cl^-/mL resin	0.18 to 0.26 mmol Cl^-/mL resin
Dynamic binding capacity	~ 42 mg BSA/mL resin[8]	~ 110 mg HSA/mL resin[2]	≥ 160 mg BSA/mL resin[9]
pH stability, operational[4]	2 to 12	2 to 12	2 to 12
pH stability, CIP[5]	2 to 14	2 to 14	2 to 14
pH ligand fully charged[6]	Entire pH range	Below 9	Entire pH range
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M NaOH[7], 8 M urea, 6 M guanidine hydrochloride, and 70% ethanol		Stable to commonly used aqueous buffers, non-ionic detergents, 1.0 M NaOH[7], 6 M guanidine hydrochloride
Avoid	Oxidizing agents, anionic detergents and buffers		
Storage	20% ethanol, 4 to 30˚C	20% ethanol, 4 to 30˚C	20% ethanol, 4 to 30˚C

[1] Median particle size of the cumulative volume distribution.

[2] DEAE 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 (DEAE) or 0.1 M acetate, 2 M NaCl (in the elution buffer), pH 5.0 (SP and CM).

[3] Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 300 cm/h in a PEEK 7.5/100 column at 10 cm bed height (2 min residence time) for Lysozyme in 50 mM Glycine-NaOH, pH 9.

[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 range.

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

[8] Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 300 cm/h in a Tricorn 5/100 at 10 cm bed height (2 min residence time) for BSA in 50mM Tris, pH 8.0.

[9] Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 300 cm/h in a PEEK 7.5/100 column at 10 cm bed height (2 min residence time) for BSA in 50 mM Tris-HCl, pH 7.5.

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 operating flow rate[1]	2–10 mL/min (60–300 cm/h)
Maximum operating 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] At room temperature using water.

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

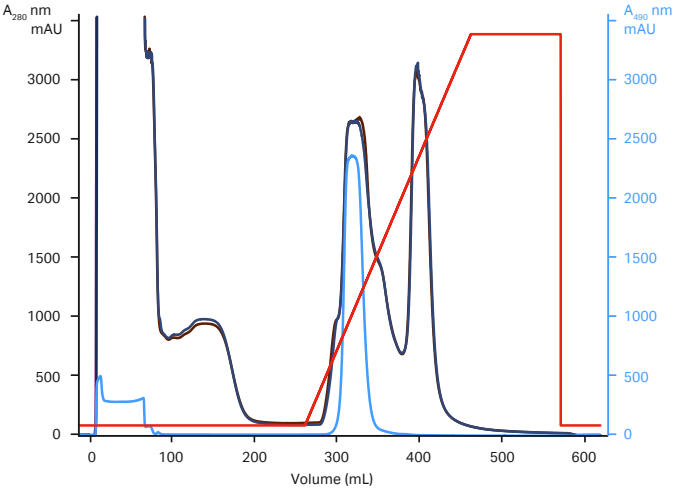

Fig 2. Repetitive purifications of GFP-(histidine)$_6$ using HiPrep Q FF 16/10 show high reproducibility (A_{280}nm = dark blue and purple curves; A_{490}nm = light blue curve).

