GE Healthcare

Instructions 71-5027-68 AF **HisTrap affinity columns**

HisTrap HP, 1 ml and 5 ml

HisTrap™ HP is a ready to use column, prepacked with precharged Ni Sepharose™ High Performance. This prepacked column is ideal for preparative purification of Histidine-tagged recombinant proteins by immobilized metal ion affinity chromatography (IMAC).

The special design of the column, together with the high-performance matrix of the Ni Sepharose medium, provides fast, simple, and easy separations in a convenient format.

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

HP columns can be operated with a syringe, peristaltic pump, or liquid chromatography system such as ÄKTAdesign™ chromatography systems or FPLC™ System.

Caution!

Contains nickel. May produce an allergic reaction.

HisTrap

Code No.	Product	No. supplied
17-5247-01	HisTrap HP 1 ml	5 × 1 ml
17-5247-05	HisTrap HP 1 ml	100 × 1 ml*
17-5248-01	HisTrap HP 5 ml	1 × 5 ml
17-5248-02	HisTrap HP 5 ml	5 × 5 ml
17-5248-05	HisTrap HP 5 ml	100 × 5 ml*

* Pack size available by special order.

Connector kit

Connectors supplied	Usage	No. supplied
1/16" male/luer female	Connection of syringe to top of HiTrap column	1
Tubing connector flangeless/M6 female	Connection of tubing (e.g. Peristaltic Pump P1) to bottom of HiTrap column*	1
Tubing connector flangeless/M6 male	Connection of tubing (e.g. Peristaltic Pump P1) to top of HiTrap column**	1
Union 1/16" female/ M6 male	Connection to original FPLC System through bottom of HiTrap column	1
Union M6 female/ 1/16" male	Connection to original FPLC System through top of HiTrap column	1
Stop plug female, 1/16"	Sealing bottom of HiTrap column	2, 5 or 7

* Union 1/16" female/M6 male is also needed.
** Union M6 female/1/16" male is also needed.

Table of contents

1. Description

Medium properties

HisTrap HP 1 ml and 5 ml columns are prepacked with Ni Sepharose High Performance, which consists of 34 µm highly cross-linked agarose beads with an immobilized chelating group. The medium has then been charged with Ni^{2+}-ions.

Several amino acids, for example histidine, form complexes with many metal ions. Ni Sepharose High Performance selectively binds proteins if suitable complex-forming amino acid residues are exposed on the protein surface. Additional histidines, such as in the case of $(histidine)_6$ -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.

Column properties

HisTrap HP columns are made of biocompatible polypropylene that does not interact with biomolecules. Columns are delivered with a stopper on the inlet and a snap-off end on the outlet. The columns have porous top and bottom frits that allow high flow rates. They cannot be opened or refilled.

Columns can be operated with either a syringe and the supplied luer adapter, a peristaltic pump, or a chromatography system such as ÄKTAdesign or FPLC System.

Note: To prevent leakage, ensure that the adapter is tight.

Table 1. HisTrap HP characteristics.

Matrix	Highly cross-linked spherical agarose, 6%
Average particle size	34 µm
Metal ion capacity	~15 µmol Ni^{2+}/ml medium
Dynamic binding capacity*	At least 40 mg $(histidine)_6$-tagged protein/ml medium
Column volumes	1 ml or 5 ml
Column dimensions	i.d. × H: 0.7 × 2.5 cm (1 ml) 1.6 × 2.5 cm (5 ml)
Recommended flow rate	1 and 5 ml/min for 1 and 5 ml column respectively
Max. flow rates	4 and 20 ml/min for 1 and 5 ml column respectively
Max back pressure††	0.3 MPa, 3 bar
Compatibility during use	Stable in all commonly used buffers, reducing agents, denaturants, and detergents. See Table 2.
Chemical stability§§§	0.01 M HCl, 0.1 M NaOH. Tested for 1 week at 40 °C. 1 M NaOH, 70% acetic acid. Tested for 12 hours. 2% SDS. Tested for 1 hour. 30% 2-propanol. Tested for 30 min.
Avoid in buffers	Chelating agents, e.g. EDTA, EGTA, citrate (see Table 2)
pH stability§§§	short term (≤ 2 hours): 2–14 long term (≤ 1 week): 3–12
Storage	20% ethanol
Storage temperature	+4 to +30 °C

* Dynamic binding capacity conditions:

Sample:	1 mg/ml $(histidine)_6$-tagged pure protein (M_r 28 000 or 43 000) in binding buffer (QB, 10% determination) or $(histidine)_6$-tagged protein 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.

