GE Healthcare

HiTrap–convenient protein purification

Column Guide

HiTrap

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.

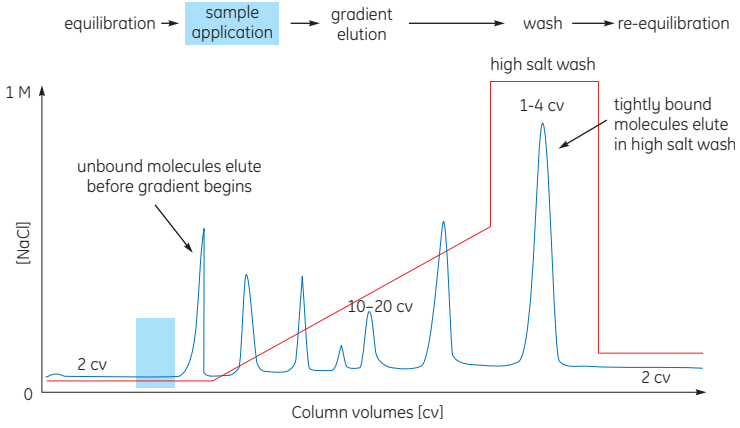


Fig 1. Typical IEX gradient elution.

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.

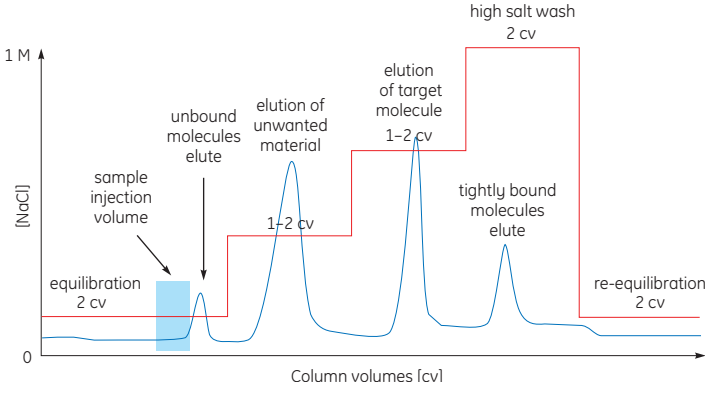


Fig 2. Typical IEX step elution.

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 $(NH_4)_2SO_4$) 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.

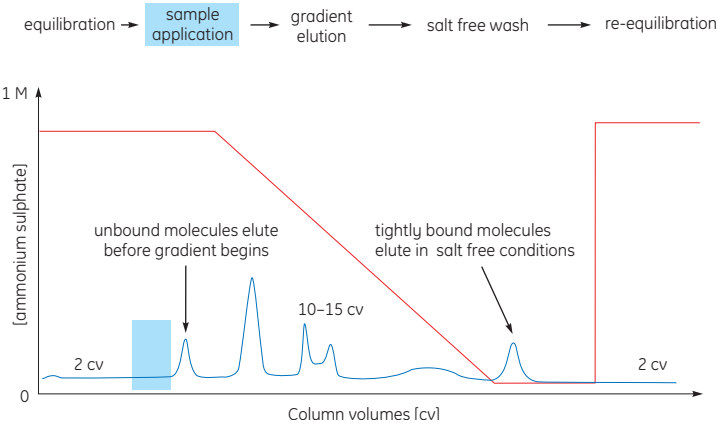


Fig 3. Typical HIC gradient elution.

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 →

ether → isopropyl → butyl → octyl → phenyl

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.

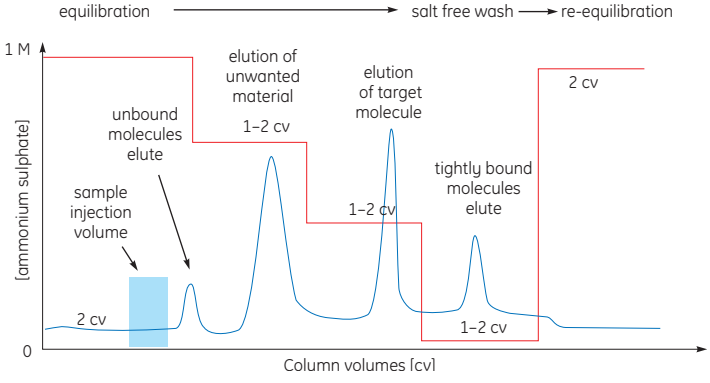


Fig 4. Typical HIC step elution.

