Sepharose Fast Flow ion exchange resins and prepacked column formats

ION EXCHANGE CHROMATOGRAPHY

The popularity of Sepharose™ Fast Flow ion exchange (IEX) chromatography resins reflects the important role they play in protein purification today. The reliability and well-documented performance of the resins have made them a common choice for capture and intermediate purification of proteins in both research and industry.

Sepharose Fast Flow ion exchangers offer many practical advantages:

- high binding capacity and good flow properties
- high chemical and physical stabilities
- reliable and reproducible performance
- easy and effective cleaning-in-place (CIP)/sanitization
- various, convenient prepacked column formats
- predictable scale-up

IEX chromatography

IEX is probably the most frequently used and versatile method for fractionating proteins and peptides, even those with small differences in charge. Furthermore, binding and elution conditions are easy to optimize, resulting in fast separations that are reproducible and cost-effective to scale up.

The technique is based on reversible interactions between charged molecules and immobilized ion exchange groups of opposite charge. The charged molecules are allowed to bind to the separation resin at low ionic strength and are eluted with a salt or pH gradient. Continuous gradient elution is most often used when good resolution is needed, while simple stepwise gradient elution is employed for sample preparation, group separation, or concentration.

Fig 1. Sepharose Fast Flow ion exchangers, available in different formats for process development to production scale, are a common choice in preparative protein separations.

Sepharose Fast Flow ion exchangers include resins that are called weak (CM, DEAE, and ANX) or strong (SP and Q). The binding capacity of weak ion exchangers varies considerably more with pH than that of strong ion exchangers, which might affect selectivity. In contrast, the ligands of strong ion exchangers remain charged, with a consistently high capacity maintained over a broad working pH range.

Chromatography resin characteristics

Sepharose Fast Flow ion exchange resins comprise SP Sepharose Fast Flow, CM Sepharose Fast Flow, Q Sepharose Fast Flow, DEAE Sepharose Fast Flow, and ANX Sepharose 4 Fast Flow (high sub).

SP, CM, Q, and DEAE are based on a robust, cross-linked, spherical, 6% agarose matrix with very good flow properties and high loading capacity. Typical flow velocities of 300 to 400 cm/h through a 15 cm high bed at a pressure of 1 bar (14.5 psi, 0.1 MPa), give fast separation cycles, which is especially important early in purifications when rapid enrichment is required. For washing and equilibration, flow velocities can be extended up to 750 cm/h.

ANX Sepharose 4 Fast Flow (high sub) is based on a cross-linked, spherical, 4% agarose matrix, resulting in a resin with higher porosity, which is particularly useful for purifying high molecular weight proteins. Like the other Sepharose Fast Flow ion exchange resins, ANX has high flow rates for processing.

Table 1. Characteristics of Sepharose Fast Flow ion exchangers

Cation exchangers

	SP Sepharose Fast Flow	CM Sepharose Fast Flow
Matrix	Cross-linked agarose, 6%, spherical	Cross-linked agarose, 6%, spherical
Particle size, d_{50v}[1]	~ 90 µm	~ 90 µm
Type of resin	Strong cation	Weak cation
Charged group	$-CH_2-CH_2-CH_2SO_3^-$	$-O-CH_2COO^-$
Ionic capacity	0.18 to 0.25 mmol H^+/mL resin	0.09 to 0.13 mmol H^+/mL resin
Dynamic binding capacity	~ 70 mg ribonuclease A/mL resin[2]	~ 50 mg ribonuclease A/mL resin[2]
Pressure/flow characteristics[3]	400 to 700 cm/h at < 0.1 MPa in a XK 50/30 column with 5 cm diameter and 15 cm bed height (at 25°C using buffers with the same viscosity as water)	300 to 600 cm/h at < 0.1 MPa in a XK 50/30 column with 5 cm diameter and 15 cm bed height (at 25°C using buffers with the same viscosity as water)
pH stability, operational[4]	4 to 13	4 to 13
pH stability, CIP[5]	3 to 14	2 to 14
pH ligand fully charged[6]	Entire pH range	Above 6
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M NaOH[7], 8 M urea, 6 M guanidine hydrochloride, and 70% ethanol	
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

