HisTrap™ FF crude, 1 ml and 5 ml

HisTrap FF crude is a ready to use HiTrap™ column, prepacked with precharged Ni Sepharose™ 6 Fast Flow. This prepacked column is intended for purification of histidine-tagged recombinant proteins by immobilized metal affinity chromatography (IMAC). After thorough cell disruption, it is possible to load the unclarified lysate on the column without precentrifugation and filtration of the sample. Extending the duration of the mechanical treatment of the sample to ensure a more complete lysis is recommended.

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. The special design of the column in combination with the medium, provide fast, simple, and convenient purifications. Short purification time generally minimizes deleterious effects, such as degradation and oxidation of sensitive target proteins, and is therefore of great importance.

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

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Please 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, please 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 × 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 crude 1 ml and 5 ml columns are prepacked with the affinity medium Ni Sepharose 6 Fast Flow, which consists of highly cross-linked agarose beads with an immobilized chelating group. The medium has been precharged 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. An added histidine tag increases the affinity for Ni^{2+} and generally makes the histidine-tagged protein the strongest binder among other proteins in samples such as *E. coli* lysates.

Table 2. HisTrap FF crude 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*.
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*)
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 (not prepacked) or 1 ml
Flow rate:	0.25 ml/min or 1 ml/min, for 0.25 ml or 1 ml column, respectively
Binding buffer:	20 mM sodium phosphate, 500 mM NaCl, 5 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4

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

2 H_2O at room temperature. For calculation of pressure limits, see *Section 7 Adjusting pressure limits in chromatography system software, on page 16*.

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*).

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% Triton™ 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[3]
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 crude 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 crude 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, **20-40 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 crude 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 crude is compatible with reducing agents as listed in *Table 3*. 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

Column	Code No.	Loading volume	Elution volume	Comments	Application
HiPrep™ 26/10 Desalting	17-5087-01	2.5 to 15 ml	7.5 to 20 ml	Prepacked with Sephadex™ G-25 Fine. Requires a laboratory pump or a chromatography system to run.	For desalting and buffer exchange of protein extracts ($M_r > 5000$).
HiTrap Desalting	17-1408-01	0.25 to 1.5 ml	1.0 to 2.0 ml	Prepacked with Sephadex G-25 Superfine. Requires a syringe or pump to run.	
PD-10 Desalting	17-0851-01	1.0 to 2.5 ml[1] 1.75 to 2.5 ml[2]	3.5 ml[1] up to 2.5 ml[2]	Prepacked with Sephadex G-25 Medium. Runs by gravity flow or centrifugation	For desalting, buffer exchange, and cleanup of proteins and other large biomolecules ($M_r > 5000$).
PD MiniTrap™ G-25	28-9180-07	0.1 to 0.5 ml[1] 0.2 to 0.5 ml[2]	1.0 ml[1] up to 0.5 ml[2]		
PD MidiTrap™ G-25	28-9180-08	0.5 to 1.0 ml[1] 0.75 to 1.0 ml[2]	1.5 ml[1] up to 1.0 ml[2]		

[1] Volumes with gravity elution
[2] Volumes with centrifugation

3 Preparation

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 and induction, please refer to established protocols.

Recommended four-step protocol for cell lysis

The protocol below has been used successfully in our own laboratories, but other established procedures may also work.

Step	Action
1	**Dilution of cell paste:** Add 5 to 10 ml of binding buffer for each gram of cell paste. To prevent the binding of host cell proteins with exposed histidines, it is essential to include imidazole at a low concentration in the sample and binding buffer (see *Section 5 Optimization of purification performance, on page 14*).
2	**Enzymatic lysis:** 0.2 mg/ml lysozyme, 20 µg/ml DNAse, 1 mM $MgCl_2$, 1 mM Pefabloc™ SC or PMSF (final concentrations). Stir for 30 min at room temperature or 4°C depending on the sensitivity of the target protein.
3	**Mechanical lysis** [1]: Sonication on ice, approx. 10 min or homogenization with a French press or other homogenizer or freeze/thaw, repeated at least five times.
4	**Adjust the pH of the lysate:** Do not use strong bases or acids for pH-adjustment (precipitation risk). Apply the unclarified lysate on the column directly after preparation.

1 Mechanical lysis time may have to be extended compared with standard protocols to secure an optimized lysate for sample loading (to prevent clogging of the column and back pressure problems). Different proteins have different sensitivity to cell lysis and caution has to be taken to avoid frothing and overheating of the sample.

