GE Healthcare

Instructions 28-4020-89 AD **Affinity media**

Ni Sepharose™ 6 Fast Flow

CAUTION!
Contains nickel.
May produce an allergic reaction.

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. Ni Sepharose 6 Fast Flow is a BioProcess™ IMAC medium that consists of 90 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 6 Fast Flow has low Ni^{2+} leakage, high protein-binding capacity, and is compatible with a wide range of additives used in protein purification. Its high flow properties make it excellent for scale-up.

Ni Sepharose 6 Fast Flow is available in 5, 25, 100, 500 ml, 1L, and 5L bulk packs. Ni Sepharose 6 Fast Flow is also available prepacked in 1 and 5-ml HisTrap™ FF, 1 and 5-ml HisTrap FF crude and 20-ml HisPrep™ FF 16/10 columns.

Sepharose

Contents

1. Product description 3
2. General considerations 5
3. Packing columns 6
4. Packing lab-scale columns 7
5. Packing process-scale columns 8
6. Evaluation of column packing 8
7. Preparation before purification 11
8. Purification procedure 13
9. Optimization 14
10. Troubleshooting 15
11. Regenerating the medium 18
12. Cleaning-in-Place (CIP) 19
13. Storage 20
14. Further information 20
15. Ordering information 20

1. Product description

Ni Sepharose 6 Fast Flow 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, and its high flow properties make it excellent for scaling-up. The key characteristics of the medium are listed in Table 1. A variety of compounds that are compatible with Ni Sepharose 6 Fast Flow are listed in Table 2.

Table 1. Medium characteristics

Matrix	Highly cross-linked 6% spherical agarose
Dynamic binding capacity*	Approx. 40 mg histidine-tagged protein/ml medium
Metal ion capacity	Approx. 15 µmol Ni^{2+}/ml medium
Average particle size	90 µm
Max. linear flow rate†	600 cm/h (20 ml/min) using XK 16/20 column with 5 cm bed height
Recommended flow rate†	150 cm/h
Max. operating pressure† columns.	0.1 MPa, 1 bar (when packed in XK columns. May vary if used in other columns)
Chemical stability‡	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‡	Short term (at least2 hours) 2–14 Long term (≤1 week) 3–12
Storage	20% ethanol
Storage temperature	4°C to 30°C

* Dynamic binding capacity conditions:

Sample: 1 mg/ml histidine-tagged pure proteins (Mr 43 000) in binding buffer (capacity at 10% breakthrough) or histidine-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, 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, 0.5 M imidazole, pH 7.4

Note: Dynamic binding capacity is protein-dependent.

† H_2O at room temperature.

‡ Ni^{2+}-stripped medium.

Table 2 Ni Sepharose 6 Fast Flow 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 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 Na2SO4
	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 General considerations.

† Tested for 1 week at 40°C.

‡ The strong chelator EDTA has been used succesfully in some cases at 1 mM. Generally, chelating agents should be used with caution (and only in the sample, not in 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

Ni Sepharose 6 Fast Flow is supplied precharged with Ni^{2+} ions. In general, Ni^{2+} is the preferred metal ion for purification of recombinant histidine-tagged proteins and imidazole is used for elution. Elution using reduced pH (linear or stepwise decrease in pH) is a alternative elution procedure.

Imidazole at low concentrations is commonly used in the binding and wash buffer to minimize binding of unwanted 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 unwanted proteins) and high yield (binding of all of the histidine-tagged protein). The concentration of imidazole that will give optimal purification results is protein-dependent, 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 and Optimization).

Leakage of Ni^{2+} from Ni Sepharose 6 Fast Flow is low under all normal conditions (lower than for other IMAC media tested). For applications where very low leakage during purification is critical, leakage can be diminished even further by performing a blank run or alternatively performing an acidic wash (see Purification procedure, page 12.)

Ni Sepharose 6 Fast Flow is compatible with reducing agents (see Table 2). However, do not leave Ni Sepharose 6 Fast Flow with buffers containing reducing agents when not in use.

