Sepharose™ Fast Flow ion exchange media and prepacked column formats

The popularity of Sepharose Fast Flow ion exchange (IEX) chromatography media (resins) reflects the important role they play in protein purification today. The reliability and well-documented performance of the media, 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 medium 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 media 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 media characteristics

Sepharose Fast Flow ion exchange media 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, 6% highly cross-linked, beaded agarose matrix with very good flow properties and high loading capacity. Typical linear flow velocities of 300 to 400 cm/h through a 15 cm high bed at a pressure of 1 bar, 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 4% highly cross-linked agarose matrix, resulting in a medium with higher porosity, which is particularly useful for purifying high molecular weight proteins. Like the other Sepharose Fast Flow ion exchange media, 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	6% highly cross-linked agarose	6% highly cross-linked agarose
Average particle size	90 µm	90 µm
Type of medium	Strong cation	Weak cation
Charged group	$-CH_2-CH_2-CH_2SO_3^-$	$-O-CH_2COO^-$
Total ionic capacity	0.18 to 0.25 mmol H^+/mL medium	0.09 to 0.13 mmol H^+/mL medium
Dynamic binding capacity[1]	70 mg ribonuclease A/mL medium	50 mg ribonuclease A/mL medium
Flow velocity[2]	400 to 700 cm/h	300 to 600 cm/h
pH stability		
CIP[3]	3 to 14	2 to 14
Working[4]	4 to 13	4 to 13
Storage temperature	4°C to 30°C	4°C to 30°C
Storage buffer	20% ethanol, 0.2 M sodium acetate	20% ethanol
Chemical stability	All commonly used buffers, 1 M NaOH, 8 M urea, 6 M guanidine hydrochloride, and 70% ethanol	
Avoid	Oxidizing agents, cationic detergents, and buffers	

Anion exchangers

	Q Sepharose Fast Flow	DEAE Sepharose Fast Flow	ANX Sepharose 4 Fast Flow (high sub)
Matrix	6% highly cross-linked agarose	6% highly cross-linked agarose	4% highly cross-linked agarose
Average particle size	90 µm	90 µm	90 µm
Type of medium	Strong anion	Weak anion	Weak anion
Charged group	$-N^+(CH_3)_3$	$-N^+(C_2H_5)_2H$*	$-N^+(C_2H_5)_2H$*
Total ionic capacity	0.18 to 0.25 mmol Cl^-/mL medium	0.11 to 0.16 mmol Cl^-/mL medium	0.13 to 0.18 mmol Cl^-/mL medium
Dynamic binding capacity[1]	120 mg HSA/mL medium	110 mg HSA/mL medium	5 mg thyroglobulin/mL medium
Flow velocity[2]	400 to 700 cm/h	300 to 600 cm/h	min 200 cm/h
pH stability			
CIP[3]	1 to 14	1 to 14	2 to 14
Working[4]	2 to 12	2 to 12	3 to 13
Storage temperature	4°C to 30°C	4°C to 30°C	4°C to 30°C
Storage buffer	20% ethanol	20% ethanol	20% ethanol
Chemical stability	All commonly used buffers, 1 M NaOH, 8 M urea, 6 M guanidine hydrochloride, and 70% ethanol		
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).
ANX Sepharose 4 Fast Flow (high sub): Sample was applied at 300 cm/h until 10% breakthrough. Column: 1.6 × 13 cm. Buffer: 0.05 M Tris, 1 M NaCl (in the elution buffer), pH 7.5.

[2] 1 bar (100 kPa), XK 50/30 column, bed height 15 cm (SP, CM, Q, and DEAE) or 1 bar (100 kPa), XK 50/60 column, bed height 25 cm (ANX).

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

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

* 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 attached to the medium. DEAE Sepharose Fast Flow has a diethylaminoethyl-group bound to the agarose while ANX Sepharose 4 Fast Flow has a diethylaminopropyl-group attached.