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.

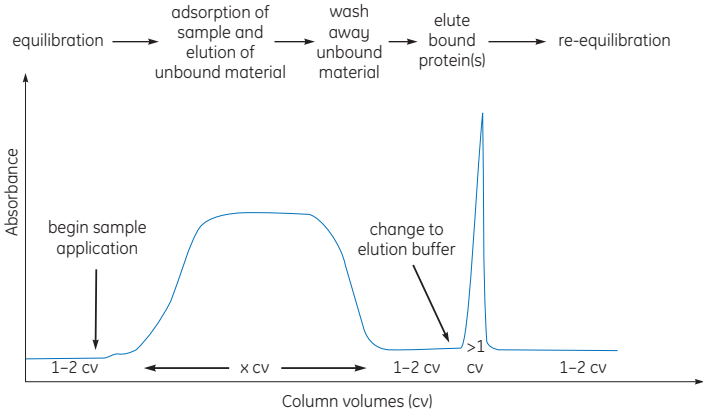


Fig 5. Typical affinity separation.

Media selection

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

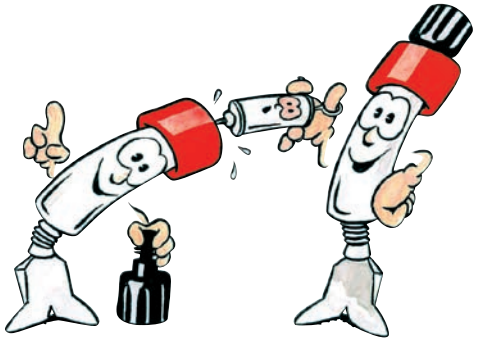
HiTrap affinity columns are ideal for method optimization or small scale purification of target proteins using well-established protocols.

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.

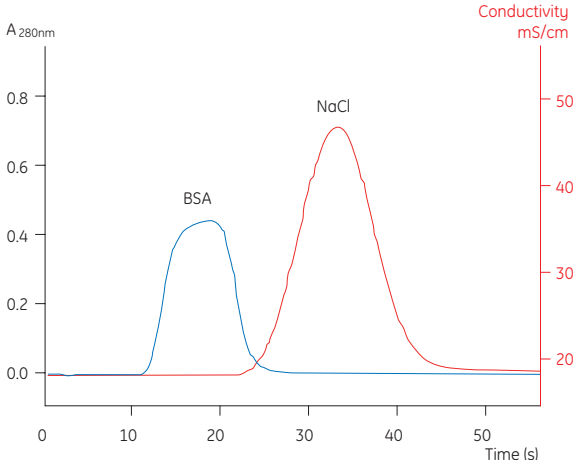


Fig 6. Typical desalting separation.

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.
2. 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.

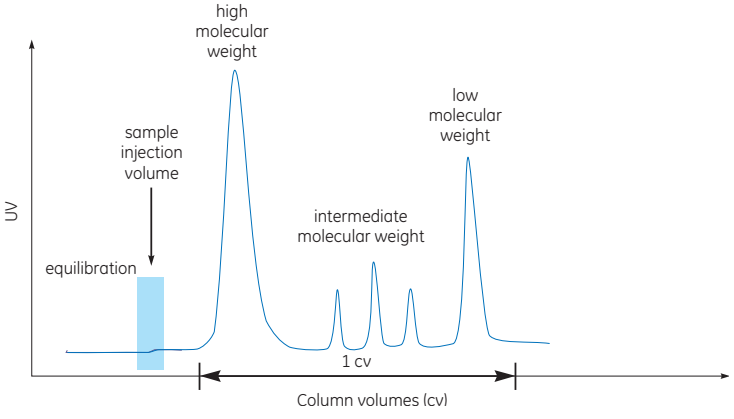
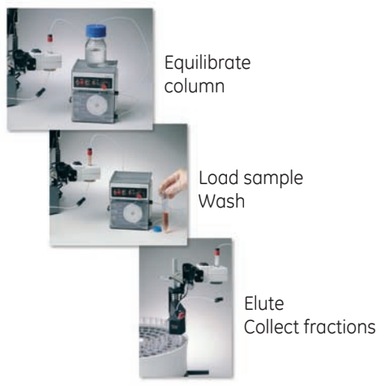


Fig 7. Typical high resolution GF separation.