3. Packing columns

Ni Sepharose 6 Fast Flow 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 3 Recommended lab-scale columns for Ni Sepharose 6 Fast Flow

Empty Column*	Packing flow rate (ml/min)		Max. recommended flow rate for chromatography (ml/min)
	first step	second step	
Tricorn™ 10/20	0.9	4.7	2
Tricorn 10/50	0.9	4.7	2
Tricorn 10/100	0.9	4.7	2
XK 16/20	2.5	8.7	5
XK 26/20	6.6	23	13
XK 50/20	24.5	85	49
XK 50/30	24.5	85	49

* For inner diameter and maximum bed volumes and bed heights, see Ordering information

Table 4 Recommended process-scale columns for Ni Sepharose 6 Fast Flow

Column	Inner diam (mm)	Bed volume (L)	Bed height max (cm)
BPG™ 100/500	100	up to 2.0 L	26
BPG 140/500	140	up to 4.0 L	26
BPG 200/500	200	up to 8.2 L	26
BPG 300/500	300	up to 18.0 L	26
BPG 450/500	450	up to 36.0 L	23
Chromaflow™ 400/100–300	400	13–37 L	30
Chromaflow 600/100–300	600	28–85 L	30

4. Packing lab-scale columns

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.
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 6 Fast Flow media are packed in XK or Tricorn columns in a two-step procedure: Do not exceed 0.5 bar (0.05 MPa) in the first step and 1.5 bar (0.15 MPa) in the second step. If the packing equipment does not include a pressure gauge, use a packing flow rate of 2.5 ml/ min (XK 16/20 column) or 0.9 ml/min (Tricorn 10/100 column) in the first step, and 8.7 ml/min (XK 16/20 column) or 4.7 ml/ min (Tricorn 10/100 column) in the second step. See Table 3 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 well-packed bed.

Note: For subsequent chromatography procedures, do not exceed 75% of the packing flow rate. See Table 3 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.
10. Connect the column to a pump or a chromatography system and start equilibration. Re-adjust the adapter if necessary.

5. Packing process-scale columns

For general process-scale column packing instructions, please visit support section at www.gelifesciences.com

6. Evaluation of column packing

Test column efficiency to check the quality of the packing. Tests should be made directly after packing and at regular intervals during the working life of the column plus when separation performance is seen to deteriorate. The best method of expressing the efficiency of a packed column is in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (A_s). These values are easily determined by applying a sample such as 1% acetone solution to the column. Sodium chloride can also be used as a test substance. Use a concentration of 2.0 M NaCl in water with 0.5 M NaCl in water as eluent.

Note: The calculated plate number will vary according to the test conditions and it should only be used as a reference value. It is important that test conditions and equipment are kept constant so that results are comparable. Changes of solute, solvent, eluent, sample volume, flow velocity, liquid pathway, temperature, etc. will influence the results.

For optimal results, the sample volume should be at maximum 2.5% of the column volume and the flow velocity between 15 and 30 cm/h. If an acceptance limit is defined in relation to column performance, the column plate number can be used as one of the acceptance criteria for column use.

Method for measuring HETP and A_s

Calculate HETP and A_S from the UV curve (or conductivity curve) as follows:

$$HETP = \frac{L}{N}$$

$$N = 5.54 \times \left(\frac{V_R}{W_h}\right)^2$$

where:

L = Bed height (cm)

N = number of theoretical plates

V_R = volume eluted from the start of sample application to the peak maximum

W_h = peak width measured as the width of the recorded peak at half of the peak height

V_R and W_h are in the same units

The concept of reduced plate height is often used for comparing column performance.

The reduced plate height is calculated as follows:

$$\frac{HETP}{d}$$

where:

d is the diameter of the bead

As a guideline, a value of <3 is normally acceptable.

The peak should be symmetrical, and the asymmetry factor as close to 1 as possible (values between 0.8–1.5 are usually acceptable).

A change in the shape of the peak is usually the first indication of bed deterioration due to use.

Peak asymmetry factor calculation:

$$A_s = \frac{b}{a}$$

where:

a = 1st half peak width at 10% of peak height

b = 2nd half peak width at 10% of peak height.

Figure 1 shows a UV trace for acetone in a typical test chromatogram in which the HETP and A_s values are calculated.