Anion exchangers

	Q Sepharose Fast Flow	DEAE Sepharose Fast Flow	ANX Sepharose 4 Fast Flow (high sub)
Matrix	cross-linked agarose, 6%, spherical	cross-linked agarose, 6%, spherical	cross-linked agarose, 4%, spherical
Particle size, d_{50v}[1]	~ 90 µm	~ 90 µm	~ 90 µm
Type of resin	Strong anion	Weak anion	Weak anion
Charged group	$-N^+(CH_3)_3$	$-N^+(C_2H_5)_2H$[10]	$-N^+(C_2H_5)_2H$[10]
Ionic capacity	0.18 to 0.24 mmol Cl^-/mL resin	0.11 to 0.16 mmol Cl^-/mL resin	0.13 to 0.18 mmol Cl^-/mL resin
Dynamic binding capacity	~ 42 mg BSA/mL resin[8]	~ 110 mg HSA/mL resin[2]	~ 5 mg/thyroglobulin/mL resin[9]
Pressure/flow characteristics[3]	400 to 700 cm/h at < 0.1 MPa in a XK 50/30 column with 5 cm diameter and 15 cm bed height (at 25°C using buffers with the same viscosity as water)	300 to 600 cm/h at < 0.1 MPa in a XK 50/30 column with 5 cm diameter and 15 cm bed height (at 25°C using buffers with the same viscosity as water)	≥ 200 cm/h at 0.1 MPa in a XK50/60 column with 5 cm diameter and 25 cm bed height (at 20°C using buffers with the same viscosity as water)
pH stability, operational[4]	2 to 12	2 to 12	3 to 13
pH stability, CIP[5]	2 to 14	2 to 14	2 to 14
pH ligand fully charged[6]	Entire pH range	Below 9	Below 9
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M NaOH[7], 8 M urea, 6 M guanidine hydrochloride, and 70% ethanol		
Avoid	Oxidizing agents, anionic detergents and buffers		
Storage	20% ethanol, 4˚C to 30˚C	20% ethanol, 4˚C to 30˚C	20% ethanol, 4˚C to 30˚C

[1] Median particle size of the cumulative volume distribution

[2] 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] The pressure/flow characteristics describe the relationship between pressure and flow under the set circumstances. The pressure given shall not be taken as the maximum pressure of the resin.

[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 XK 16/20 column at 13 cm bed height (2.6 min residence time) for thyroglobulin in 0.05 M Tris with 1 M NaCl, pH 7.5

[10] 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.

All resins are chemically robust and withstand rigorous CIP and sanitization procedures (see "Chemical stability"). Their functional ion exchange groups are attached to the matrices via chemically stable ether linkages.

The main resin characteristics are listed in Table 1. Figure 2 shows the pH working ranges of the ion exchangers as titration curves (using sodium hydroxide), that is, the pH ranges over which the functional groups are charged.