Convenient Protein Purification HiTrap Columns

Simple protein purification

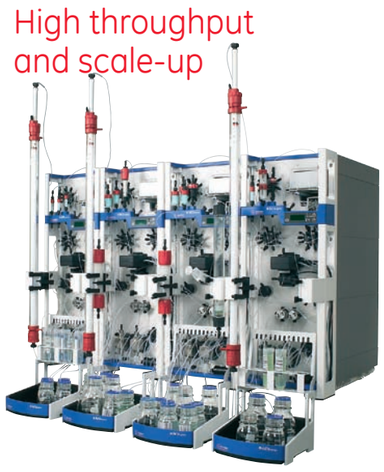


Equilibrate column

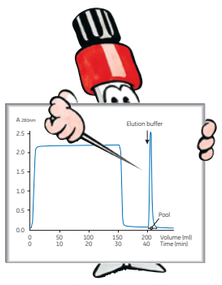
Load sample
Wash

Elute
Collect fractions

High throughput and scale-up

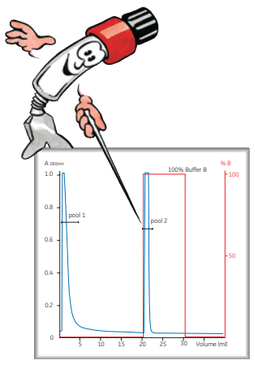


ÄKTAxpress™

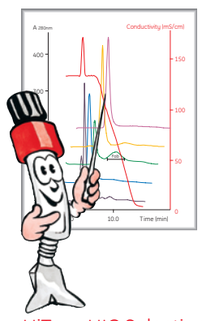


HiTrap rProtein A FF
Sample: Cell culture supernatant containing IgG_1

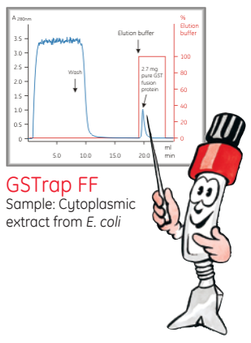
ÄKTAexplorer™



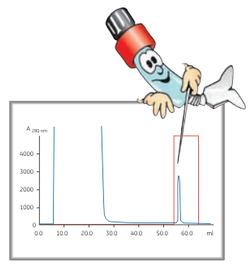
HiTrap Q HP
Sample: Human serum, conditions selected to remove IgG fraction in pool 1



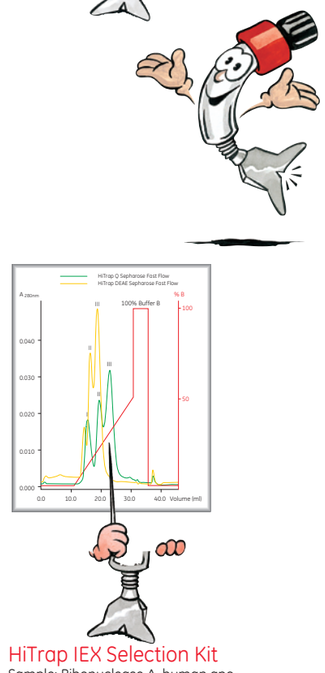
HiTrap HIC Selection Kit
Sample: Recombinant Fab fragment, media screening



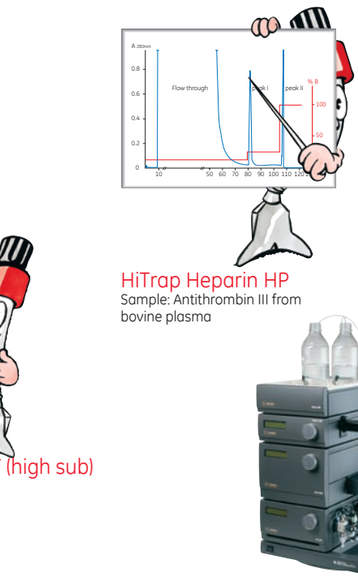
GSTrap FF
Sample: Cytoplasmic extract from *E. coli*



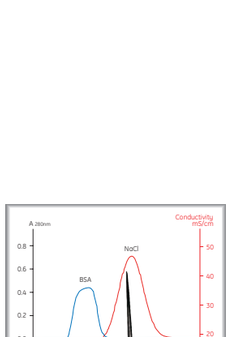
HisTrap HP
Sample: Approx. 12 mg histidine-tagged protein in *E. coli* extract