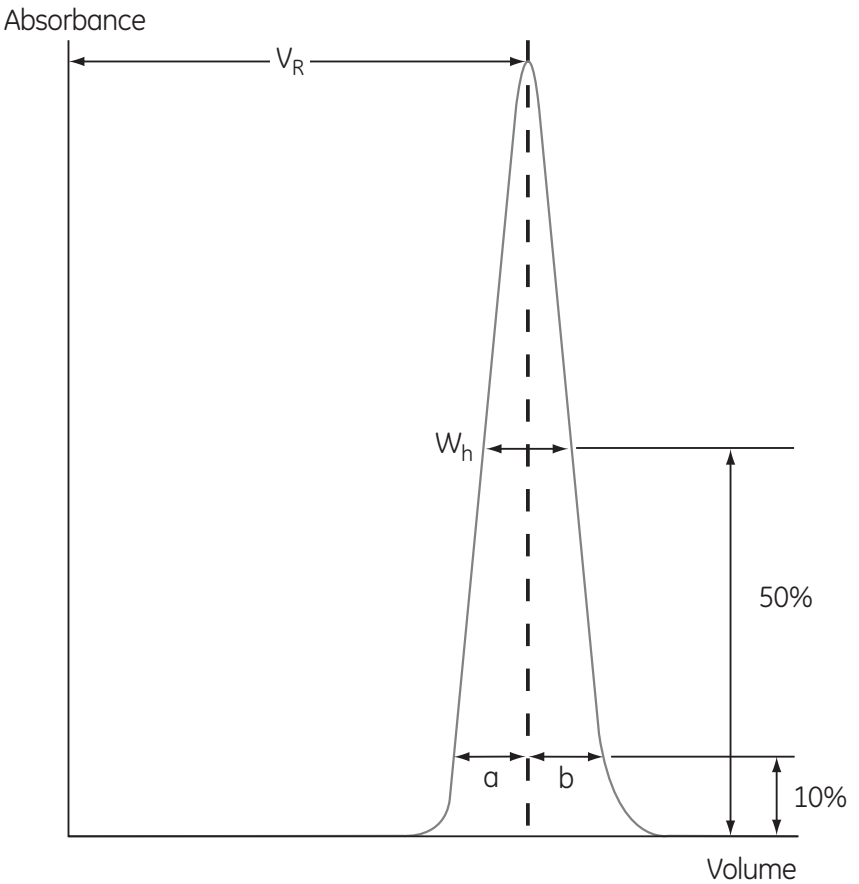

Fig 3. A typical test chromatogram showing the parameters used for HETP an A_s calculations.

7. Preparation before purification

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.

Addition of salt, for example 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.

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.

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.

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 2.5–7.5 can be used, for example. At pH values below 4, metal ions will be stripped off the medium.

EGTA and EDTA can be used for elution. Chelating agents such as EGTA and EDTA cause protein elution by stripping the metal ions from the medium. The target protein pool will then include Ni^{2+} ions.

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 for the presence of target protein and impurities. See Optimization.

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, 20–40 mM imidazole, pH 7.4 (The optimal imidazole concentration is protein-dependent; 20–40 mM is suitable for many histidine-tagged 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, 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 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 7–8. Do not use strong bases or acids for pH-adjustments (precipitation risk). ***Note:*** To prevent binding of unwanted host cell proteins with exposed histidine, add the same concentration of imidazole to the sample as to the binding buffer (see Optimization).

8. Purification procedure

Please read the chapters "General considerations" and "Preparation before purification" before performing the purification

1. If the column contains 20% ethanol, wash it with 2–5 column volumes of distilled water. Use a linear flow rate of 50–100 cm/h.
2. Equilibrate the column with at least 2 column volumes of binding buffer. Recommended linear flow rate: 150 cm/h. In some cases, a blank run or an acidic wash is recommended before final equilibration/sample application (see below).
3. Apply the pre-treated sample (see Sample preparation).
4. Wash with binding buffer until the absorbance reaches the baseline.
5. Elute with elution buffer using a stepwise or linear gradient. In most process applications, it is recommended to use a stepwise gradient.

Leakage of Ni^{2+} from Ni Sepharose 6 Fast Flow is low under all normal conditions. In order to reduce leakage even further it is recommended to perform a blank run or alternatively perform an acidic wash. Such a treatment is intended to remove any weakly bound metal ions that otherwise might be desorbed later, during the elution of the bound protein.

Acidic wash is especially recommended when binding buffer contain no or low concentration of imidazole and in applications when the proteins to be purified are not histidine-tagged. It is important to re-equilibrate the column after the acidic wash or the blank run. This is especially important in the latter case since imidazole (and similar elution agents) has affinity for metal ions and the re-equilibration might be time consuming.

Blank run:

Use buffers without reducing agents.

1 Wash the column with 5 column volumes of distilled water
2. Wash with 5 column volumes of elution buffer.
3. Equilibrate with 5–10 column volumes of binding buffer.

alternatively

Acidic wash:

Use buffers without reducing agents.

1. Wash the column with 5 column volumes of distilled water.
2. Wash the column with 5 column volumes of an acidic buffer (for example0.02 M sodium acetate, 0.5–1.0 M NaCl, pH 4.0).
3. Equilibrate the column with 2–5 column volumes of binding buffer, or until the desired pH is obtained.

