GE Healthcare Life Sciences



HiTrap–convenient protein purification

Column Guide







Ion Exchange Chromatography (IEX)

IEX separates proteins with differences in charge. The separation is based on the reversible interaction between a charged protein and an oppositely charged chromatographic medium. Proteins bind as they are loaded onto a column. Conditions are then altered so that bound substances are eluted differentially. This elution is usually performed by increases in salt concentration or changes in pH. Most commonly, samples are eluted with salt (NaCl), using a gradient elution, as shown in Figure 1. Target proteins are concentrated during binding and collected in a purified, concentrated form.

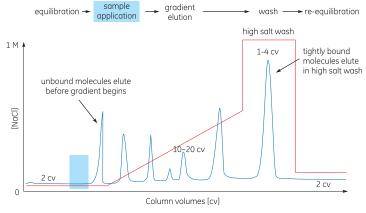


Fig 1. Typical IEX gradient elution.

Choice of ion exchanger

For most purifications it is recommended to begin with a strong exchanger to allow work over a broad pH range during method development.

Strong ion exchangers

Q (anion exchange), S or SP (cation exchange) are fully charged over a broad pH range (pH 2-12).

Weak ion exchangers

DEAE or ANX (anion exchange) and CM (cation exchange) are fully charged over a narrower pH range (pH 2-9, pH 3-10 and pH 6-10, respectively), but give alternative selectivities.

Media selection

HiTrap™ IEX Selection Kit, including seven different IEX media, is used for fast screening of IEX ligands and for method optimization.

See also Ion Exchange Columns and Media Guide, 18-1127-31.

Optimization parameters

1. Select ion exchanger.

- 2. Scout for optimum pH.
- 3. Select steepest gradient to give acceptable resolution at selected pH.
- 4. Select highest flow rate that maintains resolution and minimizes separation time.
- 5. For small scale sample clean up or large scale purifications, transfer to step elution to reduce separation times and buffer consumption, as shown in Figure 2. The different HiTrap IEX columns are ideal for small scale sample clean up.

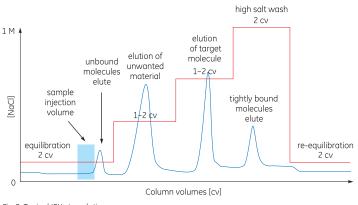


Fig 2. Typical IEX step elution.

Hydrophobic Interaction Chromatography (HIC)

HIC separates proteins with differences in hydrophobicity. The separation is based on the reversible interaction between a protein and the hydrophobic surface of a chromatographic medium. This interaction is enhanced by high ionic strength buffer, which makes HIC an ideal "next step" for purification of proteins that have been precipitated with ammonium sulphate or eluted in high salt during IEX. Samples in high ionic strength solution (e.g., 1.5 M (NH_a)₂SO_a) bind as they are loaded onto a column. Conditions are then altered so that the bound substances are eluted differentially. Elution is usually performed by decreases in salt concentration. Changes are made stepwise or with a continuously decreasing salt gradient. Most commonly, samples are eluted with a decreasing gradient of ammonium sulphate concentration. The key stages in a separation are shown in Figure 3. Target proteins are concentrated during binding and collected in a purified, concentrated form.

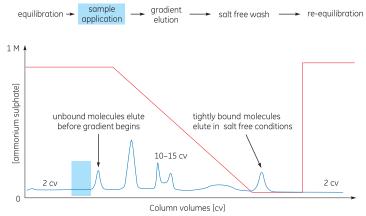


Fig. 3. Typical HIC gradient elution.

Choice of hydrophobic ligand and media selection

The hydrophobicity of a protein is difficult to determine. It is recommended to screen for the most suitable media for each application using HiTrap HIC Selection Kit.

Available hydrophobic ligands vary according to their degree of hydrophobicity:

Increasing hydrophobicity ----

Highly hydrophobic proteins bind tightly to highly hydrophobic ligands. Note that with HIC the chromatographic matrix as well as the hydrophobic ligand can affect selectivity.

Begin with a medium of low hydrophobicity if the sample is known to have hydrophobic components.

Select the medium that gives the best resolution and loading capacity at a low salt concentration

See also RPC & HIC Columns and Media Guide, 18-1149-96.

Optimization parameters

- 1. Select medium
- 2. Select optimum gradient to give acceptable resolution. For unknown samples begin with 0%B-100%B (0%B = 1 M ammonium sulphate).
- 3. Select highest flow rate that maintains resolution and minimizes separation time.
- 4. For large scale purifications, transfer to step elution to reduce separation times and buffer consumption, as shown in Figure 4.

