HisPrep™ FF 16/10

HisPrep FF 16/10 is a ready to use HiPrep™ column, prepacked with pre-charged Ni Sepharose™ 6 Fast Flow. This column is ideal for preparative purification of histidine-tagged recombinant proteins. HisPrep FF 16/10 provides fast, simple, and easy separations in a convenient format and Ni Sepharose 6 Fast Flow, a BioProcess™ medium, is ideal for scaling up.

The column is used in an optimal way with liquid chromatography systems such as ÄKTA™.

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

HiPrep column characteristics

HiPrep columns are made of biocompatible polypropylene that does not interact with biomolecules. The columns are delivered with stoppers at the inlet and outlet. The arrow on the column label indicates the column orientation and the recommended flow direction, see *Figure 1*.

Fig 1. HiPrep 16/10 column

Note: *HiPrep columns cannot be opened or refilled*

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

Table 1. Characteristics of HiPrep FF 16/10 column

Column volume (CV)	20 ml
Column dimensions	16 × 100 mm
Column hardware pressure limit [1]	5 bar (0.5 MPa)

[1] The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography medium and the column tubing used.

Medium and column data

HisPrep FF 16/10 is prepacked with Ni Sepharose 6 Fast Flow, which consists of 90 µm highly cross-linked agarose beads with an immobilized chelating group. The medium has been charged with Ni^{2+}-ions. Several amino acids, for example histidine, form complexes with many metal ions. Ni Sepharose 6 Fast Flow selectively binds proteins if suitable complex-forming amino acid residues are exposed on the protein surface. Additional histidines, such as a histidine-tag, increase affinity for Ni^{2+} and generally make the histidine-tagged protein the strongest binder among other proteins in e.g., an *E. coli* extract.

Table 2. Medium and column characteristics

Medium	Ni Sepharose 6 Fast Flow
Matrix	Highly cross-linked spherical agarose, 6%
Average particle size	90 µm
Metal ion capacity	Approx. 15 µmol Ni^{2+}/ml medium
Dynamic binding capacity [1]	Approx. 40 mg $(histidine)_6$-tagged protein/ml medium
Recommended flow rate	1 to 10 ml/min (30 to 300 cm/h)
Maximum flow rate [2]	10 ml/min (300 cm/h)
Compatibility during use	Stable in all commonly used buffers, reducing agents, denaturants, and detergents. See *Table 3*.
Chemical stability (for medium without metal ion)	1 M NaOH, 70% acetic acid; tested for 12 hours. 2% SDS; tested for 1 hour. 30% 2-propanol; tested for 30 minutes.
pH stability [3] (for medium without metal ion)	
Working range	3 to 12
Cleaning-in-place	2 to 14
Storage	4°C to 30°C in 20% ethanol

[1] Dynamic binding capacity conditions:

Sample:	1 mg/ml $(histidine)_6$-tagged pure protein (M_r 43 000) in binding buffer (at 10% breakthrough) or $(histidine)_6$-tagged protein (M_r 28 000) bound from *E. coli* extract
Column volume:	0.25 ml or 1 ml

Flow rate:	0.25 ml/min or 1 ml/min
Binding buffer:	20 mM sodium phosphate, 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] Water at room temperature. Flow rate is determined by $v \times \eta \leq 10$ ml/min where v = flow rate and η= viscosity.

[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.

2 Buffers and compatibility

Table 3. Ni Sepharose 6 Fast Flow 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 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 *Blank run, on page 10.*) before applying buffer/sample including reducing agents is recommended. Do not leave HisPrep FF 16/10 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.

3 General considerations

Introduction

This section describes important information that should be considered when using HisPrep FF 16/10 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 HisPrep FF 16/10 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 10*.
- Use binding and elution buffers without reducing agents.

Reduce discoloring when reducing agents are used

HisPrep FF 16/10 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 agents before the purification. See Section *Blank run, on page 10*.

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

4 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 to 40 mM imidazole, pH 7.4 (The optimal imidazole concentration is protein dependent; 20 to 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).

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. See Table 4 for column selection.*

Blank run

Note: *Perform a blank run without reducing agents before applying buffers/samples containing reducing agents. Likewise, a blank run is recommended for critical purifications where metal ion leakage during purification must be minimized.*

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

Step	Action
1	Wash the column with 5 column volumes (CV) of distilled water.
2	Wash with 5 CV elution buffer.
3	Equilibrate with 10 CV binding buffer.

Sample preparation

Step	Action
1	Adjust the sample to the composition of the binding buffer, using one of these methods: • Dilute the sample with binding buffer. • Exchange buffer using a desalting column, see *Table 4*.
2	Filter the sample through a 0.45 µm filter or centrifuge immediately before loading it to the column. This prevents clogging and increases the life time of the column when loading large sample volumes.