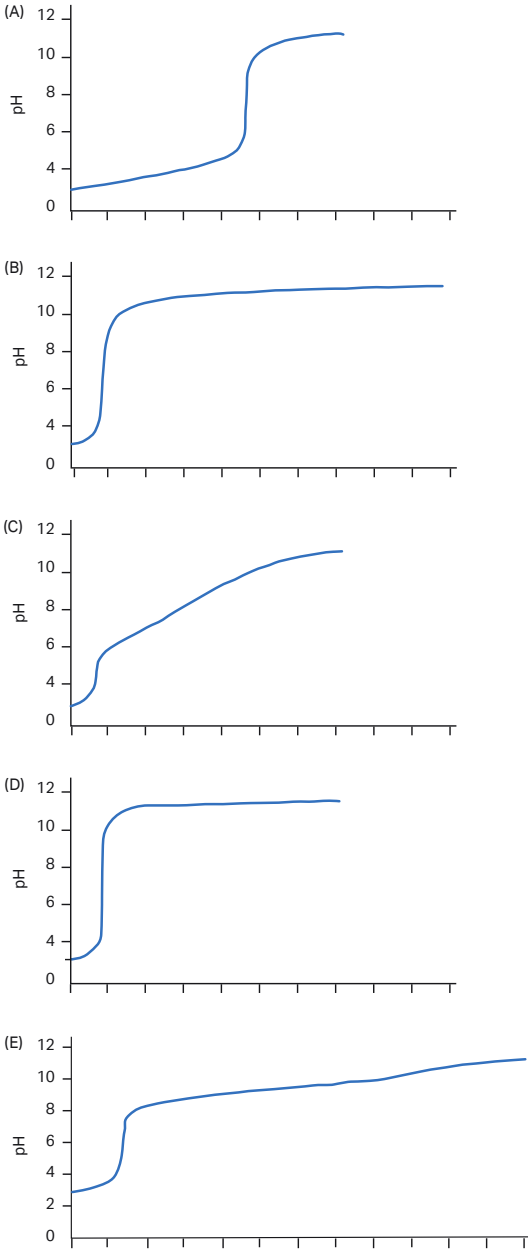


Fig 2. Titration curves showing the pH ranges over which the functional groups are charged. (A) CM Sepharose Fast Flow; (B) SP Sepharose Fast Flow; (C) DEAE Sepharose Fast Flow; (D) Q Sepharose Fast Flow; (E) ANX Sepharose 4 Fast Flow (high sub).

Packing in laboratory columns

Sepharose Fast Flow resins are supplied in laboratory packs for users who prefer the flexibility of packing columns of their choice. Straightforward and well-proven recommendations for packing, operation, and maintenance are included in the instructions supplied with each pack.

Prepacked Sepharose Fast Flow ion exchange columns

By providing extra speed, convenience, and reproducibility, prepacked formats extend the usefulness of Sepharose Fast Flow ion exchangers. In addition, ÄKTA™ chromatography systems include preset method templates for the prepacked columns, which further simplify operation, improve reproducibility, and save time.

The resins are supplied in four types of columns: HiPrep™ 16/10 (20 mL), HiScreen™ (4.7 mL), HiTrap™ (1 mL and 5 mL) and PreDictor™ RoboColumn™ units (200 µL and 600 µL). The five HiTrap 1 mL columns are also included in HiTrap IEX Selection Kit together with SP Sepharose XL and Q Sepharose XL. Q Sepharose Fast Flow and SP Sepharose Fast Flow are also available in the prefilled PreDictor 96-well filter plates for fast and easy process development. These plates are filled with either 6 µL, 20 µL, or 50 µL chromatography resin.

HiPrep 16/10 columns

HiPrep SP FF 16/10, HiPrep CM FF 16/10, HiPrep Q FF 16/10, and HiPrep DEAE FF 16/10 are prepacked, ready-to-use IEX columns for preparative, small-scale purifications. The columns are simple to operate and compatible with single pump configurations as well as ÄKTA systems. Rapid enrichment during the initial capture of proteins from the start material is a suitable application for any of the columns named above.

HiPrep 16/10 columns are made of polypropylene, which is biocompatible with biomolecules. Its bed height of 10 cm gives a volume of 20 mL. See Table 2 for the main characteristics of the HiPrep 16/10 column. Note that HiPrep columns cannot 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 to 10 mL/min (60 to 300 cm/h)
Maximum flow rate[1]	10 mL/min (300 cm/h)
Column hardware pressure limit	0.5 MPa (5 bar, 73 psi)

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

HiScreen columns

HiScreen columns are part of the process development platform available from Cytiva and designed for method optimization and parameter screening in packed bed. HiScreen columns have small bed volumes (4.7 mL), reducing the cost for sample and buffer consumption. The prepacked columns give reproducible results scalable to BioProcess™ columns packed with the same resins using the same flow velocity.

HiScreen columns are made of biocompatible polypropylene that do not interact with biomolecules. The columns can be run with peristaltic pumps or chromatography systems, such as ÄKTA systems. Table 3 lists the characteristics of HiScreen columns.

Note: HiScreen columns cannot be opened or repacked.

Table 3. Characteristics of HiScreen columns

Column volume	4.7 mL
Column dimensions	0.77 × 10 cm
Column hardware pressure limit[1]	0.8 MPa (8 bar, 117 psi)

[1] Note: 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.

HiTrap columns

HiTrap SP FF, HiTrap CM FF, HiTrap Q FF, HiTrap DEAE FF, and HiTrap ANX FF (high sub) are small, affordable, and easy-to-use prepacked 1 mL or 5 mL IEX columns.