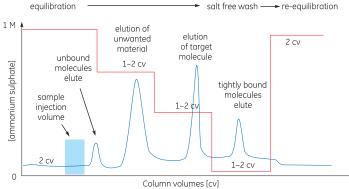


Fig 4. Typical HIC step elution

Affinity Chromatography (AC)

AC separates proteins on the basis of a reversible interaction between a protein (or group of proteins) and a specific ligand attached to a chromatographic matrix. AC can be used whenever a suitable ligand is available.

The target protein(s) is specifically and reversibly bound by a complementary binding substance (ligand). The sample is applied under conditions that favor specific binding to the ligand. Unbound material is washed away, and the bound target protein is recovered by changing conditions to those favouring desorption. Elution is performed specifically, using a competitive ligand, or non specifically, by changing the pH, ionic strength or polarity. Proteins are concentrated during binding and collected in a purified, concentrated form. The key stages in a separation are shown in Figure 5.

One important application using AC is purification of tagged recombinant proteins, for example histidine-, GST-, MBP-, and/or Strep(II)-tagged.

AC may also be used to remove specific contaminants. For example, HiTrap Benzamidine FF (high sub) removes trypsin-like serine proteases.

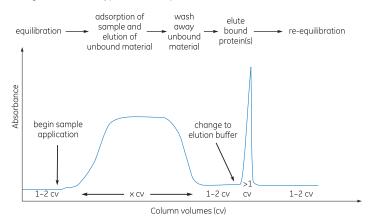


Fig 5. Typical affinity separation.

Media selection

Parameters such as scale of purification and commercial availability of affinity matrices should be considered when selecting affinity media.

HiTrap affinity columns are ideal for method optimization or small scale purification of target proteins using well-established protocols.

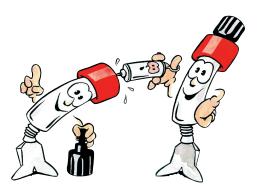
Affinity media can be prepared by coupling a ligand to a selected gel matrix. HiTrap NHS-activated HP is designed specifically to facilitate this process and is supplied with a recommended coupling procedure for coupling primary amines.

See also Affinity Chromatography Columns and Media Guide, 18-1121-86.

Optimization parameters

1. Select correct specificity for target protein.

- 2. Follow manufacturer's recommendations for binding or elution conditions.
- 3. Select optimum flow rate for sample application to achieve efficient binding.
- 4. Select optimum flow rate for elution to maximize recovery.
- 5. Select maximum flow rate for column re-equilibration to minimize run times.



Gel Filtration Chromatography (GF)

Gel filtration (size exclusion) chromatography separates proteins with differences in molecular size. Samples are eluted isocratically (single buffer, no gradient). Since buffer composition does not directly affect resolution, the buffer conditions can be varied to suit the sample type or the requirements for the next purification, analysis or storage step. Proteins are collected in purified form in the chosen buffer.

Sample clean up

Sephadex™ G-25, is ideal for rapid clean up of protein samples.

HiTrap Desalting columns (prepacked with Sephadex G-25) enable fast sample clean up in less than 5 minutes for sample volumes from 0.25 to 1.5 ml, as shown in Figure 6. To increase the maximum sample volume capacity to 3 ml simply connect two columns in series.

HiTrap Desalting columns are ideal for desalting, buffer exchange, and removal of salts, co-factors, labels or other small molecules.

Sample volumes up to 30% of total column volume are loaded when using gel filtration for desalting. The high sample volume gives a separation with minimal sample dilution. Larger sample volumes can be applied but resolution will be reduced.

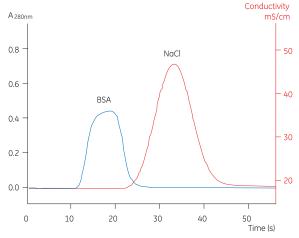


Fig 6. Typical desalting separation.

High resolution separations

For high resolution separations the technique should be used when sample volumes have been minimized. Figure 7 shows a typical high resolution gel filtration separation.

Media selection

Refer to Gel Filtration Columns and Media Guide, 18-1124-19.

Optimization parameters for high resolution separations

- 1. Select medium that gives the best separation of target proteins from contaminants.
- Select the highest flow rate that maintains resolution and minimizes separation time. Lower flow rates improve resolution of high molecular weight components, whereas faster flow rates may improve resolution of low molecular weight components.
- 3. Determine the maximum sample volume that can be loaded without significant reduction in resolution (sample volume should be 0.5 to 5% of total column volume).
- 4. To improve resolution further, increase column length by connecting two columns in series.