Note: *To minimize the co-adsorption of unwanted host cell proteins, it is essential to include imidazole at a low concentration in the sample and binding buffer, see* *Section 6 Optimization, on page 12*

5 Purification

Recommended flow rates

Flow rate: 1 to 10 ml/min (30 to 300 cm/h)

Procedure

Step	Action
1	Equilibrate with at least 5 column volumes (CV) of binding buffer. Avoid introducing air into the column. ***Note:*** *To prevent leakage, make sure that the connectors are tight. Use fingertight 1/16" connector (28-4010-81).* ***Note:*** *In some cases, a blank run is recommended before final equilibration/sample application, see Section* *Blank run, on page 10.**.*
2	Adjust the sample to the chosen starting conditions and load on the column.
3	Wash with 5 to 10 CV binding buffer until the UV trace of the effluent returns to near baseline.
4	Elute either by linear gradient elution or a step elution at recommended flow rates. • Linear gradient elution: Elute with 0% to 100% elution buffer (up to 500 mM imidazole) in 10 to 20 CV. • Step elution: Elute with 5 CV elution buffer including imidazole at chosen concentration. If necessary, repeat at higher imidazole concentrations until the target protein has been eluted.

Step	Action
5	Re-equilibrate the column with 5 to 10 CV binding buffer or until the UV baseline, eluent pH, and conductivity reach the required values. ***Note:*** *Do not exceed the maximum recommended flow and/or back pressure for the column.* ***Note:*** *If imidazole needs to be removed from the eluted protein, use a desalting column according to Table 4.*

6 Optimization

Concentration of imidazole

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

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

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

Choice of metal ion

Ni^{2+} is usually the first choice metal ion for purifying most $(histidine)_6$-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, for example 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).

7 Stripping and recharging

The column does not have to be stripped and recharged between each purification cycle if the same protein is to be purified. It may be sufficient to strip and recharge it after five to seven purifications, or when the medium starts to change color to more white. The interval depends on parameters such as the metal ion used, sample properties, sample volume, target protein, etc.

Stripping

Stripping buffer

20 mM sodium phosphate, 0.5 M NaCl, 50 mM EDTA, pH 7.4

Step	Action
1	Wash with at least 5 to 10 column volumes (CV) of stripping buffer
2	Wash with at least 5 to10 CV binding buffer
3	Wash with at least 5 to 10 CV distilled water
4	Clean the column, see *Section 8 Cleaning-in-Place (CIP), on page 15* and/or recharge with metal ions according to the method below.

Recharging

Step	Action
1	Make sure that the column is equilibrated with distilled water. If not, equilibrate with 5 CV distilled water.
2	Load 0.5 CV 0.1 M $NiSO_4$ in distilled water. ***Note:*** *Salts of other metals, chlorides or sulfates, may also be used, see Section 6 Optimization, on page 12*
3	Wash with 5 CV distilled water.
4	Wash with 5 CV binding buffer (to adjust pH).

Step	Action
5	For future use, equilibrate with 5 to 10 CV 20% ethanol and store.

8 Cleaning-in-Place (CIP)

General description

Correct preparation of samples and buffers should maintain columns in good condition. However, reduced performance, increased back pressure or blockage indicates that the column needs cleaning.

CIP removes very tightly bound, precipitated or denatured substances from the medium. If such contaminants are allowed to accumulate, they may affect the chromatographic properties of the prepacked column, reduce the capacity of the medium and, potentially, come off in subsequent runs. If the fouling is severe, it may block the column, increase back pressure and affect the flow properties.

CIP should be performed regularly to prevent the build-up of contaminants and to maintain the capacity, flow properties and general performance of prepacked columns.

It is recommended to perform a CIP:

- When an increase in back pressure is seen.
- If reduced column performance is observed.
- Between runs when the same column is used for purification of different proteins to prevent possible cross-contamination.

CIP protocol

The **metal ion stripped** column can be cleaned by the following procedures.

To remove...	Then...
Ionically bound proteins	1 Wash with several column volumes (CV) of 1.5 M NaCl. 2 Wash with at least 3 CV distilled water.
Precipitated proteins, hydrophobically bound proteins, and lipoproteins	1 Wash the column with 1 M NaOH, contact time usually 1 to 2 h (longer time may be required to inactivate endotoxins). 2 Wash with approximately 3 to 10 CV binding buffer. 3 Wash with 5 to 10 CV distilled water.
Hydrophobically bound proteins, lipoproteins, and lipids.	1 Wash with 5 to 10 CV 30% isopropanol for at least 15 to 20 min. 2 Wash with approximately 10 CV distilled water. or 1 Wash with 2 CV 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. 2 Remove residual detergent by washing with at least 5 CV 70% ethanol [1]. 3 Wash with 3 to 10 CV binding buffer.