The 1 mL column is often used for method screening to quickly establish optimized binding and elution conditions in packed bed. The fast and simple column operation is well-suited for this role, as well as for small-scale purifications. The larger 5 mL column is an excellent choice for group separation and sample concentration, and when the purification method has been established and larger amounts of protein need to be purified. For quick scale-up of purification, two or three HiTrap columns can easily be connected in series. Further scale-up can be conducted on HiPrep 16/10 columns (Fig 3).

HiTrap columns are made of polypropylene, which is biocompatible with biomolecules. Table 4 lists the characteristics of HiTrap columns. Note that HiTrap columns cannot be opened or repacked.

Table 4. Characteristics of HiTrap 1 mL and 5 mL columns

Column volumes	1 mL and 5 mL
Column dimensions	0.7 × 2.5 cm (1 mL) 1.6 × 2.5 cm (5 mL)
Recommended flow rate	1 mL/min (1 mL) 5 mL/min (5 mL)
Maximum flow rate[1]	4 mL/min (1 mL) 20 mL/min (5 mL)
Column hardware pressure limit	0.5 MPa (5 bar, 73 psi)

[1] Room temperature, aqueous buffers

PreDictor 96-well plate and PreDictor RoboColumn

Q Sepharose Fast Flow and SP Sepharose Fast Flow are available in prepacked PreDictor plates in three different volumes (6 µL, 20 µL, and 50 µL) or in PreDictor RoboColumns of two different volumes (200 µL and 600 µL).

PreDictor plates are disposable, 96-well filter plates made of polypropylene and polyethylene. Each well is prefilled with the defined amount of chromatography resin. The plates can be used with centrifugation or vacuum, manually or in automated robotic systems. PreDictor plates support high-throughput process development (HTPD) by allowing parallel screening of chromatographic conditions.

PreDictor RoboColumns are prepacked miniaturized chromatography columns.

PreDictor RoboColumns are a convenient screening format and are also part of Cytiva's tools for HTPD. These miniaturized columns support HTPD using a robotic liquid handling workstation, such as Freedom EVO™ from Tecan, for fully automated and parallel chromatographic separations. Perform HTPD work using PreDictor RoboColumn, alone, or as a complement to PreDictor plates.

Chemical stability

Good chemical stability allows the use of effective CIP schemes that result in high recoveries over many purification cycles. Likewise, it allows regular sanitization to prevent microbial growth and maintain a high level of hygiene. Thus, both CIP and sanitization promote good economy and are therefore key factors to consider when selecting ion exchange resins and prepacked columns for preparative applications.

For CIP, regular washing with 0.5 to 1.0 M sodium hydroxide should be sufficient to remove most contaminating material, although very hydrophobic molecules might bind so tightly that they need to be eluted with strong detergents or organic solvents, such as 70% ethanol or 30% isopropanol.

General CIP and sanitization protocols for SP Sepharose Fast Flow, CM Sepharose Fast Flow, Q Sepharose Fast Flow, DEAE Sepharose Fast Flow, and ANX Sepharose 4 Fast Flow (high sub) are supplied with the resins. Note that specific protocols should be developed according to the nature and condition of the starting material.

Applications

Sepharose Fast Flow ion exchangers have many applications. Their speed, reliability, and documented success make them a good choice for preparative protein separations. Separating protein mixtures early in purification schemes puts the high flow rates and capacities of the columns to very effective use.

The excellent availability of suitable equipment also contributes to effective use of the columns, especially as ÄKTA systems have method templates for HiPrep, HiScreen, and HiTrap columns.

Scaling up five-fold and twenty-fold using different prepacked Q Sepharose Fast Flow columns

As shown in Figure 3, easy scale-up is a key practical benefit of working with any Sepharose Fast Flow ion exchanger. A laboratory protein separation was scaled up first five-fold and then 20-fold on prepacked HiTrap Q FF and HiPrep Q FF 16/10 columns, respectively, with excellent reproducibility.