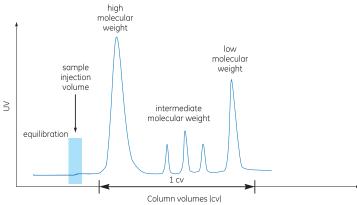
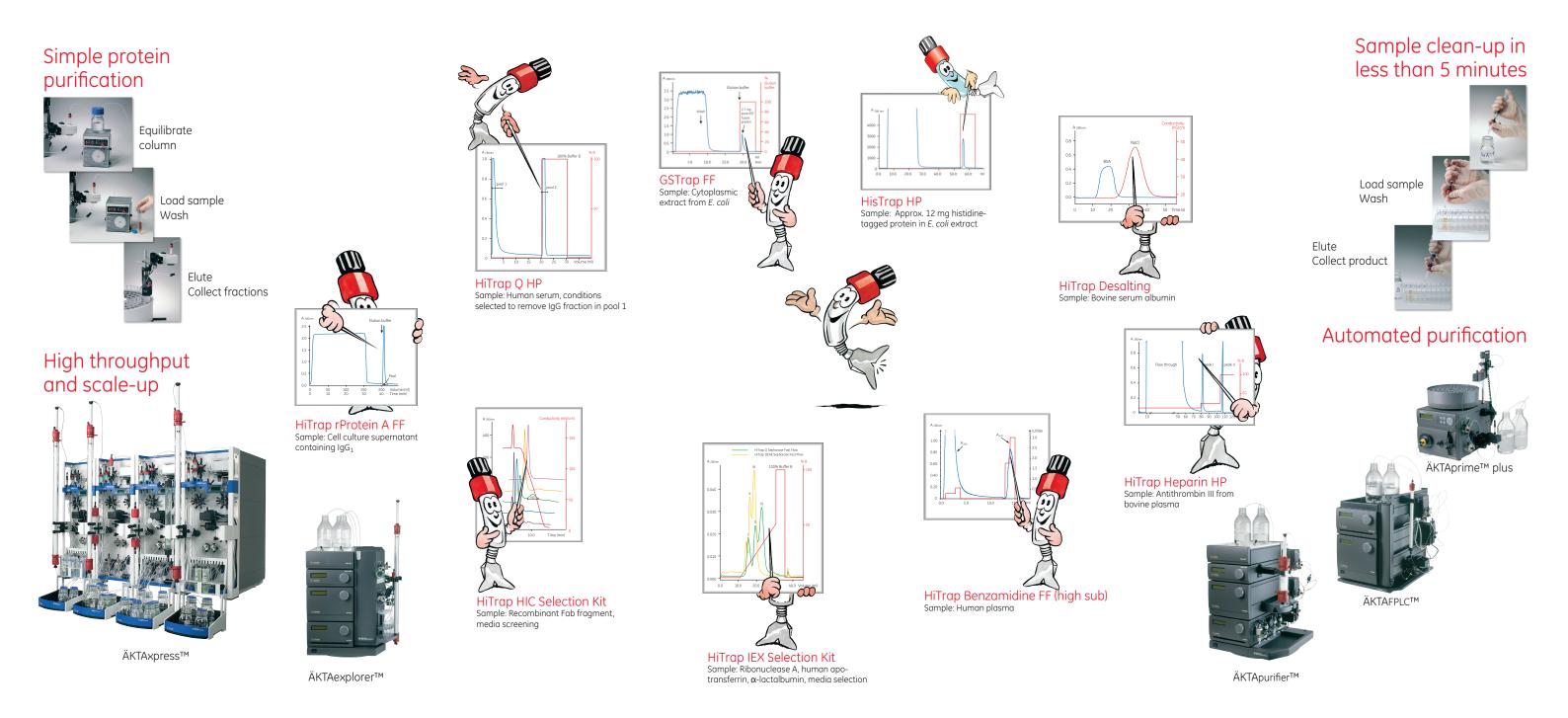