†† H_2O at room temperature

§§§ 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 2).

Table 2. Ni Sepharose High Performance is compatible with the following compounds, at least at the concentrations given.

Reducing agents*	5 mM DTE
	5 mM DTT
	20 mM ß-mercaptoethanol
	5 mM TCEP
	10 mM reduced glutathione
Denaturing agents	8 M urea[††]
	6 M guanidine hydrochloride[††]
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[§§§]
	60 mM citrate[§§§]
Buffer substances	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[††]

* See Notes and blank run, p. 10–11.

†† Tested for 1 week at +40 °C.

§§§ 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 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

HisTrap HP is supplied precharged with Ni^{2+} ions. In general, Ni^{2+} is the preferred metal ion for purification of recombinant histidine-tagged proteins. Note, however, that in some cases it may be wise to test other metal ions, e.g. Zn^{2+} and Co^{2+}, as the strength of binding depends on the nature of the histidine-tagged protein as well as the metal ion (see Optimization).

We recommend binding at neutral to slightly alkaline pH (pH 7–8) in the presence of 0.5–1.0 M NaCl. Sodium phosphate buffers are often used. Tris-HCl can generally be used, but should be avoided in cases where the metal-protein affinity is very weak, since it may reduce binding strength. Avoid chelating agents such as EDTA or citrate in buffers, see Table 2.

Including salt e.g. 0.5–1.0 M NaCl in the buffers and samples, eliminates ion-exchange effects but can also have a marginal effect on the retention of proteins.

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 (binding of histidine-tagged target protein). This optimal concentration is different for different histidine-tagged proteins, and is usually slightly higher for Ni Sepharose High Performance than for similar IMAC media on the market (see Data File 18-1174-40). Use highly pure imidazole; such imidazole gives essentially no absorbance at 280 nm.

As alternatives to imidazole elution, histidine-tagged proteins can be eluted from the medium by several other methods or combinations of methods – a lowering of pH within the range of 2.5–7.5 can be used, for example. At pH values below 4, metal ions will be stripped off the medium.

Note: If the proteins are sensitive to low pH, we recommend collection of the eluted fractions in tubes containing 1 M Tris-HCl, pH 9.0 (60–200 µl/ml fraction) to restore the pH to neutral.

Chelating agents such as EGTA or EDTA will also strip metal ions from the medium and thereby cause protein elution, but the target protein pool will then contain Ni^{2+} ions. In this case, Ni^{2+} ions can be removed by desalting on a HiTrap™ Desalting, a PD-10 Desalting Column, or HiPrep™ 26/10 Desalting, (see Table 4).

Leakage of Ni^{2+} from Ni Sepharose High Performance is very low under all normal conditions, **lower than for other IMAC media tested.** For applications where extremely low leakage during purification is critical, leakage can be even further reduced by performing a blank run (see page 11).
Likewise, a blank run should also be performed before applying buffers/ samples containing reducing agents (see page 11).

Whatever conditions are chosen, HisTrap HP columns can be operated with a syringe, peristaltic pump, or chromatography system.

Note: If Peristaltic Pump P-1 is used, the maximum flow rate that can be run on a HisTrap HP 1 ml column is 3 ml/min.

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.

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.

Recommended conditions

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).

Sample preparation

For optimal growth, induction, and cell lysis conditions for your recombinant histidine-tagged clones, 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). 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 Optimization).

Table 4. Prepacked columns for desalting and buffer exchange.

Code No	Column	Loading volume	Elution volume	Comments	Application
17-1408-01	HiTrap Desalting	0.1–1.5 ml	1.3–4.0 ml	Prepacked with Sephadex™ G-25 Superfine. Requires a syringe or pump to run.	For desalting and buffer exchange of protein extracts (M_r>5000).
17-5087-01	HiPrep 26/10 Desalting	Up to 15 ml	15–20 ml	Prepacked with Sephadex G-25 Fine. Requires a pump to run.	For desalting and buffer exchange of protein extracts (M_r>5000).
17-0851-01	PD-10 Desalting	2.5 ml	3.5 ml	Prepacked with Sephadex G-25. Requires only gravity to run.	For desalting and buffer exchange of protein extracts (M_r>5000).
17-0855-01	NICK™	0.1 ml	0.4 ml	Prepacked with Sephadex G-25.	For separation of proteins (M_r>5000) and nicktranslated DNA from radiolabelled nucleotides not shorter than 120 mers, and similar separations.
17-0853-01 17-0854-01 17-0852-01	NAP™-5 NAP-10 NAP-25	0.5 ml 1.0 ml 2.5 ml	1.0 ml 1.5 ml 3.5 ml	Prepacked with Sephadex G-25 DNA grade. Requires only gravity to run.	For purification of proteins (M_r>5000), DNA and oligo-nucleotides greater than 10 bases in length.