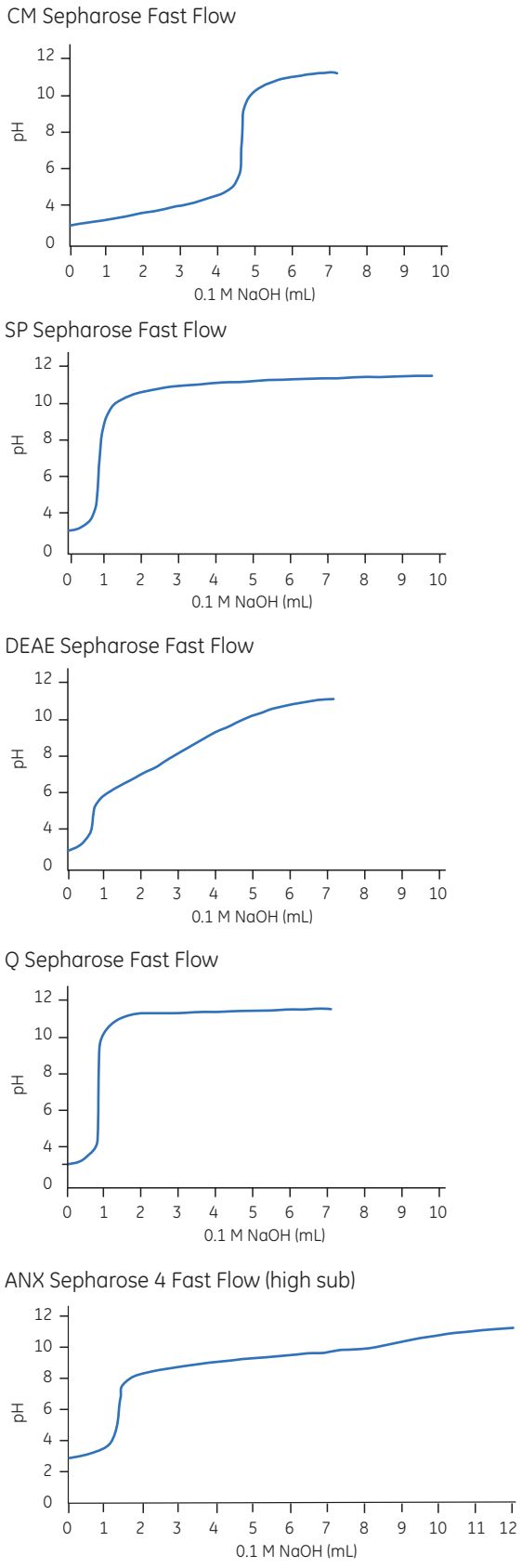


Fig 2. Titration curves for CM, SP, DEAE, Q Sepharose Fast Flow, and ANX Sepharose 4 Fast Flow (high sub) showing the pH ranges over which the functional groups are charged.

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

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

Packing in laboratory columns

Sepharose Fast Flow media 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 simplifies operation, improves reproducibility, and saves time.

The media 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 a filled with either 6 µL, 20 µL, or 50 µL chromatography media.

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)
Maximum pressure over the packed bed during operation	1.5 bar (22 psi, 0.15 MPa)
Column hardware pressure limit	5 bar (73 psi, 0.5 MPa)

[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 GE Healthcare 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 media using the same linear flow velocity.

HiScreen columns are made of biocompatible polypropylene that does not interact with biomolecules. They 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*	8 bar (0.8 MPa, 117 psi)

* Note: 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.

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 to this role, as well as to small-scale purifications. The larger 5 mL column is an excellent choice for group separations 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, see Figure 6.

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)
Maximum back-pressure	3 bar (43 psi, 0.3 MPa)

[1] Room temperature, aqueous buffers.

PreDictor 96-well plate and PreDictor RoboColumn

Q sepharsoe Fast Flow and SP Sepharose Fast Flow are available in prepacked PreDictor plates in three different volumes, 6 µL, 20 µL, and 50 µL as well as in PreDictor RoboColumn of two different volumns, 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 medium. The plates can be used with centrifugation or vacuum, manually or in automated robotic systems. PreDictor plates support HTPD by allowing parallel screening of chromatographic conditions.

PreDictor RoboColumns are prepacked miniaturized chromatography columns.

PreDictor RoboColumn is a convenient screening format and are also part of GE Healthcare's tools for high throughput process development (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 media 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 organic solvents, like 70% ethanol or 30% isopropanol, or with strong detergents.

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 media. 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 in general. Separating protein mixtures early in purification schemes puts the high flow rates and capacities of the columns to very effective use.

