cytiva

HisTrap FF, 1mL and 5mL

Instructions for Use

HisTrap™ FF is a ready to use HiTrap™ column, prepacked with precharged Ni Sepharose™ 6 Fast Flow. This prepacked column is ideal for preparative purification of histidine-tagged recombinant proteins by immobilized metal ion affinity chromatography (IMAC).

HisTrap FF columns provide fast, simple, and easy separations in a convenient format, including an excellent start for scaling up.

Ni Sepharose 6 Fast Flow has low nickel ion (Ni^{2+}) leakage and is compatible with a wide range of additives used in protein purification.

HisTrap FF columns can be operated with a syringe, peristaltic pump, or liquid chromatography system such as ÄKTA™.

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Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

1 Product description

HiTrap column characteristics

The columns are made of biocompatible polypropylene that does not interact with biomolecules.

The columns are delivered with a stopper at the inlet and a snap-off end at the outlet. Table 1 lists the characteristics of HiTrap columns.

Fig 1. HiTrap, 1 mL column.

Fig 2. HiTrap, 5 mL column

Note: *HiTrap columns cannot be opened or refilled.*

Note: *Make sure that the connector is tight to prevent leakage.*

Table 1. Characteristics of HiTrap columns

Column volume (CV)	1 mL	5 mL
Column dimensions	0.7 × 2.5 cm	1.6 x 2.5 cm
Column hardware pressure limit	5 bar (0.5 MPa)	5 bar (0.5 MPa)

Note: *The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography medium, sample/liquid viscosity and the column tubing used.*

Supplied Connector kit with HiTrap column

Connectors supplied	Usage	No. supplied
Union 1/16" male/ luer female	For connection of syringe to HiTrap column	1
Stop plug female, 1/16"	For sealing bottom of HiTrap column	2, 5 or 7

Chromatography medium properties

HisTrap FF 1 ml and 5 ml columns are prepacked with Ni Sepharose 6 Fast Flow, which consists of 90 µm highly cross-linked agarose beads with an immobilized chelating group. The medium has been charged with Ni^{2+}-ions.

Several amino acids, for example histidine, form complexes with many metal ions. Ni Sepharose 6 Fast Flow selectively binds proteins if suitable complex-forming amino acid residues are exposed on the protein surface. Additional histidines, such as a histidine-tag, increase affinity for Ni^{2+} and generally make the histidine-tagged protein the strongest binder among other proteins in e.g., an *E. coli* extract.

Table 2. HisTrap FF characteristics

Matrix	Highly cross-linked spherical agarose, 6%
Average bead size	90 µm
Metal ion capacity	~ 15 µmol Ni^{2+}/ml medium
Dynamic binding capacity[1]	Approx. 40 mg $(histidine)_6$-tagged protein/ml medium
Recommended flow rate	1 ml/min and 5 ml/min for 1 ml and 5 ml column, respectively
Max. flow rates[2]	4 ml/min and 20 ml/min for 1 ml and 5 ml column, respectively.
Compatibility during use	Stable in all commonly used buffers, reducing agents, denaturants, and detergents. See *Table 3, on page 6*.
Chemical stability[3]	0.01 M HCl, 0.1 M NaOH; Tested for one week at 40°C. 1 M NaOH, 70% acetic acid; Tested for 12 h. 2% SDS; Tested for 1 h. 30% 2-propanol; Tested for 30 min.
Avoid in buffers	Chelating agents, e.g., EDTA, EGTA, citrate (see *Table 3, on page 6*)
pH stability[3]	
short term (at least 2 h)	2 to 14
long term (< 1 week)[	3 to 12
Storage	20% ethanol
Storage temperature	4°C to 30°C

1 Dynamic binding capacity conditions:

Sample:	1 mg/ml $(histidine)_6$-tagged pure protein (M_r 43 000) in binding buffer ($Q_{B\,10\%}$ determination) or $(histidine)_6$-tagged protein M_r 28 000) bound from *E. coli* extract
Column volume:	0.25 ml or 1 ml
Flow rate:	0.25 ml/min or 1 ml/min
Binding buffer:	20 mM sodium phosphate, 0.5 M NaCl, 5 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 0.5 M NaCl, 0.5 M imidazole, pH 7.4

Note: *Dynamic binding capacity is protein-dependent.*

2 H_2O at room temperature. For calculation of pressure limits, see *Chapter 9 Adjusting pressure limits in chromatography system software, on page 18*.