Fig 7. Typical high resolution GF separation

Convenient Protein Purification HiTrap Columns



	HiTrap columns/kits		Fisher Scientific Cat. No.	Quantity/components	Maximum operating flow rate	Approximate binding capacity per ml media	Applications
	Affinity / Isolation of immunoglobulins HiTrap MabSelect™	BisProcess	45001462 45001463	5 × 1 ml 1 × 5 ml	4 ml/min 20 ml/min	Human IgG ~30 mg/ml	Purification of monoclonal IgG for fast purifications from large sample volumes. Prepacked with MabSelect.
	HiTrap MabSelect SuRe™	BieProcess	45001464 45000008 45000009	5×5ml 5×1ml 1×5ml	4 ml/min 20 ml/min	Human IgG ~30 mg/ml	Purification of monoclonal IgG with the possibility to perform Cleaning-in-Place (CIP) between runs with 0.1 to 0.5 M NaOH. Prepacked with MabSelect SuRe medium that has a alkali-stabilized protein A ligand.
	HiTrap MabSelect Xtra™	BieProcess	45000010 45001465 45001466	5 × 5 ml 5 × 1 ml 1 × 5 ml	4 ml/min 20 ml/min	Human IgG ~40 mg/ml	Purification of monoclonal IgG for fast purifications from large sample volumes. Prepacked with MabSelect Xtra giving increased dynamic binding capacity.
-	HiTrap rProtein A FF	BioProcess	45001467 45000262	5 × 5 ml 5 × 1 ml	4 ml/min	Human IgG ~50 mg/ml	Monoclonal and polyclonal IgG from ascites fluid, serum and cell culture supernatant IgG classes, fragments and subclasses.
			45000263 45000264 45000265	2 × 1 ml 1 × 5 ml 5 × 5 ml	20 ml/min		
	HiTrap Protein A HP		45000049 45000050	5 × 1 ml 2 × 1 ml	4 ml/min	Human IgG ~20 mg/ml	Monoclonal and polyclonal IgG from ascites fluid, serum and cell culture supernatant IgG classes, fragments and subclasses.
_	HiTrap Protein G HP		45000051 45000052 45000053	1 × 5 ml 5 × 5 ml 5 × 1 ml	20 ml/min 4 ml/min	Human IgG 25 mg/ml	Monoclonal and polyclonal IgG from ascites fluid, serum and cell culture supernatant IgG classes, fragments and subclasses including
			45000054 45000055	2 × 1 ml 1 × 5 ml	20 ml/min		human IgG_3 strong affinity to monoclonal mouse IgG_1 and rat IgG .
=	MAbTrap™ Kit HiTrap IgY Purification HP		45000056 45000185 45000277	5 × 5 ml HiTrap Protein G HP column (1 × 1 ml), accessories, pre-made buffers 1 × 5 ml	4 ml/min 20 ml/min	Human IgG 25 mg/ml IgY 20 mg/ml	Monoclonal and polyclonal IgG from ascites fluid, serum and cell culture supernatant IgG classes, fragments and subclasses. IgY from egg yolk.
	HiTrop IgM Purification HP Affinity / Isolation of tagged proteins		45000276	5 × 1 ml	4 ml/min	Human IgM 5 mg/ml	Monoclonal and human IgM.
	HisTrap™ HP		45000323 45000324 45000325	5 × 1 ml 1 × 5 ml 5 × 5 ml	4 ml/min 20 ml/min	(Histidine) ₆ -tagged protein (M _r 43 000) at least 40 mg/ml	Histidine-tagged proteins. HisTrap HP columns are prepacked with Ni Sepharose™ High Performance, a Ni²+ precharged medium. Optimized for high performance purifications of histidine-tagged proteins.
	HisTrap FF	BioProcess	45000326 45000326	5 x 1 ml 5 x 5 ml	4 ml/min 20 ml/min	(Histidine)_6-tagged protein (Mr 43 000) ~40 mg/ml	Histidine-tagged proteins. HisTrap FF columns are prepacked with Ni Sepharose 6 Fast Flow, a Ni²+ precharged medium. Optimized for large sample volumes and scale-up.
	HisTrap FF crude HisTrap FF crude Kit	BioProcess	45000000 45000335 45001428	5 × 1 ml 5 × 5 ml Uistean Efforcide entrance (Z - 1 ml), accessories, and muffers	4 ml/min 20 ml/min 4 ml/min	(Histidine) ₆ -tagged protein (M, 43 000) ~40 mg/ml (Histidine)6-tagged protein (Mr 43 000) at least 40 mg/ml	Histidine-tagged protein. HisTrap FF crude columns are prepacked with Ni Sepharose 6 Fast Flow and optimized for direct loading of sonicated unclarified cell lysate without any sample pretreatment such as centrifugation and filtration. Histidine-tagged proteins. HisTrap FF crude columns are prepacked with Ni Sepharose 6 Fast Flow.
	HiTrap IMAC HP (see below) HiTrap IMAC HP (see below)		45001428	HisTrap FF crude columns (3 × 1 ml), accessories, pre-made buffers	4 111/11111	(misuainejo-taggea protein (mi 43 000) at least 40 mg/mi	misuaine-tagged proteinis. His hap nn chade columnis are prepacked with Ni septial use o nast how.