Note: *If the sonicated or homogenized unclarified cell lysate is frozen before use, precipitation and aggregation may increase. New sonication of the lysate can then prevent increased back pressure problems when loading on the column.*

4 Purification

Step	Action
1	Fill the pump tubing or syringe with distilled water.
2	Remove the stopper and connect the column to the chromatography system tubing, syringe (use the connector provided) or laboratory pump "drop-to-drop" to avoid introducing air into the system.
3	Remove the snap-off end at the column outlet.
4	Wash out the ethanol 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 ml and 5 ml columns, respectively. In some cases, a blank run is recommended before final equilibration/ sample application.
6	Apply the unclarified lysate with a pump or a syringe. Continuous stirring of the sample during sample loading is recommended to prevent sedimentation. Typical loading volumes of unclarified lysate (highly dependent on specific sample, sample pretreatment, and temperature at sample loading) **HisTrap FF crude 1 ml:** Up to 100 ml **HisTrap FF crude 5 ml**: Up to 500 ml ***Note:*** *Sample loading at 4°C may increase the viscosity of the sample. An adverse effect of increased sample viscosity is that maximum back pressure for the column is reached at a lower sample volume loading on the column. Do not exceed the binding capacity of the column. Large volumes may increase back pressure, making the use of a syringe more difficult*.

Step	Action
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* *Section 5 Optimization of purification performance, on page 14**).*
8	Elute with elution buffer using a one-step procedure or a 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, can separate proteins with similar binding strengths.

Note: *Unclarified lysates may cause increased air bubble formation during purification. An attached flow restrictor in the chromatography system can prevent this if it causes problems. If a flow restrictor is attached, it is important to change the pressure limit to 0.5 MPa (5 bar) on the ÄKTA system (where the column and the flow restrictor give a pressure of 0.3 MPa and 0.2 MPa, respectively).*

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

Note: *Ni Sepharose 6 Fast Flow is compatible with reducing agents. See Table 3. However, for optimal performance 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 crude 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 reduced by performing a blank run (as described below) before loading the sample.*

Performing a blank run

Use binding and elution buffers **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.

Note: *If the column has clogged it may be possible to perform cleaning-in-place, see* *Section 8 Column cleaning and storage, on page 19**. If cleaning-in-place is unsuccessful, replace the column. Optimize sample pretreatment before the next sample loading.*

5 Optimization of purification performance

Concentration of imidazole

Imidazole at low concentrations is commonly used in the binding and the wash buffers to minimize binding of host cell proteins. Imidazole is also included in the sample (generally at the same concentration as in the wash buffer) to further minimize binding of host cell proteins. 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
(see Data File 11-0008-86).

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 high purity imidazole, which gives essentially no absorbance at 280 nm.

Choice of metal ion

Ni^{2+} is usually the first choice metal ion for purifying most $(histidine)_6$-tagged recombinant proteins from 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+}.

Stripping and recharging

Note: *The column does not have to be stripped and recharged between each purification if the same protein is to be purified. It may be sufficient to strip and recharge it after approximately two to five purifications, depending on the specific sample, sample pretreatment, sample volume, etc.*

Stripping

Recommended stripping buffer: 20 mM sodium phosphate, 500 mM 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 onto the HisTrap FF crude 1 ml and 5 ml columns, respectively. Wash with 5 column volumes of distilled water, and 5 column volumes of binding buffer (to adjust pH) before storage in 20% ethanol. Salts of other metals, chlorides, or sulfates, may also be used.

6 Scaling up

Scaling up from a HisTrap FF crude 1 ml column to a 5 ml column while keeping the same linear flow rate provides highly consistent results.

7 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.*

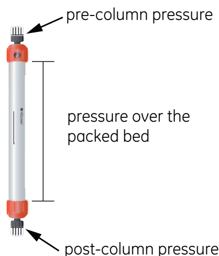

Fig 3. Pre-column and post-column measurements.

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.

Step	Action
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.*

8 Column cleaning and storage

Cleaning-in-place (CIP)

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 above.

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 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 h (12 h 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% isopropanol for about 15 to 20 min. 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 h. 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

Note: *Reversed flow may improve the efficiency of the cleaning-in-place procedure.*

9 Storage

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

10 Troubleshooting

The following tips may be of assistance. If you have any further questions about your HisTrap FF crude column, please visit www.gelifesciences.com/hitrap, contact our technical support, or your local GE representative.

Increased back pressure

- Increase the efficiency of the mechanical cell disruption (for example increase sonication time). Keep the sample on ice to avoid frothing and overheating as this may denature the target protein.

  Over-sonication can also lead to co-purification of host proteins with the target protein.
- Increase dilution of the cell paste before sonication or dilute after the sonication to reduce viscosity.
- If the lysate is very viscous due to a high concentration of host nucleic acid, continue sonication until the viscosity is reduced, and/or add an additional dose of DNAse and Mg^{2+} (see *Section 3 Preparation, on page 10*,).

  Alternatively, draw the lysate through a syringe needle several times.
- Freeze/thaw of the unclarified lysate may increase precipitation and aggregation. Sonication of the thawed lysate can prevent increased back pressure problems when loading on the column.
- If the purification has been performed at 4°C, move to room temperature if possible (sample viscosity is reduced at room temperature).
- Decrease flow rate during sample loading.

Column has clogged

- If cleaning-in-place is unsuccessful, replace the column. Optimize sample pretreatment before the next sample loading.