Sample:	1. Conalbumin, 2 mg/mL 2. α-lactalbumin, 4 mg/mL 3. Soy trypsin inhibitor, 6 mg/mL
Sample volume:	1 column volume (CV) (A) 1 mL; (B) 5 mL; (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; (C) 400 mL
Flow rate:	(A) 1 mL/min (150 cm/h); (B) and (C) 5 mL/min (150 cm/h)

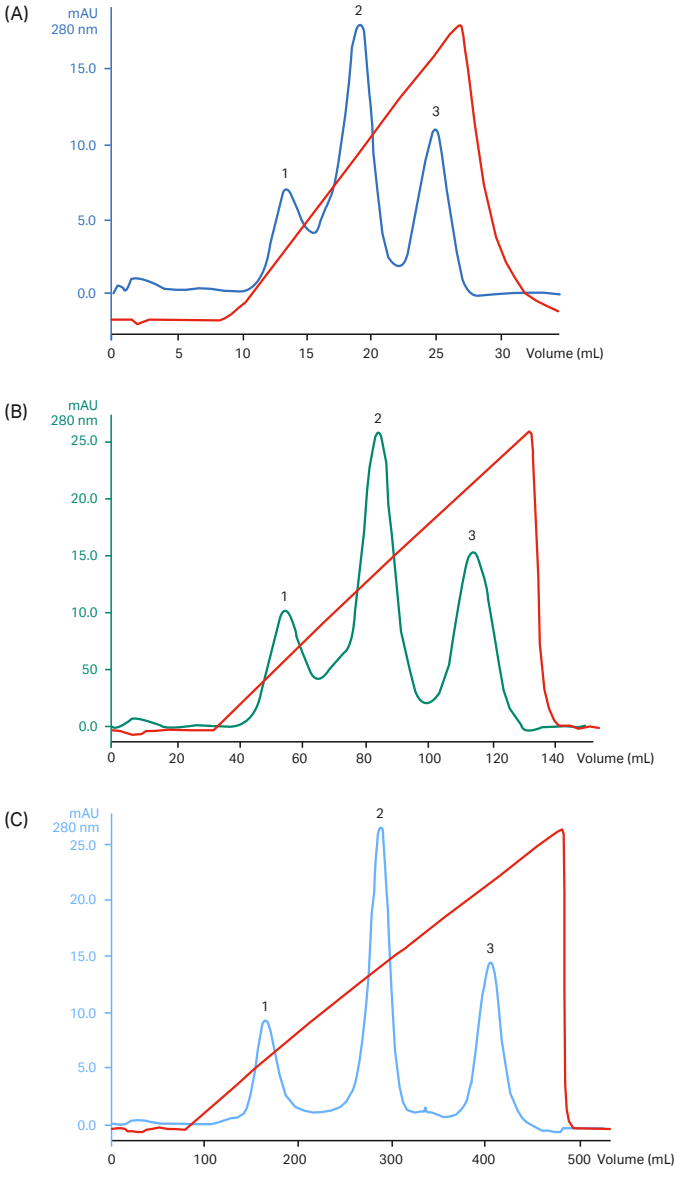
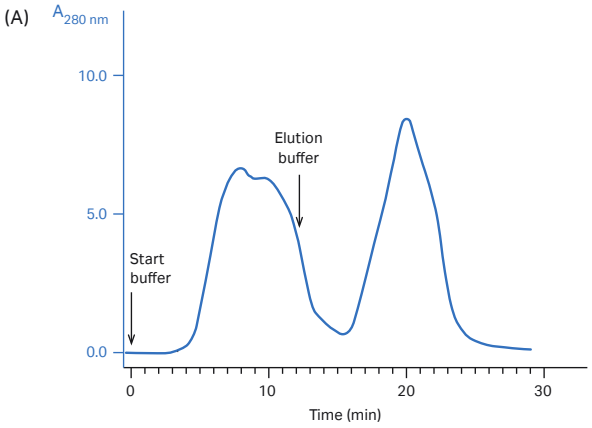


Fig 3. Five-fold and twenty-fold scale-up on prepacked Q Sepharose Fast Flow columns. (A) HiTrap Q FF, 1 mL (0.7 × 2.5 cm); (B) HiTrap Q FF, 5 mL (1.6 × 2.5 cm); (C) HiPrep Q FF 16/10, 20 mL (1.6 × 10 cm).