	HiTrap IMAC FF (see below) GSTrap™ HP		45000332	5×1ml	4 ml/min	GST-tagged protein (M _r 63 000) ~10 mg/ml	Glutathione S-transferase (GST) tagged proteins produced using the pGEX series of expression vectors,
_	GSTrap FF		45000333 45000334 45000281	1 × 5 ml 5 × 5 ml 5 × 1 ml	15 ml/min 4 ml/min	~10 mg GST/ml	other glutathione S-transferases and glutathione-dependent proteins. Prepacked with Glutathione Sepharose High Performance. Glutathione S-transferase (GST) tagged proteins produced using the pGEX series of expression vectors,
			45000282 45000283	2 × 1 ml 1 × 5 ml	15 ml/min	GST-tagged protein (M, 43 000) ~10 mg/ml	other glutathione S-transferases and glutathione-dependent proteins. Prepacked with Glutathione Sepharose 6 Fast Flow.
	GSTrap 4B		45000284 45001429 45001430	5 × 5 ml 5 × 1 ml 1 × 5 ml	1 ml/min 5 ml/min	~10 mg GST/ml	Glutathione S-transferase (GST) tagged proteins produced using the pGEX series of expression vectors, other glutathione S-transferases and glutathione-dependent proteins.
-	MBPTrap™ HP		45001431 45001540	5 × 5 ml 5 × 1 ml	4 ml/min	MBP-tagged protein (Mr \sim 70 000, multimer in solution) \sim 7 mg/ml medium	Prepacked with Glutathione Sepharose 48. Purification of MBP (Maltose Binding Protein)-tagged proteins. Prepacked HiTrap columns with Dextrin Sepharose High Performance.
_	StrepTrap™ HP		45001541 45001542 45001519	1 × 5 ml 5 × 5 m 5 × 1 ml	20 ml/min 	MBP-tagged protein (M _r ~158 000, multimer in solution) ~16 mg/ml medium Strep(II)-tagged protein (M _r 37 400) ~6 mg/ml medium	Optimized for high performance purificationss of MBP-tagged proteins Purification of Strep(II)-tagged proteins. Prepacked HiTrap columns with StrepTactin™ Sepharose High Performance.
			45001520 45001521	1 × 5 ml 5 × 5 ml	20 ml/min		Optimized for high performance purifications of Strep(II)-tagged proteins.
	HiTrap Streptavidin HP Affinity / Group specific media HiTrap IMAC HP		45000278 45000163	5×1ml	4 ml/min 4 ml/min	Biotinylated BSA 6 mg/ml (Histidine) ₆ -tagged protein (M, 43 000) ~40 mg/ml	Biotin and biotinylated molecules, such as biotin-tagged fusion proteins. Strep-tagged proteins.
-	HiTrap IMAC FF	BioProcess	45000164 45000167	5 × 5 ml 5 × 1 ml	20 ml/min 4 ml/min	$(\text{Histidine})_{6} \text{ tagged protein (N_{f} 43 000)} \sim 40 \text{ mg/ml}$ $(\text{Histidine})_{6} \text{-tagged protein (M_{f} 43 000)} \sim 40 \text{ mg/ml}$	HiTrap IMAC HP is prepacked with IMAC Sepharose High Performance. Optimization of purification of histidine-tagged proteins with usage of other metal ions than Ni ²⁺ .
-	HiTrap Chelating HP		45000168 45000060 45000061	5×5ml 5×1ml 1×5ml	20 ml/min 4 ml/min 20 ml/min	(Histidine) _c -tagged protein (M _r 27 600) ~12 mg/ml	HiTrap IMAC FF is prepacked with IMAC Sepharose 6 Fast Flow. Proteins and peptides with exposed amino acids: His (Cys, Trp), e.g., &g-macroglobulin and interferon, histidine-tagged proteins.
-	GSTrap HP (see above)		45000062	5 × 5 ml	2011//1111		Optimizing purification of histidine-tagged proteins by charging with different metal ions.
_	GSTrap FF (see above) GSTrap 4B (see above) HiTrap Blue HP		45000063	5×1ml	4 ml/min	HSA (M _r 68 000) 20 mg/ml	Albumin, nucleotide requiring enzymes, coagulation factors.
_	HiTrap Streptavidin HP		45000064 45000278	1×5ml 5×1ml	20 ml/min 4 ml/min	Biotinylated BSA 6 mg/ml	Biotin and biotinylated molecules, such as biotin-tagged proteins.
_	HiTrap Heparin HP HiTrap Benzamidine FF (high sub)		45000057 45000058 45000288	5×1ml 1×5ml 5×1ml	4 ml/min 20 ml/min 4 ml/min	Antithrombin III (bovine) ~3 mg/ml	Antithrombin III and other coagulation factors, lipoprotein lipases, DNA binding proteins, protein synthesis factors.
			45000288 45000289 45000290	2 × 1 ml 1 × 5 ml	20 ml/min	≥ 35 mg trypsin/ml	Trypsin and trypsin-like serine proteases (e.g., thrombin and factor Xa).