HiTrap IEX Selection Kit
Sample: Ribonuclease A, human apo-transferrin, α-lactalbumin, media selection



HiTrap Benzamidine FF (high sub)
Sample: Human plasma

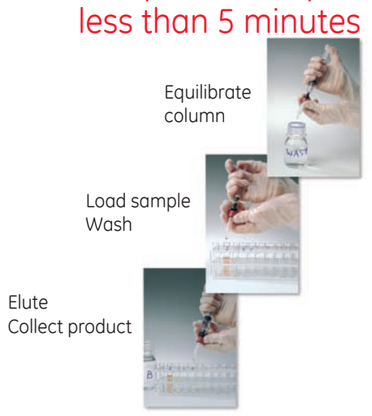


HiTrap Desalting
Sample: Bovine serum albumin

HiTrap Heparin HP
Sample: Antithrombin III from bovine plasma

ÄKTApurifier™

Sample clean-up in less than 5 minutes



Equilibrate column

Load sample
Wash

Elute
Collect product

Automated purification

ÄKTAprime™ plus

ÄKTAFPLC™

HiTrap columns/kits		Code No.	Quantity/components	Maximum operating flow rate	Approximate binding capacity per ml media	Applications
Affinity / Isolation of immunoglobulins						
HiTrap MabSelect™	BioProcess	28-4082-53 28-4082-55 28-4082-56	5 × 1 ml 1 × 5 ml 5 × 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™	BioProcess	11-0034-93 11-0034-94 11-0034-95	5 × 1 ml 1 × 5 ml 5 × 5 ml	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™	BioProcess	28-4082-58 28-4082-60 28-4082-61	5 × 1 ml 1 × 5 ml 5 × 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	17-5079-01 17-5079-02 17-5080-01 17-5080-02	5 × 1 ml 2 × 1 ml 1 × 5 ml 5 × 5 ml	4 ml/min 20 ml/min	Human IgG ~50 mg/ml	Monoclonal and polyclonal IgG from ascites fluid, serum and cell culture supernatant IgG classes, fragments and subclasses.
HiTrap Protein A HP		17-0402-01 17-0402-03 17-0403-01 17-0403-03	5 × 1 ml 2 × 1 ml 1 × 5 ml 5 × 5 ml	4 ml/min 20 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		17-0404-01 17-0404-03 17-0405-01 17-0405-03	5 × 1 ml 2 × 1 ml 1 × 5 ml 5 × 5 ml	4 ml/min 20 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 human IgG_3 strong affinity to monoclonal mouse IgG_1 and rat IgG.
MAbTrap™ Kit		17-1128-01	HiTrap Protein G HP column (1 × 1 ml), accessories, pre-made buffers	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.
HiTrap IgY Purification HP		17-5111-01	1 × 5 ml	20 ml/min	IgY 20 mg/ml	IgY from egg yolk.
HiTrap IgM Purification HP		17-5110-01	5 × 1 ml	4 ml/min	Human IgM 5 mg/ml	Monoclonal and human IgM.
Affinity / Isolation of tagged proteins						
HisTrap™ HP		17-5247-01 17-5247-05 17-5248-01 17-5248-02 17-5248-05	5 × 1 ml 100 × 1 ml* 1 × 5 ml 5 × 5 ml 100 × 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 Ni^{2+} precharged medium. Optimized for high performance purifications of histidine-tagged proteins.
HisTrap FF	BioProcess	17-5319-01 17-5319-02 17-5255-01 17-5255-02	5 × 1 ml 100 × 1 ml* 5 × 5 ml 100 × 5 ml*	4 ml/min 20 ml/min	$(Histidine)_6$-tagged protein (M_r 43 000) ~40 mg/ml	Histidine-tagged proteins. HisTrap FF columns are prepacked with Ni Sepharose 6 Fast Flow, a Ni^{2+} precharged medium. Optimized for large sample volumes and scale-up.
HisTrap FF crude	BioProcess	11-0004-58 11-0004-59 17-5286-01 17-5286-02	5 × 1 ml 100 × 1 ml* 5 × 5 ml 100 × 5 ml*	4 ml/min 20 ml/min	$(Histidine)_6$-tagged protein (M_r 43 000) ~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.
HisTrap FF crude Kit		17-5318-06	HisTrap FF crude columns (3 × 1 ml), accessories, pre-made buffers	4 ml/min	$(Histidine)_6$-tagged protein (M_r 43 000) at least 40 mg/ml	Histidine-tagged proteins. HisTrap FF crude columns are prepacked with Ni Sepharose 6 Fast Flow.