3 Ni^{2+}-stripped medium.

The Ni^{2+}-charged medium is compatible with all commonly used aqueous buffers, reducing agents, denaturants such as 6 M Gua-HCl and 8 M urea, and a range of other additives (see *Table 3, on page 6*).

Table 3. Ni Sepharose 6 Fast Flow is compatible with the following compounds at the concentrations given

Reducing agents[1]	5 mM DTE
	5 mM DTT
	20 mM β-mercaptoethanol
	5 mM TCEP
	10 mM reduced glutathione
Denaturing agents[2]	8 M urea
	6 M Gua-HCl
Detergents	2% Trition™ X-100 (nonionic)
	2% Tween™ 20 (nonionic)
	2% NP-40 (nonionic)
	2% cholate (anionic)
	1% CHAPS (zwitterionic)
Other additives	500 mM imidazole
	20% ethanol
	50% glycerol
	100 mM Na_2SO_4
	1.5 M NaCl
	1 mM EDTA[3]
	60 mM citrate

Buffer	50 mM sodium phosphate, pH 7.4
	100 mM Tris-HCl, pH 7.4
	100 mM Tris-acetate, pH 7.4
	100 mM HEPES, pH 7.4
	100 mM MOPS, pH 7.4
	100 mM sodium acetate, pH 4[2]

[1] Ni Sepharose 6 Fast Flow is compatible with reducing agents. However, for optimal performance, removal of any weakly bound Ni^{2+} ions by performing a blank run without reducing agents (as described in Section *Purification*) before applying buffer/sample including reducing agents is recommended. Do not leave HisTrap FF columns with buffers including reducing agents when not in use.

[2] Tested for one week at 40°C.

[3] The strong chelator EDTA has been used successfully in some cases at 1 mM. Generally, chelating agents should be used with caution (and only in the sample, not in the buffers). Any metal-ion stripping may be counteracted by addition of a small excess of $MgCl_2$ before centrifugation/filtration of the sample. Note that stripping effects may vary with applied sample volume.

2 General considerations

Introduction

This chapter describes important information that should be considered when using HisTrap FF in order to achieve the best results. The actions for minimizing nickel leakage and discoloring are normally not needed but can be performed for sensitive applications.

Imidazole concentration

The recommended binding buffer is:

- 20 mM sodium phosphate, 500 mM NaCl, **20 to 40 mM imidazole**, pH 7.4

The imidazole concentration in sample and binding buffer can be further increased if there is a need for higher final purity. If, on the other hand, there is a need for higher yield the imidazole concentration can be lowered (this may result in lower final purity).

Minimize nickel-ion leakage

- Leakage of Ni-ions from HisTrap FF is very low under all normal conditions. For applications where extremely low leakage during purification is critical, leakage can be diminished by performing a blank run.
- Use binding and elution buffers without reducing agents.

Step	Action
1	Wash the column/chromatography medium with 5 column volumes of distilled water.
2	Wash with 5 column volumes of elution buffer.
3	Equilibrate with 10 column volumes of binding buffer.

Reduce discoloring when reducing agents are used

HisTrap FF is compatible with reducing agents as listed in *Table 3, on page 6*. Discoloring is always seen when using high concentrations of reducing agents. In most cases this does not affect the performance of the chromatography medium. To minimize the discoloring, perform a blank run using buffers without reducing as described above before the purification.