9. Optimization

Concentration of imidazole in binding/wash buffer

Imidazole at low concentrations is commonly used in the binding/wash buffer 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 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 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 6 Fast Flow often requires a slightly higher concentration of imidazole in the binding/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 20–40 mM in the binding/wash buffer is a good starting point

for many proteins. Prepacked HisTrap FF 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-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 and position of the affinity tag on the protein, the type of ion used, and the pH of buffers. Some proteins may therefore be easier to purify with ions other than Ni^{2+}, for example Zn^{2+} or Co^{2+}.

Prepacked HiTrap™ IMAC FF columns or IMAC Sepharose 6 Fast Flow (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+}.

A study to compare the purification of six histidine-tagged recombinant proteins, including three variants of histidine-tagged maltose binding protein, with different metal ions has indicated that Ni^{2+} generally gives best selectivity between histidine-tagged proteins and nontagged host cell proteins (see Application Note 18-1145-18).

10. Troubleshooting

The following tips may be of assistance. If you have any further questions about Ni Sepharose 6 Fast Flow, please visit *www.gelifesciences.com* or contact our technical support, or your local GE Healthcare representative.

Column has clogged:

- Cell debris in the sample may clog the column. Clean the column according to the section Cleaning-in-Place.
- 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 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 minutes. 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 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 4–6 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. 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 6 Fast Flow is exceeded:** Increase the volume of Ni Sepharose 6 Fast Flow 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 4–8 M urea or 4–6 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 change 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. Ensure 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:** Elute stepwise or with a linear imidazole gradient to determine the optimal imidazole concentrations to use for binding and washing; add imidazole to the sample to the same concentration as in the binding buffer. Wash before elution with binding buffer containing as high a concentration of imidazole as possible, without causing elution of the the target 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 and/or gel filtration 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), change the NaCl concentration or add glycerol (up to 50%) to the wash buffer to disrupt nonspecific interactions.

11. Regenerating the medium

When performing repeated purification cycles, the need for stripping and recharging is highly dependent on the sample properties, sample volumes, metal ion, etc. To recharge Ni Sepharose 6 Fast Flow, first 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.

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 Optimization).

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.

12. Cleaning-in-Place (CIP)

When an increase in back-pressure is seen, the column should be cleaned. Before cleaning, strip off the Ni^{2+} ions using the recommended procedure (see Regenerating the medium on the previous page). 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 methods:

- Remove ionically bound proteins by washing with several column volumes of 1.5 M NaCl. Then wash with several 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 (longer time may be required to inactivate endotoxins). Then wash with approximately 10 column volumes of binding buffer, followed by 10 column volumes of distilled water.
- Remove hydrophobically bound proteins, lipoproteins and lipids by washing with 5–10 column volumes of 30% isopropanol for about 15–20 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, 0.1–0.5% nonionic detergent in 0.1 M acetic acid, contact time 1–2 hours. After treatment, always remove residual detergent by washing with 5–10 column volumes of 70% ethanol. Then wash with approximately 10 column volumes of distilled water.

13. Storage

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

14. Further information

Check *www.gelifesciences.com* for further information. Several handbooks also contain useful information, see Ordering information.

15. Ordering information

Product	Quantity	Code No.
Ni Sepharose 6 Fast Flow	5 ml	17-5318-06
Ni Sepharose 6 Fast Flow	25 ml	17-5318-01
Ni Sepharose 6 Fast Flow	100 ml	17-5318-02
Ni Sepharose 6 Fast Flow	500 ml	17-5318-03
Ni Sepharose 6 Fast Flow	1L	17-5318-04
Ni Sepharose 6 Fast Flow	5L	17-5318-05

Larger quantities are available. Please contact GE Healthcare for more information.

Prepacked columns	Quantity	Code No.
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	1 × 20 ml	17-5256-01
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

* Special pack delivered on specific customer order.

Empty lab-scale columns	Quantity	Code No.
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
XK 50/20 column, 50 mm i.d.	1	18-1000-71
XK 50/30 column, 50 mm i.d.	1	18-8751-01

Literature	Quantity	Code No.
Recombinant Protein Purification Handbook, Principles and Methods	1	18-1142-75
Affinity Chromatography Handbook, Principles and Methods	1	18-1022-29
Affinity Chromatography Columns and Media Product Profile	1	18-1121-86
Ni Sepharose and IMAC Sepharose Selection Guide	1	28-4073-92
Datafile BPG columns	1	18-1115-23
Datafile BPG 450 columns	1	18-1160-59
Datafile Chromaflow columns	1	18-1138-92

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

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

www.gelifesciences.com/protein-purification

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

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

imagination at work

28-4020-89 AD 01/2010