Separation of bovine plasma components on SP Sepharose Fast Flow at laboratory and industrial scales

Figure 4 shows a protein separation scaled up from laboratory scale (50 mL sample) to industrial production level (6.5 L sample) on SP Sepharose Fast Flow, again with high reproducibility. Analysis of the separated proteins showed no significant differences in the pattern or purity of the individual peaks at either scale. Note that the height equivalent to the theoretical plate (HETP) values are essentially the same for both packed columns, despite their widely differing sizes.

Column:	XK 26/20, 15 cm bed height, 80 mL bed volume
Sample:	Filtered bovine plasma in 0.1 M sodium acetate, pH 5.2
Sample volume:	50 mL sample (25 g/L)
Start buffer:	0.1 M sodium acetate, pH 5.2
Elution buffer:	0.4 M sodium acetate, pH 8.0
Flow rate:	530 mL/h (100 cm/h)



Column:	BPG 300/500, 15 cm bed height, 10.5 L bed volume
Sample:	Filtered bovine plasma in 0.1 M sodium acetate, pH 5.2
Sample volume:	6.5 L sample (25 g/L)
Start buffer:	0.1 M sodium acetate, pH 5.2
Elution buffer:	0.4 M sodium acetate, pH 8.0
Flow rate:	70 L/h (100 cm/h)

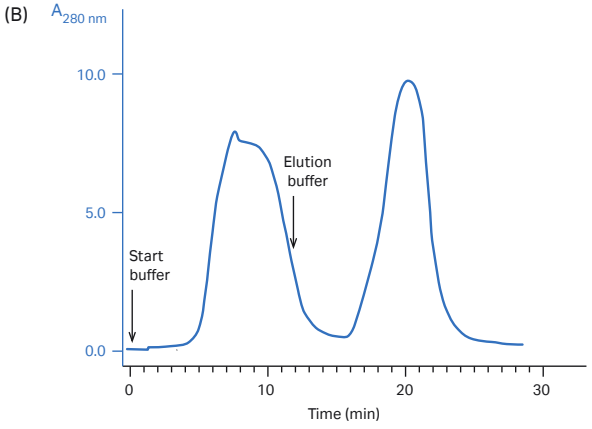


Fig 4. Scale-up from (A) a laboratory column (bed volume 80 mL) packed with SP Sepharose Fast Flow to (B) a column suitable for industrial production (bed volume 10.5 L) with retained packing and separation characteristics.

Effect of pH on the separation of standard proteins on a HiPrep CM FF 16/10 column

In Figure 5, the effect of pH on the separation of standard proteins on a prepacked HiPrep CM FF 16/10 column is shown. As can be seen, pH 7.5 gives the most distinct separation.

Sample: HiPrep CM FF 16/10, 20 mL
Sample: 10 mg apotransferrin, ribonuclease A, and cytochrome C in 1 mL
Buffer: CIEX pH 3 to 7.5 BufferPrep kit
Gradient: 0% to 50% B in 300 mL (15 CV) where 50% B = 0.5 M NaCl
Flow rate: 10 mL/min (300 cm/h)