Table 4. Prepacked columns for desalting and buffer exchange

Column	Loading volume	Elution volume
HiPrep 26/10 Desalting[1]	2.5 to 15 mL	7.5 to 20 mL
HiTrap Desalting[2]	0.25 to 1.5 mL	1.0 to 2.0 mL
PD-10 Desalting[3]	1.0 to 2.5 mL[4]	3.5 mL
	1.75 to 2.5 mL[5]	Up to 2.5 mL
PD MiniTrap™ G-25	0.1 to 2.5 mL[4]	1.0 mL
	0.2 to 0.5 mL[5]	Up to 0.5 mL
PD MidiTrap™ G-25	0.5 to 1 mL[4]	1.5 mL
	0.75 to 1 mL[5]	Up to 1 mL

1 Prepacked with Sephadex™ G-25 Fine and requires a pump or a chromatography system to run.

2 Prepacked with Sephadex G-25 Superfine and requires a syringe or pump to run.

3 Prepacked with Sephadex G-25 and can be run by the gravity flow or centrifugation.

4 Volumes with gravity elution.

5 Volumes with centrifugation.

3 Operation

Buffer preparation

Water and chemicals used for buffer preparation should be of high purity. Filter buffers through a 0.22 µm or a 0.45 µm filter before use.

Use high purity imidazole as this will give very low or no absorbance at 280 nm.

Recommended buffers

Binding buffer:	20 mM sodium phosphate, 0.5 M NaCl, 20–40 mM imidazole, pH 7.4 (The optimal imidazole concentration is protein dependent; 20–40 mM is suitable for many proteins.)
Elution buffer:	20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4 (The imidazole concentration required for elution is protein dependent).

If the recombinant histidine-tagged protein is expressed as inclusion bodies, include 6 M Gua-HCl or 8 M urea in all buffers and sample. On-column refolding of the denatured protein may be possible.

Note: *When using high concentrations of urea or Gua-HCl, protein unfolding generally takes place. Refolding on-column (or after elution) is protein-dependent.*

Tip: *Samples containing urea can be analyzed directly by SDS-PAGE whereas samples containing Gua-HCl must be buffer-exchanged to a buffer with urea before SDS-PAGE.*

Sample preparation

For optimal growth, induction, and cell lysis conditions, please refer to established protocols.

Adjust the sample to the composition and pH of the binding buffer by:

- Adding buffer, NaCl, imidazole, and additives from concentrated stock solutions,
- by diluting the sample with binding buffer, or
- by buffer exchange, (see *Table 4, on page 9*).

Do not use strong bases or acids for pH-adjustment (precipitation risk). Filter the sample through a 0.22 µm or a 0.45 µm filter and/or centrifuge it immediately before applying it to the column.

To prevent the binding of host cell proteins with exposed histidine, it is essential to include imidazole at a low concentration in the sample and binding buffer (see *Chapter 5 Optimization, on page 13*).

4 Purification

Step	Action
1	Fill the syringe or pump tubing with distilled water.
2	Remove the stopper and connect the column to the syringe (use the adapter provided), laboratory pump or chromatography system tubing "drop-to-drop" to avoid introducing air into the system.
3	Remove the snap-off end at the column outlet.
4	Wash the column with 3 to 5 column volumes of distilled water.
5	Equilibrate the column with at least 5 column volumes of binding buffer. Recommended flow rates are 1 ml/min or 5 ml/min for the 1 and 5 ml columns respectively. In some cases a blank run is recommended before final equilibration/sample application (see instructions below).

Step	Action
6	Apply the pretreated sample using a syringe or a pump.
7	Wash with binding buffer until the absorbance reaches a steady baseline (generally, at least 10 to 15 column volumes). ***Note:*** *Purification results are improved by using imidazole in sample and binding buffer (see Chapter 5 Optimization, on page 13).*
8	Elute with elution buffer using a one-step or linear gradient. For step elution, five column volumes of elution buffer is usually sufficient. A shallow gradient, e.g., a linear gradient over 20 column volumes or more, may separate proteins with similar binding strengths.