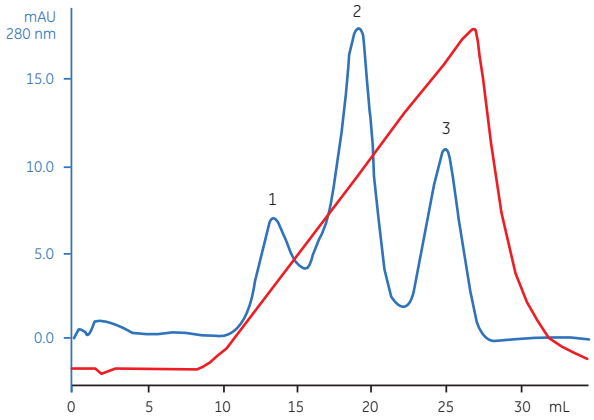
The excellent availability of suitable equipment also contributes to effective use of the columns, especially as ÄKTA systems have method templates for both HiPrep, HiScreen, and HiTrap columns.

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

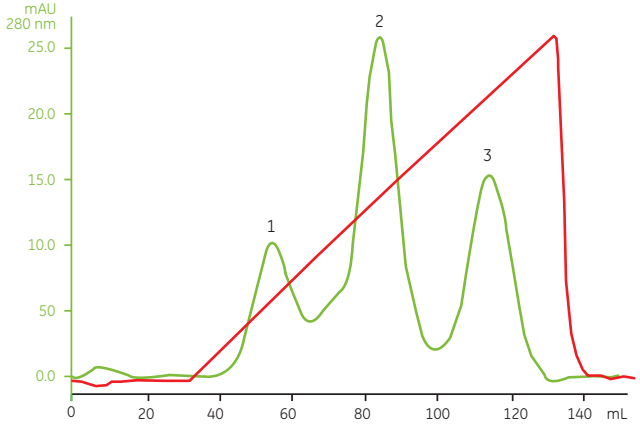
As shown in Figure 6, easy scale-up is a key practical benefit of working with any Sepharose Fast Flow ion exchanger. A laboratory protein separation was scaled up first five-fold and then twenty-fold on prepacked HiTrap Q FF and HiPrep Q FF 16/10 columns, respectively, with excellent reproducibility.

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)

(A) HiTrap Q FF, 1 mL



(B) HiTrap Q FF, 5 mL



(C) HiPrep Q FF 16/10, 20 mL

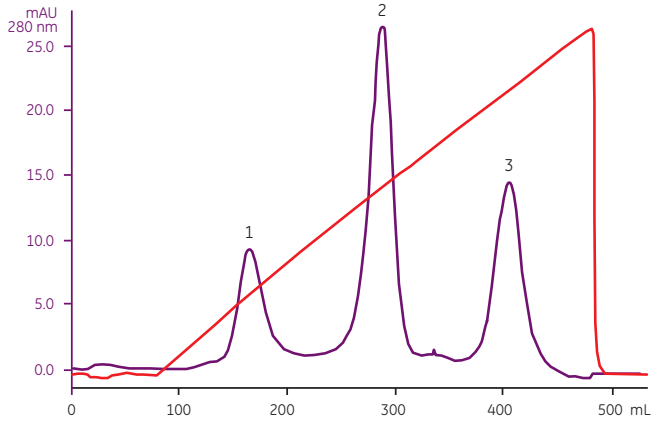
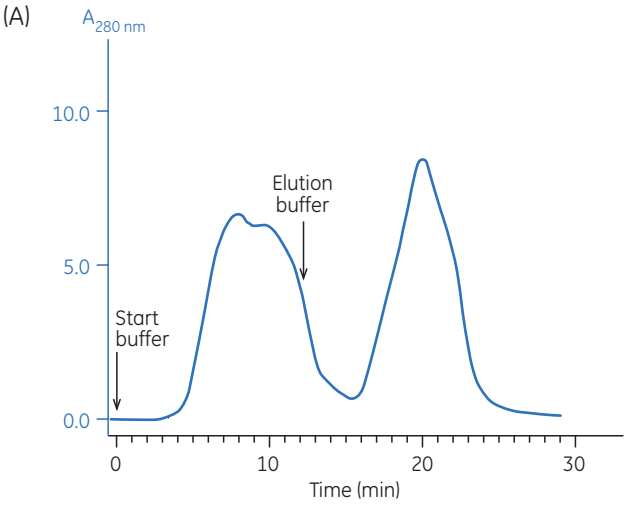


Fig 6. Five-fold and twenty-fold scale-up on prepacked Q Sepharose Fast Flow columns. (A) HiTrap Q FF, 1 mL (0.7 × 2.5 cm), (B) HiTrap Q FF, 5 mL (1.6 × 2.5 cm), and (C) HiPrep Q FF 16/10, 20 mL (1.6 × 10 cm).

