Ni Sepharose™ High Performance

Immobilized metal ion affinity chromatography (IMAC) exploits the interaction between chelated transition metal ions and side-chains of certain amino acids (mainly histidine) on proteins. In general, Ni^{2+} is the preferred metal ion for purification of histidine-tagged proteins. The IMAC medium Ni Sepharose High Performance consists of 34 µm beads of highly cross-linked agarose, to which a chelating group has been coupled. This chelating group has then been charged with nickel (Ni^{2+}) ions.

Ni Sepharose High Performance has low Ni^{2+} leakage, high protein-binding capacity and stability, and is compatible with a wide range of additives used in protein purification. This make Ni Sepharose High Performance the first-choice medium for high performance purification of histidine-tagged proteins.

Ni Sepharose High Performance is available in 25 and 100 ml lab packs and prepacked in:

- HisTrap™ HP 1 and 5 ml columns
- His MultiTrap™ HP 96-well filter plate
- His SpinTrap™ 100 µl single-use spin column

Table of Contents

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

Ni Sepharose High Performance is highly stable and compatible with a wide range of common additives. This helps to maintain biological activity and increase product yield, while at the same time greatly expanding the range of suitable operating conditions.

In addition, the medium is easy to pack and use.

The key characteristics of the medium are listed in *Table 1*.

A variety of compounds that are compatible with Ni Sepharose High Performance are listed in *Table 2*.

Table 1. Medium characteristics

Matrix	Highly cross-linked spherical agarose, 6%
Metal ion capacity	~ 15 µmol Ni^{2+}/ml medium
Average particle size	34 µm
Dynamic binding capacity [1]	At least 40 mg $(histidine)_6$-tagged protein/ml medium
Max. linear flow rate [2]	300 cm/h
Recommended flow rate[2]	< 150 cm/h
Max. operating pressure[2]	0.3 MPa, 3 bar
Chemical stability (for medium without metal ion)	Stable in: 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.
pH stability [3] (for medium without metal ion)	
Working range	3 to 12
Cleaning-in-place	2 to 14
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 28 000 or 43 000) in binding buffer (capacity at 10% breakthrough) 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, respectively
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, 500 mM imidazole, pH 7.4

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

[2] H_2O at room temperature

[3] Working range: pH interval where the medium can be handled without significant change in function.

Cleaning-in-place: pH interval where the medium can be subjected to cleaning-in-place without significant change in function.

Table 2. Ni Sepharose High Performance is compatible with the following compounds up to 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
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 High Performance 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 *Blank run, on page 12*.) before applying buffer/sample including reducing agents is recommended. Do not leave Ni Sepharose High Performance 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 section describes important information that should be considered when using Ni Sepharose High Performance 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 Ni Sepharose High Performance 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. See Section *Blank run, on page 12*.
- Use binding and elution buffers without reducing agents.

Reduce discoloring when reducing agents are used

Ni Sepharose High Performance is compatible with reducing agents as listed in *Table 2*. 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 agents before the purification. See Section *Blank run, on page 12*.

Table 3. 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 Column packing

Ni Sepharose High Performance is supplied preswollen in 20% ethanol. Prepare a slurry by decanting the 20% ethanol solution and replacing it with distilled water in a ratio of 75% settled medium to 25% distilled water.

Table 4. Recommended lab-scale columns forNi Sepharose High Performance

Empty Column [1]	Packing flow rate (ml/min) First step	Second step	Max. recommended flow rate for chromatography (ml/min)
Tricorn™ 5/20	0.5	1	0.5
Tricorn 5/50	0.5	1	0.5
Tricorn 10/20	2	4	2
Tricorn 10/50	2	4	2
Tricorn 10/100	2	4	2
XK 16/20	5	10	5
XK 26/20	13	27	13

[1] For inner diameter and maximum bed volumes and bed heights, see *Section 12 Ordering information, on page 22*.

Packing a column

Step	Action
1	Assemble the column (and packing reservoir if necessary).
2	Remove air from the end-piece and adapter by flushing with water. Make sure no air has been trapped under the column bed support. Close the column outlet leaving the bed support covered with water.
3	Resuspend the medium and pour the slurry into the column in a single continuous motion. Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.