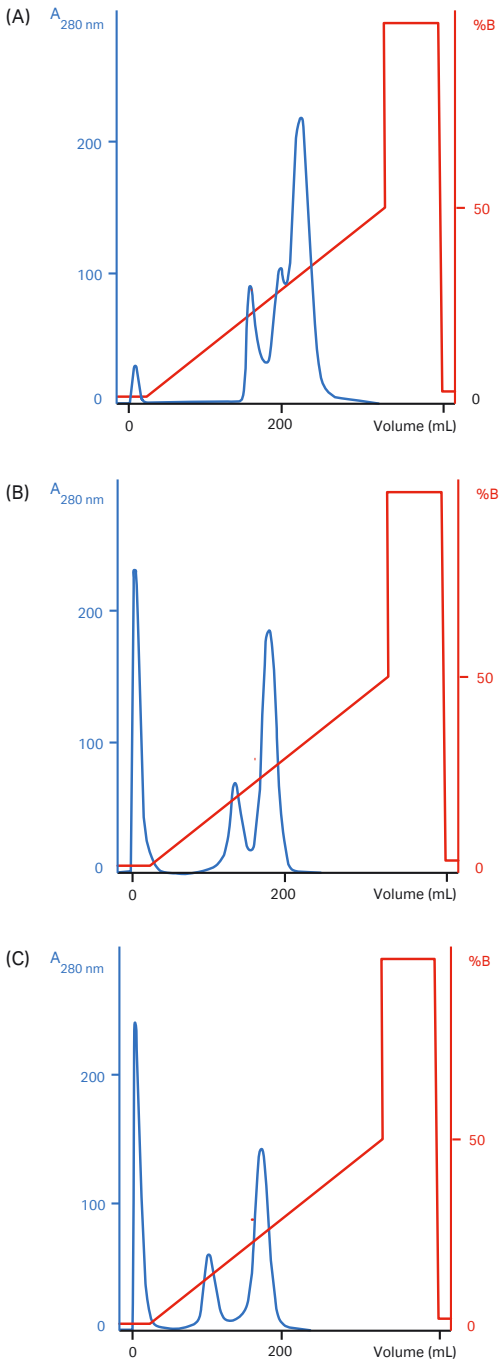


Fig 5. Separations of standard proteins on HiPrep CM FF 16/10 at: (A) pH 5.0; (B) pH 6.5; (C) pH 7.5.

Influence of different anion functional groups on retention times for alkaline phosphatase

Not only the elution pH affects the separation profile. As Figure 6 illustrates, different ligands, in this case anion groups, can give small but significant differences in retention times. After sample application, the columns were washed and eluted with the same linear gradient.

Sample: (A) HiTrap DEAE FF, 1 mL, (B) HiTrap Q FF, 1 mL, and (C) HiTrap ANX FF (high sub), 1 mL
Sample: 2 mL *E. coli* lysate clarified by centrifugation
Sample application: 2 mL
Start buffer: 20 mM Tris-HCl, pH 7.4
Elution buffer: 20 mM Tris-HCl, 0.5 M NaCl, pH 7.4
Equilibration: 20 mL start buffer
Wash: 10 mL start buffer
Elution: 40 mL, linear gradient, 0% to 100% elution buffer
Flow rate: 1 mL/min (150 cm/h)

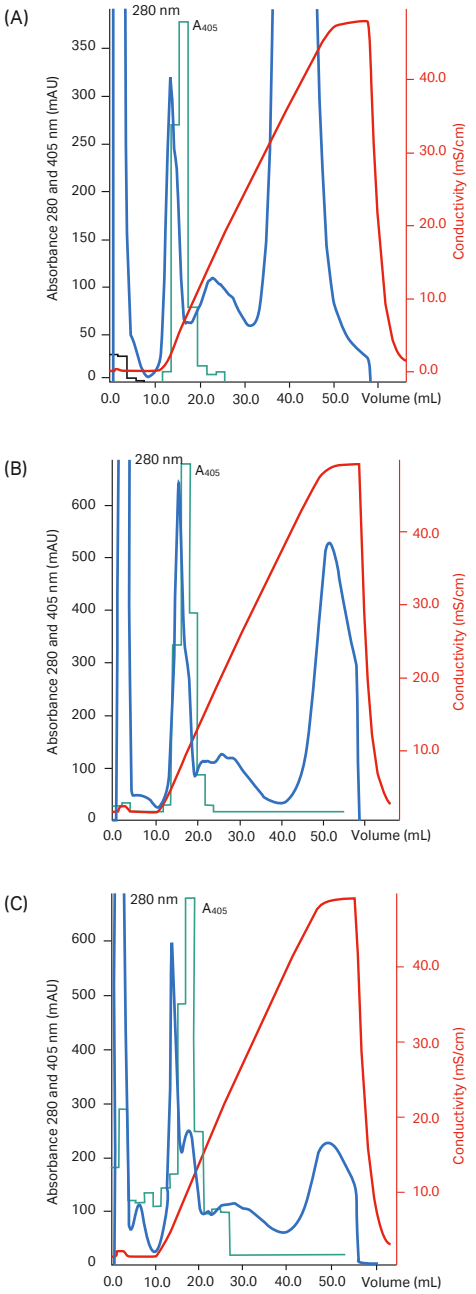


Fig 6. Clarified *E. coli* lysate separated on: (A) HiTrap DEAE FF, 1 mL; (B) HiTrap Q FF, 1 mL; (C) HiTrap ANX FF (high sub), 1 mL. Alkaline phosphatase activity measured at A_{405}.