HiTrap Chelating HP (see below) HiTrap IMAC HP (see below) HiTrap IMAC FF (see below)						
GSTrap™ HP		17-5281-01 17-5281-05 17-5282-01 17-5282-02 17-5282-05	5 × 1 ml 100 × 1 ml* 1 × 5 ml 5 × 5 ml 100 × 5 ml*	4 ml/min 15 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, other glutathione S-transferases and glutathione-dependent proteins. Prepacked with Glutathione Sepharose High Performance.
GSTrap FF		17-5130-01 17-5130-02 17-5130-05 17-5131-01 17-5131-02 17-5131-05	5 × 1 ml 2 × 1 ml 100 × 1 ml* 1 × 5 ml 5 × 5 ml 100 × 5 ml*	4 ml/min 15 ml/min	~10 mg GST/ml GST-tagged protein (M_r 43 000) ~10 mg/ml	Glutathione S-transferase (GST) tagged proteins produced using the pGEX series of expression vectors, other glutathione S-transferases and glutathione-dependent proteins. Prepacked with Glutathione Sepharose 6 Fast Flow.
GSTrap 4B		28-4017-45 28-4017-46 28-4017-47 28-4017-48 28-4017-49	5 × 1 ml 100 × 1 ml* 1 × 5 ml 5 × 5 ml 100 × 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. Prepacked with Glutathione Sepharose 4B.
MBPTrap™ HP		28-9187-78 28-9187-79 28-9187-80	5 × 1 ml 1 × 5 ml 5 × 5 m	4 ml/min 20 ml/min	MBP-tagged protein (M_r ~70 000, multimer in solution) ~7 mg/ml medium MBP-tagged protein (M_r ~158 000, multimer in solution) ~16 mg/ml medium	Purification of MBP (Maltose Binding Protein)-tagged proteins. Prepacked HiTrap columns with Dextrin Sepharose High Performance. Optimized for high performance purificationss of MBP-tagged proteins
StrepTrap™ HP		28-9075-46 28-9075-47 28-9075-48	5 × 1 ml 1 × 5 ml 5 × 5 ml	4 ml/min 20 ml/min	Strep(II)-tagged protein (M_r 37 400) ~6 mg/ml medium	Purification of Strep(II)-tagged proteins. Prepacked HiTrap columns with StrepTactin™ Sepharose High Performance. Optimized for high performance purifications of Strep(II)-tagged proteins.
HiTrap Streptavidin HP		17-5112-01	5 × 1 ml	4 ml/min	Biotinylated BSA 6 mg/ml	Biotin and biotinylated molecules, such as biotin-tagged fusion proteins. Strep-tagged proteins.
Affinity / Group specific media						
HiTrap IMAC HP		17-0920-03 17-0920-05	5 × 1 ml 5 × 5 ml	4 ml/min 20 ml/min	$(Histidine)_6$-tagged protein (M_r 43 000) ~40 mg/ml	Optimization of purification of histidine-tagged proteins with usage of other metal ions than Ni^{2+}. HiTrap IMAC HP is prepacked with IMAC Sepharose High Performance.
HiTrap IMAC FF	BioProcess	17-0921-02 17-0921-04	5 × 1 ml 5 × 5 ml	4 ml/min 20 ml/min	$(Histidine)_6$-tagged protein (M_r 43 000) ~40 mg/ml	Optimization of purification of histidine-tagged proteins with usage of other metal ions than Ni^{2+}. HiTrap IMAC FF is prepacked with IMAC Sepharose 6 Fast Flow.
HiTrap Chelating HP		17-0408-01 17-0409-01 17-0409-03 17-0409-05	5 × 1 ml 1 × 5 ml 5 × 5 ml 100 × 5 ml*	4 ml/min 20 ml/min	$(Histidine)_6$-tagged protein (M_r 27 600) ~12 mg/ml	Proteins and peptides with exposed amino acids: His (Cys, Trp), e.g., α_2-macroglobulin and interferon, histidine-tagged proteins. Optimizing purification of histidine-tagged proteins by charging with different metal ions.
GSTrap HP (see above) GSTrap FF (see above) GSTrap 4B (see above)						