Purification

1. Fill the syringe or pump tubing with distilled water. 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.
2. Remove the snap-off end at the column outlet.
3. Wash the column with 3–5 column volumes of distilled water.
4. 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 page 11).
5. Apply the pretreated sample using a syringe or a pump.
6. Wash with binding buffer until the absorbance reaches a steady baseline (generally, at least 10–15 column volumes).

Note: Purification results are improved by using imidazole in sample and binding buffer (see Optimization).

7. Elute with elution buffer using a one-step or linear gradient.
   Five column volumes are usually sufficient if the protein of interest is eluted by a one-step gradient. 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).

Note: Ni Sepharose High Performance is compatible with reducing agents. However, removal of any weakly bound Ni^{2+} ions by performing a blank run without reducing agents (as described on page 11) before applying buffer/sample including reducing agents is recommended. Do not leave HisTrap HP columns with buffers including reducing agents when not in use.

Note: Leakage of Ni^{2+} from Ni Sepharose High Performance is low under all normal conditions. The leakage is lower than for other IMAC media tested (see Data File Ni Sepharose High Performance, 18-1174-40). 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.

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.

4. Optimization

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 (binding of histidine-tagged target protein). This optimal concentration is different for different histidine-tagged proteins, and is usually slightly higher for Ni Sepharose High Performance than for similar IMAC media on the market (see Data File Ni Sepharose High Performance, 18-1174-40). Finding the optimal imidazole concentration for a specific histidine-tagged protein is a trial-and-error effort, but 20–40 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.

Ni^{2+} is usually the first choice metal ion for purifying most $(histidine)_6$-tagged recombinant proteins from nontagged host cell proteins, and also the ion most generally used. Nevertheless, it is not always possible to predict which

metal ion will be best for a given protein. 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 IMAC HP 1 ml columns, which are packed with IMAC 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 nontagged host-cell proteins (see Application Note 18-1145-18).

5. 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 5–7 purifications, depending on the cell extract, extract volume, target protein, etc.

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 5–10 column volumes of stripping buffer. Wash with at least 5–10 column volumes of binding buffer and 5–10 column volumes of distilled water before recharging the column.

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 HP 1 ml and 5 ml column, respectively. Salts of other metals, chlorides, or sulfates, may also be used (see Optimization). Wash with 5 column volumes distilled water, and 5 column volumes binding buffer (to adjust pH) before storage in 20% ethanol.

6. Cleaning-in-place

When an increase in back-pressure is seen, the column can 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 methods;

- Remove ionically bound proteins by washing the column with several column volumes of 1.5 M NaCl; then wash the column with approx. 10 column volumes of distilled water.
- Remove precipitated proteins, hydrophobically bound proteins, and lipoproteins by washing the column with 1 M NaOH, contact time usually 1–2 hours (12 hours or more for endotoxin removal). Then wash the column with approx. 10 column volumes of binding buffer, followed by 5–10 column volumes of distilled water.
- Remove hydrophobically bound proteins, lipoproteins, and lipids by washing the column with 5–10 column volumes 30% isopropanol for about 15–20 minutes. Then wash the column with approx. 10 column volumes of distilled water.

  Alternatively, wash the column with 2 column volumes of detergent in a basic or acidic solution. Use, for example, 0.1–0.5% nonionic detergent in 0.1 M acetic acid, contact time 1–2 hours. After treatment, always remove residual detergent by washing with at least 5 column volumes of 70% ethanol. Then wash the column with approx. 10 column volumes of distilled water.

7. Scaling-up

Two or three HisTrap HP 1 ml or 5 ml columns can be connected in series for quick scale-up (note that back-pressure will increase).

Ni Sepharose High Performance, the medium prepacked in HisTrap HP columns, is supplied preswollen in 25 and 100 ml lab packs (see ordering information). An alternative scale-up strategy is thus to pack the medium in empty columns – Tricorn™ and XK columns are suitable for this purpose.