Process development and scale-up to production

The excellent performance of Sepharose Fast Flow ion exchangers for laboratory-scale, preparative applications naturally lends itself to process development and scale-up. The resins are well supported for this task.

As members of the BioProcess family, all ion exchangers are supported with special services and documentation to facilitate the development, scale-up, and routine operation of production applications. Validated manufacture, secure supply, and regulatory support constitute just part of this package.

Ordering information

Product	Quantity	Product code
Q Sepharose Fast Flow	25 mL	17051010
	300 mL	17051001
	5 L	17051004
	10 L	17051005
	60 L	17051060
SP Sepharose Fast Flow	25 mL	17072910
	300 mL	17072901
	5 L	17072904
	10 L	17072905
	60 L	17072960
DEAE Sepharose Fast Flow	25 mL	17070910
	500 mL	17070901
	10 L	17070905
	60 L	17070960
CM Sepharose Fast Flow	25 mL	17071910
	500 mL	17071901
	10 L	17071905
	60 L	17071960
ANX Sepharose 4 Fast Flow (high sub)	25 mL	17128710
	500 mL	17128701
	5 L	17128704
	10 L	17128705
	60 L	17128760

Prepacked formats	Quantity	Product code
HiPrep DEAE FF 16/10	1 × 20 mL	28936541
HiPrep CM FF 16/10	1 × 20 mL	28936542
HiPrep Q FF 16/10	1 × 20 mL	28936543
HiPrep SP FF 16/10	1 × 20 mL	28936544
HiScreen Q FF	1 × 4.7 mL	28950510
HiScreen SP FF	1 × 4.7 mL	28950513
HiScreen DEAE FF	1 × 4.7 mL	28978245
HiTrap Q FF	5 × 1 mL	17505301
	5 × 5 mL	17515601
HiTrap SP FF	5 × 1 mL	17505401
	5 × 5 mL	17515701

Prepacked formats	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 ANX 4 FF (high sub)	5 × 1 mL	17516201
	5 × 5 mL	17516301
HiTrap IEX Selection Kit	7 × 1 mL	17600233
PreDictor Q Sepharose Fast Flow, 6 µL	4 × 96-well plates	28943269
PreDictor Q Sepharose Fast Flow, 20 µL	4 × 96-well plates	28943270
PreDictor Q Sepharose Fast Flow, 50 µL	4 × 96-well plates	28943271
PreDictor SP Sepharose Fast Flow, 6 µL	4 × 96-well plates	28943272
PreDictor SP Sepharose Fast Flow, 20 µL	4 × 96-well plates	28943273
PreDictor SP Sepharose Fast Flow, 50 µL	4 × 96-well plates	28943274
PreDictor RoboColumn Q Sepharose Fast Flow, 200 µL	1 × 8-row columns	28986086
PreDictor RoboColumn SP Sepharose Fast Flow, 200 µL	1 × 8-row columns	28986087
PreDictor RoboColumn Q Sepharose Fast Flow, 600 µL	1 × 8-row columns	28986180
PreDictor RoboColumn SP Sepharose Fast Flow, 600 µL	1 × 8-row columns	28986181

Accessories	Quantity	Product code
1/16" male/Luer female*	2	18111251
Tubing connector flangeless/ M6 female	2	18100368
Tubing connector flangeless/ M6 male	2	18101798
Union 1/16" female/M6 male	6	18111257
Union M6 female/1/16" male	5	18385801
Union luerlock female/ M6 female	2	18102712
HiTrap/HiPrep, 1/16" male connector for ÄKTA systems	8	28401081
Stop plug female, 1/16"†	5	11000464
Fingertight stop plug, 1/16"‡	5	11000355

* One connector included in each HiTrap package.
† Two, five, or seven stop plugs female included in HiTrap packages depending on products.
‡ One fingertight stop plug is connected to the top of each HiTrap column at delivery.

Related literature	Product code
Handbook: Ion Exchange Chromatography: Principles and Methods	11000421
Selection guide: Ion Exchange Columns and Media	18112731
Selection guide: Prepacked chromatography columns for ÄKTA systems	28931778
Data file: AxiChrom columns	28929041

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