Protein is difficult to dissolve or precipitates during purification

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

  - 2% Triton 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 Triton X-100 and NP-40 (but not Tween) have a high absorbance at 280 nm. Furthermore, detergents cannot be easily removed by buffer exchange.

- **The protein might 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 well 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.

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:** Decrease amount of sample, or decrease protein concentration by eluting with linear imidazole gradient instead of imidazole steps. Try detergents or change 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% Triton X- 100) or increase the NaCl concentration.
- **Protein found in the flowthrough:** Concentration of imidazole in the sample and/or binding buffer is too high; decrease imidazole concentration.
- **Protein found in the flowthrough:** Histidine tag may be insufficiently exposed; 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.
- **Protein found in the flowthrough:** Buffer/sample composition is not optimal; check pH and composition of sample and binding buffer. Ensure that the concentration of chelating or strong reducing agents in the sample as well as the concentration of imidazole is not too high.

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*).

- 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 thoroughly before elution with binding buffer containing the highest possible imidazole concentration (chosen imidazole concentration must not cause elution of the histidine-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% Triton 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 not optimal:** Check pH and composition of sample and binding buffer. Make sure that the concentration of chelating or strong reducing agents in the sample, as well as 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:** If HisTrap FF crude 1 ml columns have been used, change to a larger column, HisTrap FF crude 5 ml.

Unwanted air bubble formation

- Unclarified lysates may cause increased air bubble formation during purification. An attached flow restrictor in the chromatography system can prevent this.

11 Ordering information

Product	No. Supplied	Code No.
HisTrap FF crude	1 × 1 ml	29-0486-31
	5 × 1 ml	11-0004-58
	100 × 1 ml [1]	11-0004-59
	5 × 5 ml	17-5286-01
	100 × 5 ml [1]	17-5286-02

[1] Pack size available by special order.

Related products	No. Supplied	Code No.
HisTrap FF crude kit	3 × 1 ml + buffers	28-4014-77
HisTrap FF	5 × 1 ml	17-5319-01
	100 × 1 ml [1]	17-5319-02
	5 × 5 ml	17-5255-01
	100 × 5 ml [1]	17-5255-02
HisPrep™ FF 16/10	1 × 20 ml	28-9365-51
Ni Sepharose 6 Fast Flow	5 ml	17-5318-06
	25 ml	17-5318-01
	100 ml	17-5318-02
	500 ml	17-5318-03
HiTrap Desalting	1 × 5 ml	29-0486-84
	5 × 5 ml	17-1408-01
	100 × 5 ml [1]	11-0003-29
PD-10 Desalting Column	30	17-0851-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02

[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	18-1112-51
Tubing connector flangeless/M6 female *(For connection of tubing to bottom of HiTrap column)*	2	18-1003-68
Tubing connector flangeless/M6 male *(For connection of tubing to top of HiTrap column)*	2	18-1017-98
Union 1/16” female/M6 male *(For connection to original FPLC System through bottom of HiTrap column)*	6	18-1112-57
Union M6 female /1/16” male *(For connection to original FPLC System through top of HiTrap column)*	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep, 1/16" male connector for ÄKTA design	8	28-4010-81
Stop plug female, 1/16” *(For sealing bottom of HiTrap column)*	5	11-0004-64
Fingertight stop plug, 1/16"	5	11-0003-55

Related literature	Code No.
Recombinant Protein Purification Handbook, Principles and Methods	18-1142-75
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
Affinity Chromatography Columns and Media, Selection Guide	18-1121-86
Ni Sepharose and IMAC Sepharose, Selection Guide	28-4070-92
Prepacked chromatography columns for ÄKTA systems, Selection guide	28-9317-78

Appendix A Preparing phosphate buffers with imidazole

Phosphate buffer (containing imidazole for binding and elution buffers) (*20 mM sodium phosphate, 500 mM NaCl, 20 to 500 mM imidazole in 1 l*).

To 1.78 g $Na_2HPO_4 \times 2H_2O$ (177.99 g/mol), 1.38 g $NaH_2PO_4 \times H_2O$ (137.99 g/mol) and 29.22 g NaCl (58.44 g/mol) and X g imidazole (depending on the chosen imidazole binding and elution concentrations, see Table below) add distilled water to 900 ml and dissolve completely.

Adjust pH from basic to 7.4 with HCl. Add distilled water to 1000 ml and filter through a 0.45-µm filter.

This gives a final concentration of 20 mM sodium phosphate, 500 mM NaCl, 10–500 mM imidazole, pH 7.4.

Use high purity imidazole as this will give very low or no absorbance at 280 nm (imidazole, 68.08 g/mol).

Imidazole concentration in buffer (mM)	Weight of imidazole in Phosphate buffer (g)
10	1.4
30	2.0
40	2.7
50	3.4
60	4.1
70	4.8
80	5.4
90	6.1
100	6.8
200	13.6
300	20.4
400	27.2
500	34.0

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