Note: *If imidazole needs to be removed from the protein, use HiTrap Desalting, a PD-10 Desalting Column, or HiPrep 26/10 Desalting depending on the sample volume (see Table 4, on page 9).*

Note: *Ni Sepharose 6 Fast Flow is compatible with reducing agents. See Table 3, on page 6. However, removal of any weakly bound Ni^{2+} ions by performing a blank run without reducing agents (as described below) before applying buffer/sample including reducing agents is recommended. Do not leave HisTrap FF columns with buffers including reducing agents when not in use.*

Note: *Leakage of Ni^{2+} from Ni Sepharose 6 Fast Flow is low under all normal conditions. For very critical applications, leakage during purification can be even further diminished by performing a blank run (as described below) before loading sample.*

Blank run:

Use binding buffer and elution buffer **without** reducing agents.

Step	Action
1	Wash the column with 5 column volumes of distilled water (to remove the 20% ethanol).
2	Wash with 5 column volumes of elution buffer.
3	Equilibrate with 10 column volumes of binding buffer.

5 Optimization

Concentration of imidazole

Imidazole at low concentrations is commonly used in the binding and the wash buffers to minimize binding of host cell proteins. For the same reason, it is important to also include imidazole in the sample (generally, at the same concentration as in the wash buffer). At somewhat higher concentrations, imidazole may also decrease the binding of histidine-tagged proteins.

The imidazole concentration must therefore be optimized to ensure the best balance of high purity (low binding of host cell proteins), and high yield (strong binding of histidine-tagged target protein). This optimal concentration is different for different histidine-tagged proteins, and is usually slightly higher for Ni Sepharose 6 Fast Flow than for similar IMAC media on the market.

Finding the optimal imidazole concentration for a specific histidine-tagged protein is a trial-and-error effort, but 20 to 40 mM in the binding and wash buffers is a good starting point for many proteins. Use a high purity imidazole, such imidazole gives essentially no absorbance at 280 nm.

Choice of metal ion

Ni^{2+} is usually the first choice metal ion for purifying most histidine-tagged recombinant proteins from non-tagged host cell proteins, and also the ion most generally used. The strength of binding between a protein and a metal ion is affected by several factors, including the length, position, and exposure of the affinity tag on the protein, the type of ion used, and the pH of buffers, so some proteins may be easier to purify with ions other than Ni^{2+}.

A quick and efficient way to test this possibility and optimize separation conditions is to use HiTrap Chelating HP 1 ml columns, which are packed with Chelating Sepharose High Performance (not charged with metal ions). Each column can be charged with different metal ions, e.g., Cu^{2+}, Co^{2+}, Zn^{2+}, Ca^{2+}, or Fe^{2+}. Instructions are included with each column.

A study to compare the purification of six (histidine)$_6$-tagged recombinant proteins, including three variants of maltose-binding protein, with different metal ions has indicated that Ni^{2+} generally gives best selectivity between histidine-tagged and non-tagged host-cell proteins (see Application Note 18114518).

6 Stripping and recharging

Note: *The column does not have to be stripped and recharged between each purification if the same protein is going to be purified; it is sufficient to strip and recharge it after approximately five purifications, depending on the cell extract, extract volume, target protein, etc.*

Stripping

Recommended stripping buffer: 20 mM sodium phosphate, 0.5 M NaCl, 50 mM EDTA, pH 7.4

Strip the column by washing with at least 5 to 10 column volumes of stripping buffer. Wash with at least 5 to 10 column volumes of binding buffer and 5 to 10 column volumes of distilled water before recharging the column.

Recharging

Recharge the water-washed column by loading 0.5 ml or 2.5 ml of 0.1 M $NiSO_4$ in distilled water on HisTrap FF 1 ml and 5 ml column, respectively. Salts of other metals, chlorides, or sulfates, may also be used (see *Chapter 5 Optimization, on page 13*). Wash with 5 column volumes of distilled water, and 5 column volumes of binding buffer (to adjust pH) before storage in 20% ethanol.

7 Cleaning-in-place

When an increase in back pressure is seen, the column should be cleaned. Before cleaning, strip off Ni^{2+} ions using the recommended procedure described in *Chapter 6 Stripping and recharging, on page 15* .

After cleaning, store in 20% ethanol (wash with 5 column volumes) or recharge with Ni^{2+} prior to storage in ethanol.