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

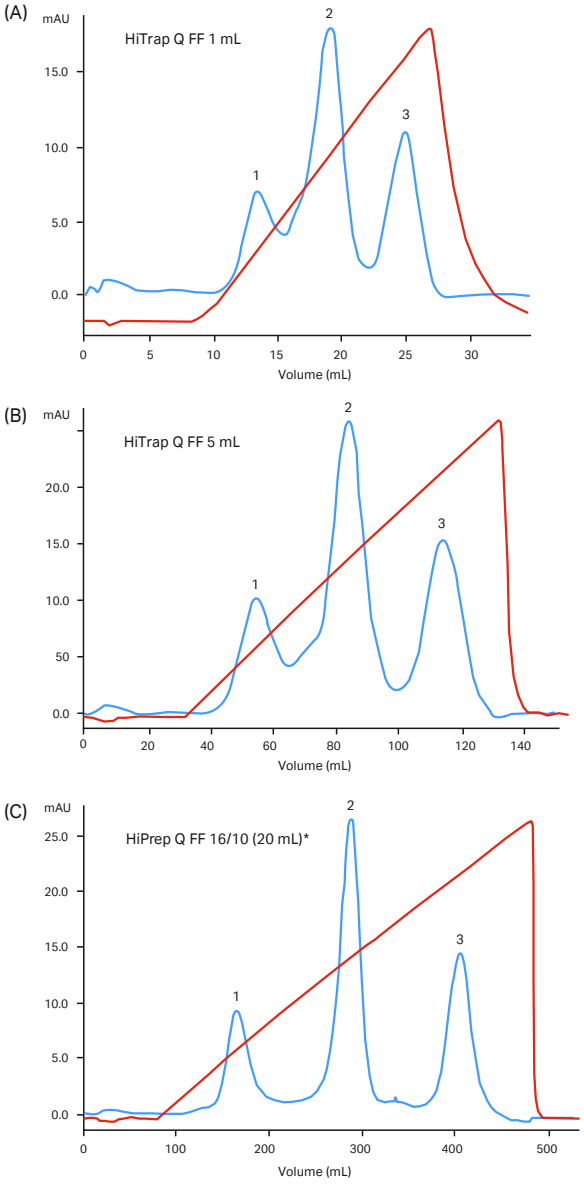

Fig 3. Scale-up from HiTrap columns to HiPrep Q FF 16/10.
(A) HiTrap Q FF 1 mL (0.7 × 2.5 cm),
(B) HiTrap Q FF 5 mL (1.6 × 2.5 cm), and
(C) HiPrep Q FF 16/10 20 mL (1.6 × 10 cm).

* 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.

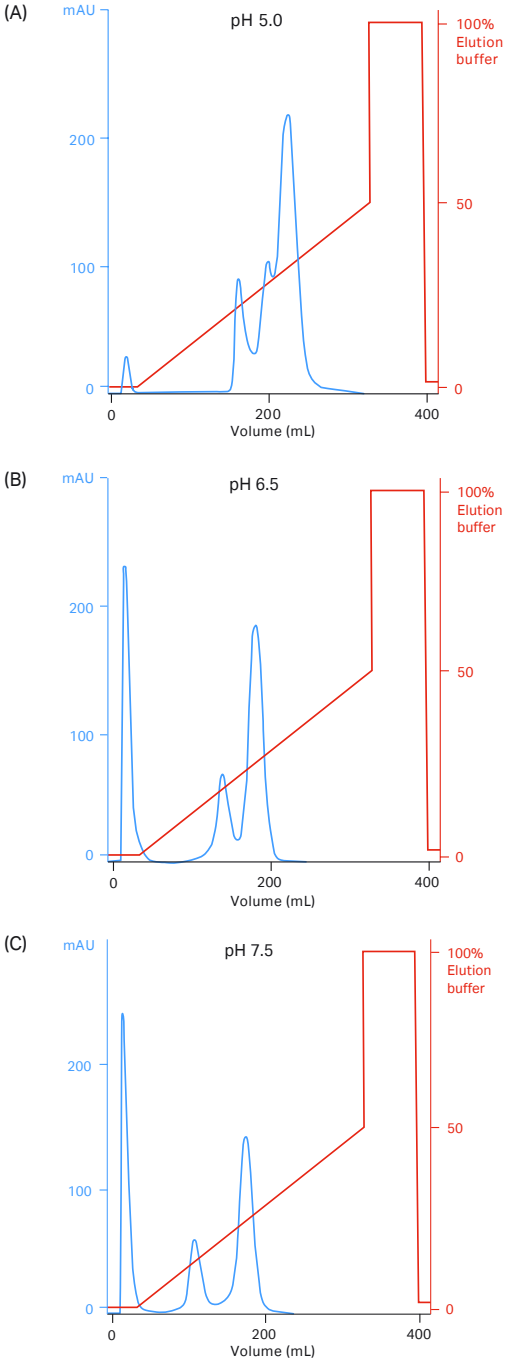

Fig 4. Separations of standard proteins on HiPrep CM FF 16/10 at (A) pH 5.0, (B) pH 6.5, and (C) pH 7.5. In this case, pH 7.5 gave the most distinct separation.