-	Affinity / Matrix for preparation of affinity media HiTrap NHS-activated HP		45000137 45000138	5×1ml 1×5ml	4 ml/min 20 ml/min	Ligand specific	For coupling of primary amines.
	IEX HiTrap IEX Selection Kit	BioProcess	45000368	7 × 1 ml columns	4 ml/min	As listed below	Media selection, method scouting.
_	Litzen O.F.	BiaProvisa	45000250	HiTrap Q FF, HiTrap SP FF, HiTrap DEAE FF, HiTrap CM FF, HiTrap ANX FF (high sub), HiTrap Q XL, HiTrap SP XL		HSA (M, 68 000) 120 mg/ml	Small scale, fast separation of sample, ideal for scale-up.
_	HiTrap Q FF	and total	45000258 45000293	5 × 1 ml		HSA (M _r 68 000) 120 mg/mi	
	HIITOD SP FF	BioProcess	45000259	5 × 5 ml 5 × 1 ml	4 ml/min 20 ml/min 4 ml/min	Ribonuclease A (Mr 13 700) 70 mg/ml	
-	HiTrap SP FF HiTrap DEAE FF	BioProcess	45000259 45000294 45000260	5 × 1 ml 5 × 5 ml 5 × 1 ml	20 ml/min 4 ml/min 20 ml/min 4 ml/min	Ribonuclease A (M, 13 700) 70 mg/ml HSA (M, 68 000) 110 mg/ml	Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up.
		BitProcess BitProcess BitProcess BitProcess BitProcess	45000294 45000260 45000291 45000261	5 × 1 ml 5 × 5 ml 5 × 1 ml 5 × 5 ml 5 × 1 ml	20 ml/min 4 ml/min 20 ml/min 4 ml/min 20 ml/min 4 ml/min		Small scale, fast separation of sample, ideal for scale-up.
	HITrap DEAE FF HITrap CM FF HITrap ANX FF (high sub)	His Process His Process His Process His Process His Process His Process	45000294 45000260 45000291 45000291 45000292 45000299 45000299	5 × 1 ml 5 × 5 ml 5 × 5 ml 5 × 5 ml 5 × 1 ml 5 × 5 ml 5 × 1 ml 5 × 5 ml	20 ml/min 4 ml/min 20 ml/min 4 ml/min 20 ml/min 4 ml/min 20 ml/min 4 ml/min 20 ml/min 20 ml/min	HSA (M _r 68 000) 110 mg/ml Ribonuclease A (M _r 13 700) 50 mg/ml BSA (M _r 67 000) 43 mg/ml	Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up, particulary useful for separation of high molecular mass proteins.
	HiTrap DEAE FF HiTrap CM FF HiTrap ANX FF (high sub) HiTrap Q XL	Bafforces Baffor	45000294 45000260 45000291 45000291 45000299 45000299 45000299 45000295 45000295	5 × 1 ml 5 × 5 ml 5 × 1 ml	20 ml/min 4 ml/min 20 ml/min 4 ml/min 20 ml/min 20 ml/min 20 ml/min 4 ml/min 20 ml/min 4 ml/min 20 ml/min 20 ml/min 20 ml/min	HSA (M, 68 000) 110 mg/ml Ribonuclease A (M, 13 700) 50 mg/ml BSA (M, 67 000) 43 mg/ml BSA (M, 67 000) >130 mg/ml	Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up, particulary useful for separation of high molecular mass proteins. Small scale, fast separation of sample, ideal for scale-up, in certain applications very high loading capacity.
	HITrap DEAE FF HITrap CM FF HITrap ANX FF (high sub)	Barborers Barborers Barborers Barborers Barborers Barborers Barborers Barborers Barborers Barborers Barborers	45000294 45000260 45000291 45000292 45000299 45000299 45000299 45000295 45000295 45000296 45000297 45000298 45000193	5 × 1 ml 5 × 5 ml 5 × 1 ml 5 × 5 ml 5 × 5 ml 5 × 1 ml 5 × 5 ml 5 × 1 ml 5 × 5 ml 5 × 1 ml	20 ml/min 4 ml/min 20 ml/min 4 ml/min 20 ml/min 4 ml/min 20 ml/min 4 ml/min 20 ml/min 4 ml/min 4 ml/min	HSA (M _r 68 000) 110 mg/ml Ribonuclease A (M _r 13 700) 50 mg/ml BSA (M _r 67 000) 43 mg/ml	Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up, particulary useful for separation of high molecular mass proteins.
	HiTrap DEAE FF HiTrap CM FF HiTrap ANX FF (high sub) HiTrap Q XL HiTrap SP XL		45000294 45000291 45000291 45000292 45000292 45000299 45000295 45000295 45000295 45000295 45000298 45000193 45000191	5 × 1 ml 5 × 5 ml 5 × 1 ml 5 × 1 ml 5 × 5 ml 5 × 1 ml 5 × 5 ml 5 × 1 ml 5 × 5 ml 5 × 1 ml 5 × 1 ml 5 × 5 ml 5 × 1 ml 5 × 5 ml	20 ml/min 4 ml/min 20 ml/min 4 ml/min 20 ml/min 20 ml/min 20 ml/min 20 ml/min 20 ml/min 20 ml/min 4 ml/min 20 ml/min 4 ml/min 20 ml/min 4 ml/min 20 ml/min 4 ml/	HSA (M, 68 000) 110 mg/ml Ribonuclease A (M, 13 700) 50 mg/ml BSA (M, 67 000) 43 mg/ml BSA (M, 67 000) >130 mg/ml Lysozyme (M, 14 300) >160 mg /ml	Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up, particulary useful for separation of high molecular mass proteins. Small scale, fast separation of sample, ideal for scale-up, particulary useful for separation of high molecular mass proteins. Small scale, fast separation of sample, ideal for scale-up, in certain applications very high loading capacity. Small scale, fast separation of sample, ideal for scale-up, in certain applications very high loading capacity.