The Ni^{2+}-stripped column can be cleaned by the following Cleaning-in-place (CIP) protocols:

CIP protocols

Ionically bound proteins	Wash with several column volumes of 1.5 M NaCl; then wash with approx. 10 column volumes of distilled water.
Precipitated proteins, hydrophobically bound proteins, and lipoproteins	Wash the column with 1 M NaOH, contact time usually 1 to 2 hours (12 hours or more for endotoxin removal). Then wash with approx. 10 column volumes of binding buffer, followed by 5 to 10 column volumes of distilled water.
Hydrophobically bound proteins, lipoproteins, and lipids	Wash with 5 to 10 column volumes of 30% iso-propanol for about 15 to 20 minutes. Then wash with approx. 10 column volumes of distilled water. Alternatively, wash with 2 column volumes of detergent in a basic or acidic solution. Use, for example, 0.1 to 0.5% nonionic detergent in 0.1 M acetic acid, contact time 1 to 2 hours. After treatment, always remove residual detergent by washing with at least 5 column volumes of 70% ethanol[1]. Then wash with approx. 10 column volumes of distilled water.

[1] Specific regulations may apply when using 70% ethanol since the use of explosion proof areas and equipment may be required.

8 Scaling up

Two or three HisTrap FF 1 ml or 5 ml columns can be connected in series for quick scale-up (note that back pressure will increase). Use HisPrep™ FF 16/10 (20 ml) prepacked column if further scale-up is necessary.

Ni Sepharose 6 Fast Flow, the medium prepacked in HisTrap FF and HisPrep FF 16/10 columns, is supplied preswollen in 25, 100, and 500 ml lab packs (see *Chapter 12 Ordering information, on page 25*). An alternative scaleup strategy is thus to pack the medium in empty columns. Tricorn™ and XK columns are suitable for this purpose.

9 Adjusting pressure limits in chromatography system software

Pressure generated by the flow through a column affects the packed bed and the column hardware, see Figure below. Increased pressure is generated when running/using one or a combination of the following conditions:

- High flow rates
- Buffers or sample with high viscosity
- Low temperature
- A flow restrictor

Note: *Exceeding the flow limit (see Table 2) may damage the column.*

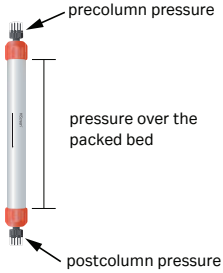

Fig 3. Pre-column and post-column measurements.

ÄKTA avant and ÄKTA pure

The system will automatically monitor the pressures (pre-column pressure and pressure over the packed bed, Δp). The pre-column pressure limit is the column hardware pressure limit (see Table 1).

The maximum pressure the packed bed can withstand depends on media characteristics and sample/liquid viscosity. The measured value also depends on the tubing used to connect the column to the instrument.

ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC and other systems with pressure sensor in the pump

To obtain optimal functionality, the pressure limit in the software may be adjusted according to the following procedure:

Step	Action
1	Replace the column with a piece of tubing. Run the pump at the maximum intended flow rate. Note the pressure as *total system pressure*, P1.
2	Disconnect the tubing and run the pump at the same flow rate used in step 1. Note that there will be a drip from the column valve. Note this pressure as P2.
3	Calculate the new pressure limit as a sum of P2 and the column hardware pressure limit (see Table 1). Replace the pressure limit in the software with the calculated value. The actual pressure over the packed bed (Δp) will during run be equal to actual measured pressure - *total system pressure* (P1).

Note: *Repeat the procedure each time the parameters are changed.*

10 Storage

Store HisTrap FF columns in 20% ethanol at 4°C to 30°C.

11 Troubleshooting

The following tips may be of assistance. If you have any further questions about your HisTrap FF column, please visit *http://cytiva.com/hitrap*, contact our technical support, or your local Cytiva representative.

Column has clogged

- Cell debris in the sample may clog the column. Clean the column according to the *Chapter 7 Cleaning-in-place, on page 16*.
- It is important to centrifuge and/or filter the sample through a 0.22 µm or a 0.45 µm filter, see Sub section *Sample preparation, on page 10*.