HiTrap Blue HP		17-0412-01 17-0413-01	5 × 1 ml 1 × 5 ml	4 ml/min 20 ml/min	HSA (M_r 68 000) 20 mg/ml	Albumin, nucleotide requiring enzymes, coagulation factors.
HiTrap Streptavidin HP		17-5112-01	5 × 1 ml	4 ml/min	Biotinylated BSA 6 mg/ml	Biotin and biotinylated molecules, such as biotin-tagged proteins.
HiTrap Heparin HP		17-0406-01 17-0407-01	5 × 1 ml 1 × 5 ml	4 ml/min 20 ml/min	Antithrombin III (bovine) ~3 mg/ml	Antithrombin III and other coagulation factors, lipoprotein lipases, DNA binding proteins, protein synthesis factors.
HiTrap Benzamidine FF (high sub)		17-5143-01 17-5143-02 17-5144-01	5 × 1 ml 2 × 1 ml 1 × 5 ml	4 ml/min 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		17-0716-01 17-0717-01	5 × 1 ml 1 × 5 ml	4 ml/min 20 ml/min	Ligand specific	For coupling of primary amines.
IEX						
HiTrap IEX Selection Kit	BioProcess	17-6002-33	7 × 1 ml columns HiTrap Q FF, HiTrap SP FF, HiTrap DEAE FF, HiTrap CM FF, HiTrap ANX FF (high sub), HiTrap Q XL, HiTrap SP XL	4 ml/min	As listed below	Media selection, method scouting.
HiTrap Q FF	BioProcess	17-5053-01 17-5156-01	5 × 1 ml 5 × 5 ml	4 ml/min 20 ml/min	HSA (M, 68 000) 120 mg/ml	Small scale, fast separation of sample, ideal for scale-up.
HiTrap SP FF	BioProcess	17-5054-01 17-5157-01	5 × 1 ml 5 × 5 ml	4 ml/min 20 ml/min	Ribonuclease A (M, 13 700) 70 mg/ml	Small scale, fast separation of sample, ideal for scale-up.
HiTrap DEAE FF	BioProcess	17-5055-01 17-5154-01	5 × 1 ml 5 × 5 ml	4 ml/min 20 ml/min	HSA (M, 68 000) 110 mg/ml	Small scale, fast separation of sample, ideal for scale-up.
HiTrap CM FF	BioProcess	17-5056-01 17-5155-01	5 × 1 ml 5 × 5 ml	4 ml/min 20 ml/min	Ribonuclease A (M, 13 700) 50 mg/ml	Small scale, fast separation of sample, ideal for scale-up.
HiTrap ANX FF (high sub)	BioProcess	17-5162-01 17-5163-01	5 × 1 ml 5 × 5 ml	4 ml/min 20 ml/min	BSA (M, 67 000) 43 mg/ml	Small scale, fast separation of sample, ideal for scale-up, particulary useful for separation of high molecular mass proteins.
HiTrap Q XL	BioProcess	17-5158-01 17-5159-01	5 x 1ml 5 x 5 ml	4 ml/min 20 ml/min	BSA (M, 67 000) >130 mg/ml	Small scale, fast separation of sample, ideal for scale-up, in certain applications very high loading capacity.
HiTrap SP XL	BioProcess	17-5160-01 17-5161-01	5 × 1 ml 5 × 5 ml	4 ml/min 20 ml/min	Lysozyme (M_r 14 300) >160 mg /ml	Small scale, fast separation of sample, ideal for scale-up, in certain applications very high loading capacity.
HiTrap Q HP	BioProcess	17-1153-01 17-1154-01	5 × 1 ml 5 × 5 ml	4 ml/min 20 ml/min	HSA (M, 68 000) 50 mg/ml	Small scale, high resolution separation of sample.
HiTrap SP HP	BioProcess	17-1151-01 17-1152-01	5 × 1 ml 5 × 5 ml	4 ml/min 20 ml/min	Ribonuclease A (M, 13 700) 55 mg/ml	Small scale, high resolution separation of sample.
HiTrap Capto™ Q	BioProcess	11-0013-02 11-0013-03	5 × 1 ml 5 × 5 ml	4 ml/min 20 ml/min	Minimum 100 mg BSA/ml (10% breakthrough, 1 min residence time)	Fast separation of sample, ideal for scale-up, high binding capacity at high flow rate.