	HiTrap DEAE FF HiTrap CM FF HiTrap ANX FF (high sub) HiTrap Q XL HiTrap SP XL HiTrap Q HP		45000294 45000260 45000291 45000292 45000299 45000299 45000299 45000295 45000296 45000296 45000296 45000298 45000193	5 × 1 ml 5 × 5 ml 5 × 1 ml 5 × 1 ml 5 × 5 ml 5 × 1 ml 5 × 5 ml 5 × 1 ml 5 × 5 ml 5 × 1 ml 5 × 5 ml 5 × 1 ml 5 × 5 ml	20 ml/min 4 ml/min 20 ml/min 20 ml/min 20 ml/min 4 ml/min 20 ml/min 20 ml/min 20 ml/min 20 ml/min 4 ml/min 20 ml/min 4 ml/min 20 ml/min 20 ml/min 20 ml/min 20 ml/min 20 ml/min 20 ml/min	HSA (Mr 68 000) 110 mg/ml Ribonuclease A (Mr, 13 700) 50 mg/ml BSA (Mr, 67 000) 43 mg/ml BSA (Mr, 67 000) >130 mg/ml Lysozyme (Mr, 14 300) >160 mg /ml HSA (Mr, 68 000) 50 mg/ml	Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up, particulary useful for separation of high molecular mass proteins. Small scale, fast separation of sample, ideal for scale-up, particulary useful for separation of high molecular mass proteins. Small scale, fast separation of sample, ideal for scale-up, in certain applications very high loading capacity. Small scale, fast separation of sample, ideal for scale-up, in certain applications very high loading capacity. Small scale, high resolution separation of sample.
	HiTrap DEAE FF HiTrap CM FF HiTrap ANX FF (high sub) HiTrap Q XL HiTrap SP XL HiTrap Q HP HiTrap SP HP HiTrap Capto™ Q HiTrap Capto DEAE		45000294 45000260 45000291 45000292 45000299 45000299 45000295 45000296 45000296 45000296 45000296 45000193 45000193 45000192 4500002 4500002 4500002 4500002 4500002 4500002 45001525 45001526	5 × 1 ml 5 × 5 ml 5 × 1 ml 5 × 1 ml 5 × 5 ml 5 × 5 ml 5 × 1 ml 5 × 5 ml 5 × 5 ml 5 × 1 ml 5 × 1 ml 5 × 5 ml	20 ml/min 4 ml/min 20 ml/min 4 ml/min 20 ml/min 4 ml/min 20 ml/min 20 ml/min 20 ml/min 20 ml/min 20 ml/min 4 ml/min 20 ml/min	HSA (M, 68 000) 110 mg/ml Ribonuclease A (M, 13 700) 50 mg/ml BSA (M, 67 000) 43 mg/ml BSA (M, 67 000) >130 mg/ml Lysozyme (M, 14 300) >160 mg /ml HSA (M, 68 000) 50 mg/ml Ribonuclease A (M, 13 700) 55 mg/ml Minimum 100 mg BSA/ml (10% breakthrough, 1 min residence time) Ovalbumin (M, 67 000) > 90 mg	Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up, particulary useful for separation of high molecular mass proteins. Small scale, fast separation of sample, ideal for scale-up, particulary useful for separation of high molecular mass proteins. Small scale, fast separation of sample, ideal for scale-up, in certain applications very high loading capacity. Small scale, fast separation of sample, ideal for scale-up, in certain applications very high loading capacity. Small scale, high resolution separation of sample. Small scale, high resolution separation of sample. Small scale, high resolution separation of sample. Fast separation of sample, ideal for scale-up, high binding capacity at high flow rate. Fast separation of sample, ideal for scale-up, high binding capacity at high flow rate.
	HiTrap DEAE FF HiTrap CM FF HiTrap ANX FF (high sub) HiTrap Q XL HiTrap SP XL HiTrap Q HP HiTrap SP HP HiTrap Capto™ Q		45000294 45000260 45000291 45000292 45000299 45000299 45000299 45000295 45000296 45000296 45000296 45000298 45000193 45000193 45000192 45000002 45000002 45000003	5 × 1 ml 5 × 5 ml 5 × 1 ml 5 × 5 ml 5 × 1 ml 5 × 5 ml 5 × 1 ml 5 × 5 ml 5 × 1 ml 5 × 5 ml 5 × 1 ml	20 ml/min 4 ml/min 20 ml/min 20 ml/min 20 ml/min 4 ml/min 20 ml/min 4 ml/min 20 ml/min 4 ml/min 20 ml/min 4 ml/min 20 ml/min 4 ml/min 20 ml/min 4 ml/min 20 ml/min 4 ml/min 20 ml/min 3 ml/min 3 ml/min 4 ml/min 3 ml/min 4 ml/min 3 ml/min 4 ml/min 3 ml/min 3 ml/min 4 ml/min 4 ml/min 3 ml/min 