Sample is too viscous

- The lysate is very viscous due to a high concentration of host nucleic acid, continue sonication until the viscosity is reduced, and/or add DNase I to 5 µg/ml, Mg^{2+} to 1 mM, and incubate on ice for 10 to 15 min.

  Alternatively, draw the lysate through a syringe needle several times.

Protein is difficult to dissolve or precipitates during purification

- **The following additives may be used:**

  - 2% Trition X-10
  - 2% Tween 20
  - 2% NP-40
  - 2% cholate
  - 1% CHAPS
  - 1.5 M NaCl
  - 50% glycerol
  - 20 mM ß-mercaptoethanol
  - 1.3 mM DTT or DTE (up to 5 mM is possible but depends on the sample and the sample volume)
  - 5 mM TCEP
  - 10 mM reduced glutathione
  - 8 M urea, or
  - 6 M Gua-HCl

Mix gently for 30 min to aid solubilization of the tagged protein (inclusion bodies may require longer mixing). Note that Trition X-100 and NP-40 (but not Tween) have a high absorbance at 280 nm. Furthermore, detergents cannot be easily removed by buffer exchange.

No histidine-tagged protein in the purified fractions

- **Elution conditions are too mild (histidine-tagged protein still bound):** Elute with an increasing imidazole gradient or decreasing pH to determine the optimal elution conditions.
- **Protein has precipitated in the column:** For the next experiment, decrease amount of sample, or decrease protein concentration by eluting with linear imidazole gradient instead of imidazole steps. Try detergents or changed NaCl concentration, or elute under denaturing (unfolding) conditions (use 4 to 8 M urea or 4 to 6 M Gua-HCl).
- **Nonspecific hydrophobic or other interaction:** Add a nonionic detergent to the elution buffer (e.g., 0.2% Trition X-100) or increase the NaCl concentration.
- **Concentration of imidazole in the sample and/or binding buffer is too high:** The protein is found in the flowthrough material. Decrease the imidazole concentration.
- **Histidine-tag may be insufficiently exposed:** The protein is found in the flowthrough material; perform purification of unfolded protein in urea or Gua-HCl as for inclusion bodies. To minimize dilution of the sample, solid urea or Gua-HCl can be added.

- **Buffer/sample composition is incorrect:** The protein is found in the flowthrough material. Check pH and composition of sample and binding buffer. Ensure that chelating or strong reducing agents are not present in the sample at too high concentration, and that the concentration of imidazole is not too high.

Histidine-tagged protein found in the pellet

SDS-PAGE of samples collected during the preparation of the bacterial lysate may indicate that most of histidine-tagged protein is located in the centrifugation pellet. Possible causes and solutions are:

- **Sonication may be insufficient:** Cell disruption may be checked by microscopic examination or monitored by measuring the release of nucleic acids at A_{260}.

  Addition of lysozyme (up to 0.1 volume of a 10 mg/ml lysozyme solution in 25 mM Tris-HCl, pH 8.0) prior to sonication may improve results.

  Avoid frothing and overheating as this may denature the target protein. Over-sonication can also lead to copurification of host proteins with the target protein.
- **The protein may be insoluble (inclusion bodies):** The protein can usually be solubilized (and unfolded) from inclusion bodies using common denaturants such as 4 to 6 M Gua-HCl, 4 to 8 M urea, or strong detergents.

  Prepare buffers containing 20 mM sodium phosphate, 8 M urea, or 6 M Gua-HCl, and suitable imidazole concentrations, pH 7.4 to 7.6. Buffers with urea should also include 500 mM NaCl. Use these buffers for sample preparation, as binding buffer and as elution buffer.

For sample preparation and binding buffer, use 10 to 20 mM imidazole or the concentration selected during optimization trials (including urea or Gua-HCl). To minimize dilution of the sample, solid urea or Gua-HCl can be added.