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

9 Adjusting pressure limits in chromatography system software

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

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

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

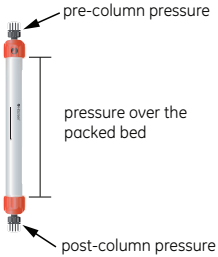

Fig 2. Pre-column and post-column measurements.

ÄKTA avant and ÄKTA pure

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

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

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

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

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

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

10 Delivery and storage

The column is supplied in 20% ethanol.

If the column is to be stored for a longer period, clean the column according to the procedure described in *Section 8 Cleaning-in-Place (CIP), on page 15*. Then equilibrate with at least 50 ml 20% ethanol.

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

11 Troubleshooting

The following tips serve as a guide.

Note: *When using high concentrations of urea or Gua-HCl (as described below), protein unfolding generally takes place. Refolding on-column (or after elution) is protein-dependent. Samples containing urea can be analyzed directly by SDS-PAGE whereas samples containing Gua-HCl must be buffer-exchanged (see Table 4) to a buffer with urea before SDS-PAGE. Solid urea or Gua-HCl can be slowly added directly to the sample to minimize dilution.*

Column has clogged

- Cell debris in the sample may clog the column. Clean the column according to *Section 8 Cleaning-in-Place (CIP), on page 15*. It is important to filter and/or centrifuge the sample before loading, see Section *Sample preparation, on page 10*.

Sample is too viscous

- If the lysate is very viscous due to the presence of 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, 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 3* for reducing agents, detergents, glycerol and denaturing agents that may be used.**

  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 the amount of sample, or decrease protein concentration by eluting with linear imidazole gradient instead of imidazole steps. Elute under denaturing (unfolding) conditions (use 4 to 8 M urea or 4 to 6 M Gua-HCl).
- **Concentration of imidazole in the binding buffer is too high:** The protein is found in the flowthrough material. Decrease the imidazole concentration in the binding buffer.
- **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. Make sure that the concentration of chelating or strong reducing agents, as well as imidazole in the sample is not too high. See *Table 3*

SDS-PAGE of samples collected during the preparation of the bacterial lysate indicates that most histidine-tagged protein is located in the centrifugation pellet

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

  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 overheating and frothing as this may denature the tagged protein.

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

  Prepare buffers containing 20 mM sodium phosphate, 8 M urea or 6 M Gua- HCl, and suitable imidazole concentrations, pH 7.4 to 7.6. Buffers with urea should also include 500 mM NaCl.

  Use these buffers for sample preparation, as binding buffer and as elution buffer. For sample preparation and binding buffer use, 20 mM imidazole or the concentration selected during the optimization trials (including urea or Gua-HCl).

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:** A shallow imidazole gradient (20 column volumes (400 ml) or more), may separate proteins with similar binding strengths.

  If optimized conditions do not remove contaminants, further purification by ion exchange chromotography (using HiTrap Q HP or HiTrap SP HP) and/or gel filtration (using 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 (for example up to 2% Triton X-100 or 2% Tween 20), or add glycerol (up to 50%) to the wash buffer to disrupt nonspecific interactions.
- **Nonspecific, hydrophobic or other interaction:** Add a nonionic detergent to the elution buffer (for example 0.2% Triton X-100) or increase the NaCl concentration.

12 Further information

For more information, please visit:

www.gelifesciences.com/protein-purification
www.gelifesciences.com/purification_techsupport

Refer to the different handbooks below, contact our technical support, or your local GE representative.

13 Ordering information

Product	Quantity	Code No.
HisPrep FF 16/10	1 x 20 ml	28-9365-51

Product	Quantity	Code No.
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
HisTrap FF crude	5 × 1 ml	11-0004-58
	100 x 1 ml[1]	11-0004-59
	5 x 5 ml	17-5286-01
	100 x 5 ml[1]	17-5286-02
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
HiPrep 26/10 Desalting	1 x 53 ml	17-5087-01
	4 x 53 ml	17-5087-02
HiTrap Desalting	5 × 5 ml	17-1408-01
	100 × 5 ml[1]	11-0003-29

[1] Pack size available by special order.

Accessories	Quantity	Code No.
To connect columns with 1/16" connectors to original FPLC System:		
Union M6 female/1/16" male	5	18-3858-01
HiTrap/HiPrep 1/16" male connector for ÄKTA design	8	28-4010-81

Related literature	Code No.
The 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
Prepacked chromatography columns for ÄKTA design, Selection guide	28-9317-78
Ni Sepharose and IMAC Sepharose, Selection guide	28-4070-92

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

28-9413-29 AE 02/2015 a16