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

Figure 7 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)

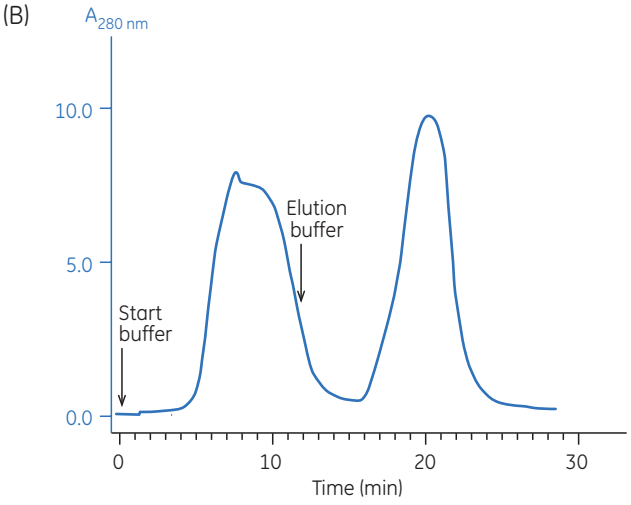


Fig 7. Scale-up from (A) a laboratory column (bed volume 80 mL) packed with SP Sepharose Fast Flow to (B) a column suitable for industrial production (bed volume 10.5 L) with retained packing and separation characteristics.

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

In Figure 8, 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.

Column: 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 recipe in ÄKTAexplorer™
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)

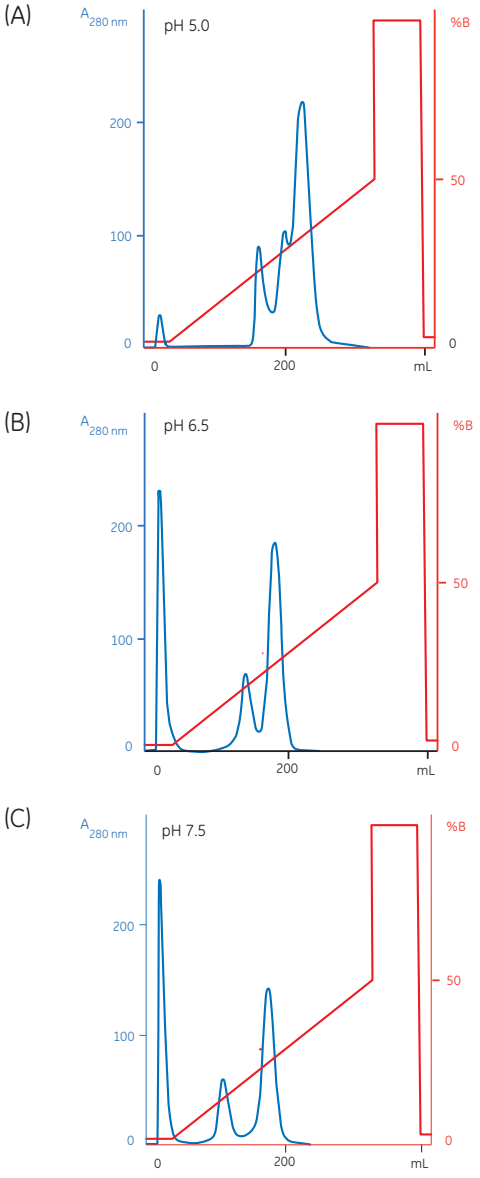


Fig 8. Separations of standard proteins on HiPrep CM FF 16/10 at (A) pH 5.0, (B) pH 6.5, and (C) pH 7.5.

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

Not only the elution pH affects the separation profile. As Figure 9 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.

Column:	(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)