Step	Action
4	If using a packing reservoir, immediately fill the remainder of the column and reservoir with water. Mount the adapter or lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.
5	Open the bottom outlet of the column and set the pump to run at the desired flow rate. Ideally, Sepharose High Performance media are packed in XK or Tricorn columns in a two-step procedure: • Do not exceed 1.0 bar (0.1 MPa) in the first step and 3.5 bar (0.35 MPa) in the second step. • If the packing equipment does not include a pressure gauge, use a packing flow rate of 5 ml/min (XK 16/20 column) or 2 ml/min (Tricorn 10/100 column) in the first step, and 9 ml/min (XK 16/20 column) or 3.6 ml/min (Tricorn 10/100 column) in the second step. See *Table 4* for packing flow rates for other columns. If the recommended pressure or flow rate cannot be obtained, use the maximum flow rate your pump can deliver. This should also give a wellpacked bed. ***Note:*** *For subsequent chromatography procedures, do not exceed 75% of the packing flow rate. See Table 4 for flow rates for chromatography.*
6	Maintain packing flow rate for at least 3 bed volumes after a constant bed height is reached. Mark the bed height on the column.
7	Stop the pump and close the column outlet.
8	If using a packing reservoir, disconnect the reservoir and fit the adapter to the column.
9	With the adapter inlet disconnected, push the adapter down into the column until it reaches the mark. Allow the packing solution to flush the adapter inlet. Lock the adapter in position.

Step	Action
10	Connect the column to a pump or a chromatography system and start equilibration. Re-adjust the adapter if necessary.

4 Preparation before purification

General recommendations

We recommend binding at neutral to slightly alkaline pH (pH 7 to 8) in the presence of 0.5 to 1.0 M NaCl. Sodium phosphate buffers are often used. Tris- HCl can generally be used, but should be avoided in cases where the metalprotein affinity is very weak, since Tris may reduce binding strength. Addition of salt, for example 0.5 to 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.

Avoid chelating agents such as EDTA or citrate in buffers, see *Table 2*.

If the recombinant histidine-tagged proteins are expressed as inclusion bodies, include up to 6 M Gua-HCl or 8 M urea in all buffers. 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.*

As an alternative to imidazole elution, histidine-tagged proteins can be eluted from the medium by several other methods or combinations of methods. Lowering pH within the range 7.5 to 2.5 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 collecting the pH-eluted fractions in tubes containing 1 M Tris-HCl, pH 9.0 (60–200 µl/ml fraction) to restore the pH to neutral. (Do not use NaOH).*

Chelating agents such as EGTA and EDTA can be used for elution. They cause protein elution by stripping the metal ions from the medium. The target protein pool will then include Ni^{2+} ions. In this case, the Ni^{2+} ions can be removed by using a desalting column. See *Table 3*.

Elution with ammonium chloride or histidine has also been reported.

Imidazole concentration in binding buffer

The purity of recombinant histidine-tagged proteins can often be increased by washing with binding buffer containing as high a concentration of imidazole as possible. However, care must be taken not to use a wash concentration of imidazole that causes elution of the histidine-tagged protein.

To obtain highest purity, first determine the optimal concentration of imidazole for sample loading, washing and elution. This can be done by eluting with a linear or stepwise gradient of imidazole from 20 to 500 mM, and testing fractions by SDS-PAGE and/or Western blotting for the presence of target protein and impurities.
See *Section 6 Optimization, on page 14*.

When maximum binding and yield of the histidine-tagged protein (rather than purity) is the main objective, choose a low imidazole concentration for binding and wash, even if that concentration (in some cases) may lead to suboptimal purity.

Buffer preparation

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

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

Recommended buffers

Binding buffer: 20 mM sodium phosphate, 0.5 M NaCl, 20 to 40 mM imidazole, pH 7.4 (The optimal imidazole concentration is protein-dependent; 20 to 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).

Ni^{2+} leakage

Leakage of Ni^{2+} from Ni Sepharose High Performance is low under all normal conditions. For very critical applications, leakage during purification can be even further decreased 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.
2	Wash with 5 column volumes of elution buffer.
3	Equilibrate with 10 column volumes of binding buffer.

Sample preparation

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

The sample should be fully dissolved. To avoid column clogging, we recommend centrifugation and filtration through a 0.45 µm or 0.22 µm filter to remove cell debris or other particulate material.

If the sample is dissolved in a buffer other than 20 mM phosphate buffer with 0.5 M NaCl pH 7.4, adjust its NaCl concentration to 0.5 M and pH to 7 to 8. This can be achieved by addition of concentrated stock solutions, by dilution with the binding buffer, or by buffer exchange (on HiTrap™ Desalting, PD-10 Desalting Column, or HiPrep 26/10 Desalting, depending on the sample volume). Do not use strong bases or acids for pH-adjustments (precipitation risk).