4 ml/min 3 ml/min 4 ml/min 3 ml/min 4 ml/min 3 ml/min 4 ml/min 4 ml/min 3 ml/min 3 ml/min 3 ml/min 3 ml/min 3 ml/min 4 ml/min 3 ml/min 3 ml/min 3 ml/min 3 ml/min 3 ml/min 3 ml/min 4 ml/min 3 ml/min 4 ml/min 3 ml/min 4 ml/min 3 ml/min 4 ml/min 3 ml/min 4 ml/min 3 ml/min 4 ml/min 3 ml/	HSA (M, 68 000) 110 mg/ml Ribonuclease A (M, 13 700) 50 mg/ml BSA (M, 67 000) >130 mg/ml Lysozyme (M, 14 300) >160 mg /ml HSA (M, 68 000) 50 mg/ml Ribonuclease A (M, 13 700) 55 mg/ml Minimum 100 mg BSA/ml (10% breakthrough, 1 min residence time)	Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up, particulary useful for separation of high molecular mass proteins. Small scale, fast separation of sample, ideal for scale-up, in certain applications very high loading capacity. Small scale, fast separation of sample, ideal for scale-up, in certain applications very high loading capacity. Small scale, fast separation of sample, ideal for scale-up, in certain applications very high loading capacity. Small scale, fast separation of sample, ideal for scale-up, in certain applications very high loading capacity. Small scale, high resolution separation of sample. Small scale, high resolution separation of sample. Fast separation of scale-up, high binding capacity at high flow rate.
	HiTrap DEAE FF HiTrap CM FF HiTrap ANX FF (high sub) HiTrap Q XL HiTrap SP XL HiTrap Q HP HiTrap Capto™ Q HiTrap Capto S		45000294 45000260 45000261 45000291 45000299 45000299 45000296 45000296 45000296 45000193 45000193 45000194 45000195 45000193 45000194 45000192 45000192 450001525 450001525 450001525 450000338 45000004 45000005	5 × 1 ml 5 × 5 ml 5 × 5 ml 5 × 1 ml 5 × 1 ml 5 × 5 ml	20 ml/min 20 ml/min	HSA (M, 68 000) 110 mg/ml Ribonuclease A (M, 13 700) 50 mg/ml BSA (M, 67 000) 43 mg/ml BSA (M, 67 000) >130 mg/ml Lysozyme (M, 14 300) >160 mg /ml HSA (M, 68 000) 50 mg/ml Ribonuclease A (M, 13 700) 55 mg/ml Minimum 100 mg BSA/ml (10% breakthrough, 1 min residence time) Ovalbumin (M, 67 000) > 90 mg Minimum 120 mg lysozyme/ml medium (10% breakthrough, 1 min residence time)	Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up. Small scale, fast separation of sample, ideal for scale-up, particulary useful for separation of high molecular mass proteins. Small scale, fast separation of sample, ideal for scale-up, in certain applications very high loading capacity. Small scale, fast separation of sample, ideal for scale-up, in certain applications very high loading capacity. Small scale, fast separation of sample, ideal for scale-up, in certain applications very high loading capacity. Small scale, high resolution separation of sample. Small scale, high resolution separation of sample. Fast separation of sample, ideal for scale-up, high binding capacity at high flow rate. Fast separation of sample, ideal for scale-up, high binding capacity at high flow rate. Fast separation of sample, ideal for scale-up, high binding capacity at high flow rate. Fast separation of sample, ideal for scale-up, high binding capacity at high flow rate. Fast separation of sample, ideal for scale-up, high binding capacity at high flow rate. Fast separation of sample, ideal for scale-up, high binding capacity at high flow rate. Fast separation of sample, ideal for scale-up, high binding capacity at high flow rate. Fast separation
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Media: FF – Sepharose Fast Flow HP – Sepharose High Performance

BioProcess™ media includes regulatory support.

*Available by special order. Please contact your local GE Healthcare representative.

Recommended separation conditions: All HiTrap columns are supplied with a detailed protocol to ensure an optimal result.

Fast and easy scale-up: For fast scaling-up, two or more HiTrap columns can be connected in series by screwing the end of one into the top of the next (backpressure will increase).

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