8. Storage

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

9. Troubleshooting

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

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

Tips: To minimize dilution of the sample, solid urea or Gua-HCl can be added.

Tips: 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.

Column has clogged:

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

Sample is too viscous:

- 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 DNase I to 5 µg/ml, Mg^{2+} to 1 mM, and incubate on ice for 10–15 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% Triton™ X-100, 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.

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 (add 4–8 M urea or 4–6 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.
- **Concentration of imidazole in the sample and/or binding buffer is too high:** The protein is found in the flow-through 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.
- **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.

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 4–6 M Gua-HCl, 4–8 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 7.4–7.6. 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 10–20 mM imidazole or the concentration selected during optimization trials (including urea or Gua-HCl).

The protein is collected but 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 2).
- **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 chromotography

(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 incorrect:** Check pH and composition of sample and binding buffer. Ensure 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 4–8 M urea or 4–6 M Gua-HCl).
- **Column capacity is exceeded:** Join two or three HisTrap HP 1 ml columns together or change to a HisTrap HP 5 ml column.

10. Further information

Visit www.gehealthcare.com/hitrap for further information. Several handbooks also contain useful information, see ordering information.

11. Ordering Information

Product	No. supplied	Code No.
HisTrap HP	5 × 1 ml	17-5247-01
HisTrap HP	100 × 1 ml *	17-5247-05
HisTrap HP	1 × 5 ml	17-5248-01
HisTrap HP	5 × 5 ml	17-5248-02
HisTrap HP	100 × 5 ml *	17-5248-05
Related products	**No. supplied**	**Code No.**
Ni Sepharose High Performance	25 ml	17-5268-01
Ni Sepharose High Performance	100 ml	17-5268-02
HiTrap Desalting	5 × 5 ml	17-1408-01
HiTrap Desalting	100 × 5 ml *	11-0003-29
PD-10 Desalting Column	30	17-0851-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
HiPrep 26/10 Desalting	4 × 53 ml	17-5087-02
HisTrap FF	5 × 1 ml	17-5319-01
HisTrap FF	100 × 1 ml *	17-5319-02
HisTrap FF	5 × 5 ml	17-5255-01
HisTrap FF	100 × 5 ml *	17-5255-02
HisTrap FF crude	5 × 1 ml	11-0004-58
HisTrap FF crude	100 × 1 ml *	11-0004-59
HisTrap FF crude	5 × 5 ml	17-5286-01
HisTrap FF crude	100 × 5 ml *	17-5286-02
HisTrap FF crude Kit	1 kit	28-4014-77
HisPrep™ FF 16/10	1 × 20 ml	17-5256-01

* Pack size available by special order.

Accessories	No. Supplied	Code No.
1/16" male/luer female*	2	18-1112-51
Tubing connector flangeless/M6 female*	2	18-1003-68
Tubing connector flangeless/M6 male*	2	18-1017-98
Union 1/16" female/M6 male*	6	18-1112-57
Union M6 female /1/16" male*	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep, 1/16" male connector for ÄKTAdesign	8	28-4010-81
Stop plug female, 1/16"†	5	11-0004-64
Fingertight stop plug, 1/16"‡	5	11-0003-55

* One connector included in each HiTrap package.

† Two, five, or seven stop plugs female included in HiTrap packages depending on the product.

‡ One fingertight stop plug is connected to the top of each HiTrap column at delivery.

Literature

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
Convenient Protein Purification, HiTrap Column Guide	18-1129-81

www.gehealthcare.com/hitrap

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

GE Healthcare Europe GmbH
Munzinger Strasse 5
D-79111 Freiburg
Germany

GE Healthcare UK Ltd
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
UK

GE Healthcare Bio-Sciences Corp
800 Centennial Avenue
P.O. Box 1327
Piscataway, NJ 08855-1327
USA

GE Healthcare Bio-Sciences KK
Sanken Bldg.
3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan

Licensing information
Purification and preparation of fusion proteins and affinity peptides comprising at least two adjacent histidine residues may require a license under US pat 5,284,933 and US pat 5,310,663, including corresponding foreign patents (assigne: Hoffman La Roche, Inc). The Tricorn column and components are protected by US design patents USD500856, USD506261, USD500555, USD495060 and their equivalents in other countries.

imagination at work

71-5027-68 AF 11/2006

Elanders Östervåla 2006