To prevent binding of host cell proteins with exposed histidine, add the same concentration of imidazole to the sample as to the binding buffer.

5 Purification

Note: *Please read the sections Section 2 General considerations, on page 6 and Section 4 Preparation before purification, on page 10 before performing the purification!*

Procedure

Step	Action
1	If the column contains 20% ethanol, wash it with 5 column volumes of distilled water. Use a linear flow rate of 50 to 100 cm/h.
2	Equilibrate the column with 5 to 10 column volumes of binding buffer. Recommended linear flow rate: 150 cm/h. In some cases, a blank run is recommended before final equilibration/ sample application (see Section *Blank run, on page 12*).
3	Apply the pretreated sample.
4	Wash with binding buffer until the absorbance reaches the baseline.
5	Elute with elution buffer using a step or linear gradient. • For step elution, 5 column volumes of elution buffer are usually sufficient. • For linear gradient, a shallow gradient over 20 column volumes, may separate proteins with similar binding strengths.

Note: *Use the elution buffer as blank when measuring absorbance manually. If imidazole needs to be removed from the protein, use HiTrap Desalting, a PD-10 Desalting Column, or HiPrep 26/10 Desalting. See Table 3.*

6 Optimization

Concentration of imidazole in binding/wash buffer

Imidazole at low concentrations is commonly used in the binding and wash buffers to minimize binding of unwanted host cell proteins. It is important to include imidazole also 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 can therefore be optimized to ensure the best balance of high purity (low binding of unwanted proteins), and high yield (binding of all of the histidine-tagged protein). This optimal concentration is different for different histidine-tagged proteins. Note that Ni Sepharose High Performance often requires a slightly higher concentration of imidazole in the wash buffer than 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. Prepacked HisTrap HP columns (1 or 5 ml) are ideal for optimization.

Choice of metal ion

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

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+}.

Prepacked HiTrap Chelating HP and HiTrap IMAC HP columns or IMAC Sepharose High Performance (not metal-ion charged) can be used to test this possibility. These products can be charged with different metal ions, e.g, Cu^{2+}, Co^{2+}, Zn^{2+}, Ca^{2+}, Ni^{2+} or Fe^{3+}.

7 Troubleshooting

The following tips may be of assistance. If you have any further questions about Ni Sepharose High Performance, please visit www.gelifesciences.com/protein-purificaton or contact our technical support, or your local GE representative.

Column has clogged

- Cell debris in the sample may clog the column. Clean the column according to the *Section 9 Cleaning-in-Place (CIP), on page 20*.
- Centrifuge and/or filter the sample through a 0.22 µm or a 0.45 µm filter, see *Sample preparation, on page 12*.

Sample is too viscous

- If the lysate is very viscous due to 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 minutes. Alternatively, draw the lysate through a syringe needle several times.

Protein is difficult to dissolve or precipitates during purification

- **See *Table 2* for reducing agents, detergents, glycerol and denaturing agents that may be used.**

  Mix gently for 30 minutes to aid solubilization of the tagged protein (inclusion bodies may require much 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.

Histidine-tagged protein found in the pellet

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

- **Sonication may be insufficient:** Check cell disruption by microscopic examination or monitor 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. Oversonication can also lead to co-purification 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. Use these buffers for sample preparation, as binding buffer, and as elution buffer. For sample preparation and binding buffer, use 10 mM imidazole or the concentration selected during optimization trials (including urea or Gua-HCl). To minimize sample dilution, solid urea or Gua-HCl can be added.

Histidine-tagged protein is found in the flowthrough and purified fractions

- **Capacity of Ni Sepharose High Performance is exceeded:** Increase the volume of Ni Sepharose High Performance used for your purification.

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.
- **The protein has precipitated in the column:** 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) to remove precipitated proteins. For the next experiment, decrease amount of sample, or decrease protein concentration by eluting with a linear imidazole gradient instead of imidazole steps.
- **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 flowthrough material. Decrease the imidazole concentration.
- **Target protein may not be histidine-tagged as expected:** Verify DNA sequence of the gene. Analyze samples taken before and after induction of expression with, for example, anti-His antibodies in Western blotting.
- **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, solid urea or Gua-HCl can be added to the sample.
- **Buffer/sample composition is incorrect:** The protein is found in the flowthrough material. Check pH and composition of sample and binding buffer. Make sure that the concentration of chelating or strong reducing agents, as well as imidazole, in the solution 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 2*).
- **Contaminants have high affinity for nickel ions:** Optimize imidazole concentration for binding buffer, see *Section 6 Optimization, on page 14*. 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 the cells. Increase the 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.