HiTrap Capto ViralQ	BioProcess	28-9078-09	5 × 5 ml	20 ml/min	Minimum 100 mg BSA/ml (10% breakthrough, 1 min residence time)	Fast separation of sample, ideal for scaling-up, high binding capacity at high flow rate. HiTrap Capto ViralQ is in all respects identical to HiTrap Capto Q, but in addition it provides a license to be used for virus purification as described in the section "License statement".
HiTrap Capto DEAE	BioProcess	28-9165-37 28-9165-40	5 × 1 ml 5 × 5 ml	4 ml/min 20 ml/min	Ovalbumin (M, 67 000) > 90 mg	Fast separation of sample, ideal for scaling-up, high binding capacity at high flow rate.
HiTrap Capto S	BioProcess	17-5441-22 17-5441-23	5 × 1 ml 5 × 5 ml	4 ml/min 20 ml/min	Minimum 120 mg lysozyme/ml medium (10% breakthrough, 1 min residence time)	Fast separation of sample, ideal for scale-up, high binding capacity at high flow rate.
HiTrap Capto MMC	BioProcess	11-0032-73 11-0032-75	5 × 1 ml 5 × 5 ml	4 ml/min 20 ml/min	BSA (M_r 67 000) >45 mg at 30 mS/cm	Fast separation of sample, ideal for scale-up, high binding capacity at high flow rate. Capto MMC is a multimodel cation exchanger that gives different selectivity compared with traditional ion exchangers.
HiTrap Capto adhere	BioProcess	28-4058-44 28-4058-46	5 × 1 ml 5 × 5 ml	4 ml/min 20 ml/min	Not available	Fast separation of sample, ideal for scaling-up, high binding capacity at high flow rate. HiTrap Capto adhere is a multimodal anion exchanger that gives different selectivity compared with traditional ion exchangers.
HIC						
HiTrap HIC Selection Kit	BioProcess	11-0034-53	7 × 1 ml columns HiTrap Phenyl FF (high sub), HiTrap Phenyl FF (low sub), HiTrap Phenyl HP, HiTrap Octyl FF, HiTrap Butyl FF, HiTrap Butyl-S FF, HiTrap Butyl HP	4 ml/min	Ligand densities as listed below	Media selection, method scouting.
HiTrap Phenyl FF (high sub)	BioProcess	17-1355-01 17-5193-01	5 × 1 ml 5 × 5 ml	4 ml/min 20 ml/min	40 µmol/ml	Small scale, fast separation of sample, ideal for scale-up.
HiTrap Phenyl FF (low sub)	BioProcess	17-1353-01 17-5194-01	5 × 1 ml 5 × 5 ml	4 ml/min 20 ml/min	25 µmol/ml	Small scale, fast separation of sample, ideal for scale-up.
HiTrap Phenyl HP	BioProcess	17-1351-01 17-5195-01	5 × 1 ml 5 × 5 ml	4 ml/min 20 ml/min	25 µmol/ml	Small scale, high resolution separation of sample.
HiTrap Butyl FF	BioProcess	17-1357-01 17-5197-01	5 × 1 ml 5 × 5 ml	4 ml/min 20 ml/min	40 µmol/ml	Small scale, fast separation of sample, ideal for scale-up.
HiTrap Butyl-S FF	BioProcess	17-0978-13 17-0978-14	5 × 1 ml 5 × 5 ml	4 ml/min 20 ml/min	10 µmol/ml	Small scale, fast separation of sample, ideal for scale-up.
HiTrap Butyl HP	BioProcess	28-4110-01 28-4110-05	5 × 1 ml 5 × 5 ml	4 ml/min 20 ml/min	25 µmol/ml	Small scale, fast separation of sample, ideal for scale-up.
HiTrap Octyl FF	BioProcess	17-1359-01 17-5196-01	5 × 1 ml 5 × 5 ml	4 ml/min 20 ml/min	5 µmol/ml	Small scale, high resolution separation of sample.
Desalting						
HiTrap Desalting	BioProcess	17-1408-01 11-0003-29	5 × 5 ml 100 × 5 ml*	15 ml/min	Sample loading capacity: 1.5 ml (non-binding technique)	Separation by size (biomolecules M_r > 5 000).

Affinity
IEX
HIC
GF

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).

Elanders Östervåla 2007

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