The eluted protein is not pure (multiple bands on SDS polyacrylamide gel)

- **Partial degradation of tagged protein by proteases:** Add protease inhibitors (use EDTA with caution, see *Table 3, on page 6*).
- **Contaminants have high affinity for nickel ions:** Elute with a stepwise or linear imidazole gradient to determine optimal imidazole concentrations to use for binding and for wash; add imidazole to the sample in the same concentration as the binding buffer. Wash before elution with binding buffer containing as high concentration of imidazole as possible, without causing elution of the tagged protein.

  A shallow imidazole gradient (20 column volumes or more), may separate proteins with similar binding strengths. If optimized conditions do not remove contaminants, further purification by ion exchange chromatography (HiTrap Q HP or HiTrap SP HP) and/or gel filtration (Superdex™ Peptide, Superdex 75 or Superdex 200) may be necessary.
- **Contaminants are associated with tagged proteins:** Add detergent and/or reducing agents before sonicating cells. Increase detergent levels (e.g., up to 2% Trition X-100 or 2% Tween 20), or add glycerol (up to 50%) to the wash buffer to disrupt nonspecific interactions.

Histidine-tagged protein is eluted during sample loading/wash

- **Buffer/sample composition is incorrect:** Check pH and composition of sample and binding buffer. Make sure that chelating or strong reducing agents are not present in the sample at a too high concentration, and that the concentration of imidazole is not too high.
- **Histidine-tag is partially obstructed:** Purify under denaturing conditions (use 4 to 8 M urea or 4 to 6 M Gua-HCl).
- **Column capacity is exceeded:** Join two or three HisTrap FF 1 ml columns together or change to a HisTrap FF 5 ml column or a prepacked HisPrep FF 16/10 column (20 ml).

12 Ordering information

Product	No. supplied	Code No.
HisTrap FF	5 × 1 ml	17531901
	100 × 1 ml[1]	17531902
	5 × 5 ml	17525501
	100 × 5 ml[1]	17525502

[1] Pack size available by special order.

Product	No. supplied	Code No.
HisPrep FF 16/10	1 × 20 ml	28936551
Ni Sepharose 6 Fast Flow	25 ml	17531801
	100 ml	17531802
	500 ml	17531803
HiTrap Desalting	1 × 5 ml	29048684
	5 × 5 ml	17140801
	100 × 5 ml[1]	11000329
PD-10 Desalting Column	30	17085101
HiPrep 26/10 Desalting	1 × 53 ml	17508701
	4 × 53 ml	17508702
HisTrap HP	1 × 1 ml	29051021
	5 × 1 ml	17524701
	100 × 1 ml[1]	17524705
	1 × 5 ml	17524801
	5 × 5 ml	17524802
	100 × 5 ml[1]	17524805
HisTrap FF crude	1 × 1 ml	29048631
	5 × 1 ml	11000458
	100 × 1 ml[1]	11000459
	5 × 5 ml	17528601
	100 × 5 ml[1]	17528602

[1] Pack size available by special order.

Accessories	Quantity	Code No.
1/16" male/luer female *(For connection of syringe to top of HiTrap column)*	2	18111251
Tubing connector flangeless/M6 female *(For connection of tubing to bottom of HiTrap column)*	2	18100368
Tubing connector flangeless/M6 male *(For connection of tubing to top of HiTrap column)*	2	18101798
Union 1/16" female/M6 male *(For connection to original FPLC System through bottom of HiTrap column)*	6	18111257
Union M6 female /1/16" male *(For connection to original FPLC System through top of HiTrap column)*	5	18385801
Union luerlock female/M6 female	2	18102712
HiTrap/HiPrep, 1/16" male connector for ÄKTA design	8	28401081
Stop plug female, 1/16" *(For sealing bottom of HiTrap column)*	5	11000464
Fingertight stop plug, 1/16"	5	11000355

Related literature	Code No.
Recombinant Protein Purification Handbook, Principles and Methods	18114275
Affinity Chromatography Handbook, Principles and Methods	18102229
Affinity Chromatography Columns and Media, Selection Guide	18112186
Ni Sepharose and IMAC Sepharose, Selection Guide	28407092
Prepacked chromatography columns for ÄKTA systems, Selection guide	28931778

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