8 Regenerating the medium

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

Stripping

To remove residual Ni^{2+}, wash with 5 column volumes 20 mM sodium phosphate, 0.5 M NaCl, 50 mM EDTA, pH 7.4. Remove residual EDTA by washing with at least 5 column volumes of binding buffer followed by 5 column volumes of distilled water before recharging the column.

Recharging

To recharge the water-washed column, load 0.5 column volumes of 0.1 M $NiSO_4$ in distilled water. Salts of other metals, chlorides or sulfates, may also be used (see *Section 6 Optimization, on page 14*).

Wash with 5 column volumes of distilled water followed by 5 column volumes of binding buffer (to adjust pH) before storage in 20% ethanol.

In some applications, substances such as denatured proteins or lipids cannot be eluted in the regeneration. These can be removed by Cleaning-in- Place.

9 Cleaning-in-Place (CIP)

When reduced performance or an increase in back-pressure are noted, the column should be cleaned. Before cleaning, strip off the Ni^{2+} ions using the recommended procedure (see *Section 8 Regenerating the medium, on page 19*). Use reversed flow direction for cleaning.

After cleaning, store in 20% ethanol or recharge with Ni^{2+} prior to storage in ethanol.

The Ni^{2+} stripped column can be cleaned by the following protocols:

CIP protocols

Ionically bound proteins	Wash with several column volumes of 1.5 M NaCl. Then wash with several 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 to remove endotoxins). Then wash with approximately 10 column volumes of binding buffer, followed by 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 minutes. Then wash with approximately 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 5 to 10 column volumes of 70% ethanol [1]. Then wash with approximately 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.

10 Storage

Store the medium for longer periods of time in 20% ethanol at 4°C to 30°C.

11 Further information

Check www.gelifesciences.com/protein-purification for further information. Several handbooks also contain useful information, see *Section 12 Ordering information, on page 22*.

12 Ordering information

Product	Quantity	Code No.
Ni Sepharose High Performance [1]	25 ml	17-5268-01
	100 ml	17-5268-02

[1] Larger quantities are available. Please contact GE for more information.

Prepacked columns	Quantity	Code No.
HisTrap HP	1 × 1 ml	29-0510-21
	5 × 1 ml	17-5247-01
	100 × 1 ml [1]	17-5247-02
	1 × 5 ml	17-5248-01
	5 × 5 ml	17-5248-02
	100 × 5 ml[1]	17-5248-05
His MultiTrap HP	4 × 96-well filter plates	28-4009-89
His SpinTrap	50 x 100 µl	28-4013-53

[1] Special pack delivered on specific customer order.

Related products	Quantity	Code No.
IMAC Sepharose High Performance	25 ml	17-0920-05
	100 ml	17-0920-06
HiTrap Chelating HP	5 × 1 ml	17-0408-01
	1 × 5 ml	17-0409-01
HiTrap IMAC HP	5 × 1 ml	17-0921-02
HiTrap IMAC HP	5 × 5 ml	17-0921-04
HiTrap Desalting	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

Empty lab-scale columns	Quantity	Code No.
Tricorn 5/20 column, 5 mm i.d.	1	28-4064-08
Tricorn 5/50 column, 5 mm i.d.	1	28-4064-09
Tricorn 10/20 column, 10 mm i.d.	1	28-4064-13
Tricorn 10/50 column, 10 mm i.d.	1	28-4064-14
Tricorn 10/100 column, 10 mm i.d.	1	28-4064-15
XK 16/20 column, 16 mm i.d.	1	18-8773-01
XK 26/20 column, 26 mm i.d.	1	18-1000-72

Literature	Code No.
Recombinant Protein Purification Handbook, Principles and Methods	18-1142-75
Size Exclusion Chromatography Handbook, Principles and Methods	18-1022-18

For local office contact information, visit
www.gelifesciences.com/contact

GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
United Kingdom

www.gelifesciences.com/protein-purification

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

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

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

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

71-5027-67 AF 03/2015 a19