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.

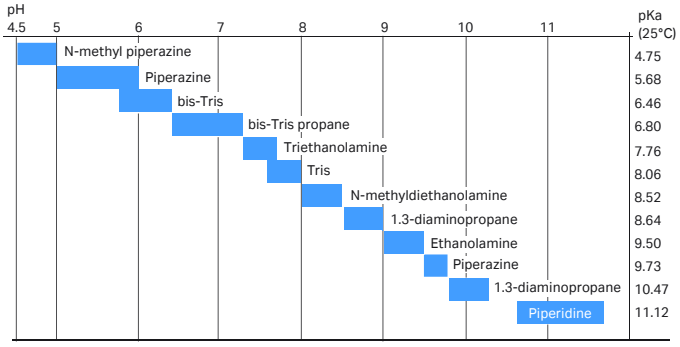

Fig 5. Recommended buffers for anion exchange chromatography.

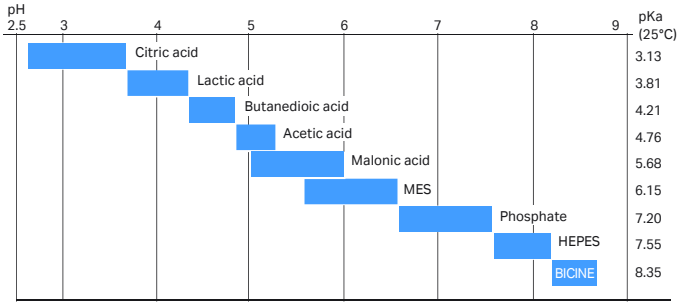

Fig 6. Recommended buffers for cation exchange chromatography.

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	Product code
HiPrep CM FF 16/10	1 × 20 mL	28936542
HiPrep SP FF 16/10	1 × 20 mL	28936544
HiPrep DEAE FF 16/10	1 × 20 mL	28936541
HiPrep Q FF 16/10	1 × 20 mL	28936543
HiPrep Q XL 16/10	1 × 20 mL	28936538
HiPrep SP XL 16/10	1 × 20 mL	28936540

Related products	Quantity	Product code
HiTrap IEX Selection Kit	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
HiTrap DEAE FF	5 × 1 mL	17505501
	5 × 5 mL	17515401
HiTrap CM FF	5 × 1 mL	17505601
	5 × 5 mL	17515501
HiTrap Q XL	5 × 1 mL	17515801
	5 × 5 mL	17515901
HiTrap SP XL	5 × 1 mL	17516001
	5 × 5 mL	17516101
Q Sepharose Fast Flow	25 mL	17051010
	300 mL†	17151001
SP Sepharose Fast Flow	25 mL	17072910
	300 mL†	17072901
DEAE Sepharose Fast Flow	25 mL	17070910
	500 mL†	17070901
CM Sepharose Fast Flow	25 mL	17071910
	500 mL†	17071901
Q Sepharose XL	300 mL	17507201
SP Sepharose XL	300 mL	17507301
Q Sepharose XL virus licensed	25 mL	17543710
	300 mL	17543701
HiTrap Desalting	5 × 5 mL	17140801
HiPrep 26/10 Desalting	1 × 53 mL	17508701
	4 × 53 mL	17508702

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

Accessories	Quantity	Product code
HiTrap/HiPrep 1/16" male connector for ÄKTA systems	8	28401081
Union M6 female 1/16" male 5	5	18385801

Related literature	Product code
Ion exchange chromatography: Principles and methods, Handbook	11000421
Ion exchange columns and media, Selection guide	18112731
Prepacked chromatography columns for ÄKTA systems, Selection guide	28931778

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