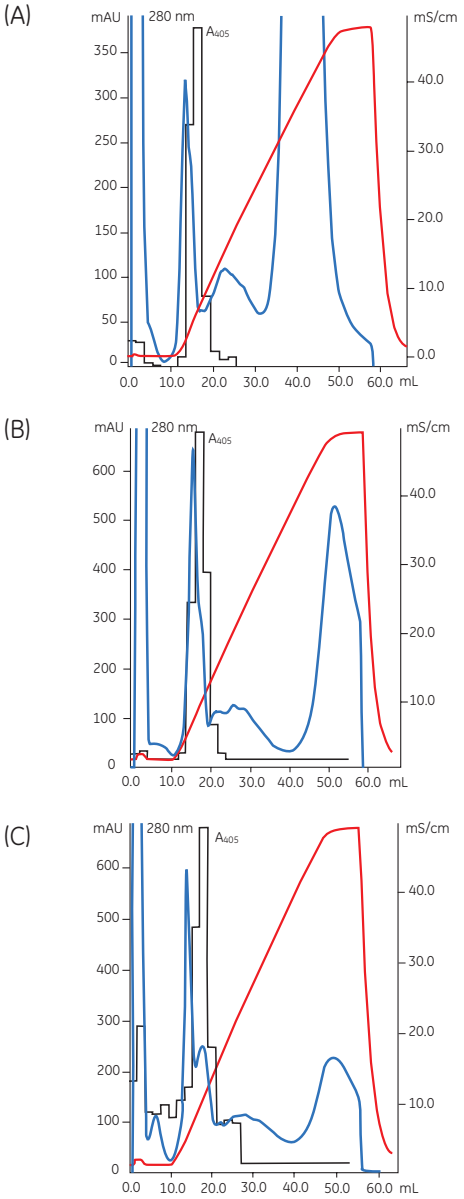


Fig 9. Clarified *E. coli* lysate separated on (A) HiTrap DEAE FF, 1 mL, (B) HiTrap Q FF, 1 mL, and (C) HiTrap ANX FF (high sub), 1 mL. Alkaline phosphatase activity measured at A_{405}.

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 media 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	Code number
Q Sepharose Fast Flow	25 mL	17-0510-10
	300 mL	17-0510-01
	5 l	17-0510-04
	10 l	17-0510-05
	60 l	17-0510-60
SP Sepharose Fast Flow	25 mL	17-0729-10
	300 mL	17-0729-01
	5 l	17-0729-04
	10 l	17-0729-05
	60 l	17-0729-60
DEAE Sepharose Fast Flow	25 mL	17-0709-10
	500 mL	17-0709-01
	10 l	17-0709-05
	60 l	17-0709-60
CM Sepharose Fast Flow	25 mL	17-0719-10
	500 mL	17-0719-01
	10 l	17-0719-05
	60 l	17-0719-60
ANX Sepharose 4 Fast Flow (high sub)	25 mL	17-1287-10
	500 mL	17-1287-01
	5 l	17-1287-04
	10 l	17-1287-05
	60 l	17-1287-60

Prepacked formats

Product	Quantity	Code number
HiPrep DEAE FF 16/10	1 × 20 mL	28-9365-41
HiPrep CM FF 16/10	1 × 20 mL	28-9365-42
HiPrep Q FF 16/10	1 × 20 mL	28-9365-43
HiPrep SP FF 16/10	1 × 20 mL	28-9365-44
HiScreen Q FF	1 × 4.7 mL	28-9505-10
HiScreen SP FF	1 × 4.7 mL	28-9505-13
HiScreen DEAE FF	1 × 4.7 mL	28-9782-45
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 ANX 4 FF (high sub)	5 × 1 mL	17-5162-01
	5 × 5 mL	17-5163-01
HiTrap IEX Selection Kit	7 × 1 mL	17-6002-33

Product	Quantity	Code number
PreDictor Q Sepharose Fast Flow, 6 µL	4 × 96-well plates	28-9432-69
PreDictor Q Sepharose Fast Flow, 20 µL	4 × 96-well plates	28-9432-70
PreDictor Q Sepharose Fast Flow, 50 µL	4 × 96-well plates	28-9432-71
PreDictor SP Sepharose Fast Flow, 6 µL	4 × 96-well plates	28-9432-72
PreDictor SP Sepharose Fast Flow, 20 µL	4 × 96-well plates	28-9432-73
PreDictor SP Sepharose Fast Flow, 50 µL	4 × 96-well plates	28-9432-74
PreDictor RoboColumn Q Sepharose Fast Flow, 200 µL	1 × 8-row columns	28-9860-86
PreDictor RoboColumn SP Sepharose Fast Flow, 200 µL	1 × 8-row columns	28-9860-87
PreDictor RoboColumn Q Sepharose Fast Flow, 600 µL	1 × 8-row columns	28-9861-80
PreDictor RoboColumn SP Sepharose Fast Flow, 600 µL	1 × 8-row columns	28-9861-81

Related literature

Handbook: Ion Exchange Chromatography & Chromatofocusing: Principles and Methods	11-0004-21
Selection guide: Ion Exchange Columns and Media	18-1127-31
Selection guide: Prepacked chromatography columns for ÄKTA systems	28-9317-78
Data file: AxiChrom columns	28-9290-41

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