GE Healthcare

Instructions 28-4046-26 AA

HiPrep IMAC FF 16/10

Introduction

HiPrep™ IMAC FF 16/10 is a ready to use column, prepacked with uncharged IMAC Sepharose™ 6 Fast Flow. This column is ideal for preparative purification of histidine-tagged recombinant proteins and untagged, naturally occurring proteins.
HiPrep IMAC FF 16/10 provides fast, simple and easy separations in a convenient format, and the IMAC Sepharose 6 Fast Flow medium is ideal for scaling up.

Column data

Medium	IMAC Sepharose 6 Fast Flow
Bead structure	Highly cross-linked 6 % agarose
Mean particle size	90 µm
Dynamic binding capacity*	Approx. 40 mg $(histidine)_6$-tagged protein/ml medium (Ni^{2+}-charged). Untagged protein: Approx. 25 mg/ml medium (Cu^{2+} charged), or approx. 15 mg/ml medium (Zn^{2+} or Ni^{2+} charged).
Metal ion capacity	Approx. 15 µmol Ni^{2+}/ml medium
Bed volume	20 ml
Bed diameter × height	16 × 100 mm
Column hardware	Polypropylene
Recommended flow rate† ‡	1–10 ml/min (30–300 cm/h)
Maximum flow rate† ‡	10 ml/min (300 cm/h)
Maximum pressure over the packed bed during operation, Δp‡	0.15 MPa, 1.5 bar, 22 psi
HiPrep column hardware pressure limit‡	0.5 MPa, 5 bar, 73 psi
Compatibility during use	See Table in Buffers and compatibility section.
Chemical stability (for medium without metal ion)	1 M NaOH, 70 % acetic acid. Tested for 12 h. 2 % SDS. Tested for 1 h. 30 % 2-propanol. Tested for 30 min.
pH stability (for medium without metal ion)	short term (≤ 2 h): 2–14 long term (≤ one week): pH 3–12
Storage	+4 to +30 °C in 20 % ethanol

* Dynamic binding capacity conditions:

Samples:	(Histidine)$_6$-tagged proteins: Capacity data were obtained for a protein (M_r 28 000) bound from an *E. coli* extract, and a pure protein (M_r 43 000; applied at 1 mg/ml in binding buffer; capacity at 10 % breakthrough). Untagged protein: Capacities determined at 10 % breakthrough for human apo-transferrin applied at 1 mg/ml in binding buffer.
Column volume:	0.25 or 1 ml
Flow rate:	0.25 or 1 ml/min, respectively
Binding buffer:	20 mM sodium phosphate, 500 mM NaCl, 5 mM imidazole, (1 mM for untagged protein) pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, (50 mM for untagged protein) pH 7.4

Note: Dynamic binding capacity is metal-ion- and protein-dependent.

† Water at room temperature. Flow rate is determined by $v \bullet \eta \leq 10$ ml/min where v = flow rate and η= viscosity.

‡ Many chromatography systems are equipped with pressure gauges to measure the pressure at a particular point in the system, usually just after the pumps. The pressure measured here is the sum of the precolumn pressure, the pressure drop over the medium bed, and the post-column pressure. This pressure is always higher than the pressure drop over the bed alone. We recommend keeping the pressure drop over the bed below 1.5 bar. Setting the upper limit of your pressure gauge to 1.5 bar will ensure the pump shuts down before the medium is over-pressured. If necessary, post-column pressure of up to 3.5 bar can be added to the limit without exceeding the column hardware limit. To determine post-column pressure, proceed as follows:

To avoid breaking the column, the post-column pressure must not exceed 3.5 bar.

1. Connect a piece of tubing in place of the column.
2. Run the pump at the maximum flow you intend to use for chromatography. Use a buffer with the same viscosity as you intend to use for chromatography. Note the backpressure as total pressure.
3. Disconnect the tubing and run at the same flow rate used in step 2. Note this backpressure as precolumn pressure.
4. Calculate the post-column pressure as total pressure minus precolumn pressure.

If the post-column pressure is higher than 3.5 bar, take steps to reduce it (shorten tubing, clear clogged tubing, or change flow restrictors) and perform steps 1–4 again until the post-column pressure is below 3.5 bar. When the post-column pressure is satisfactory, add the post-column pressure to 1.5 bar and set this as the upper pressure limit on the chromatography system.

HiPrep™

First-time use

1. Charge with metal ions (see below).
2. Set an appropriate pressure limit.
3. Equilibrate the column with 100 ml binding buffer.

HiPrep IMAC FF 16/10 can be used directly on ÄKTAdesign™ systems without the need for extra connectors.

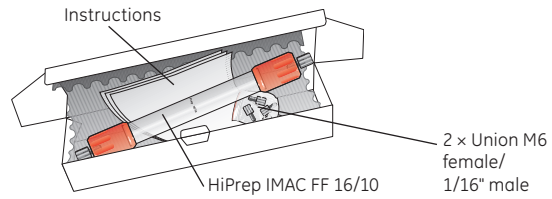
Try these conditions first

Binding buffer for histidine-tagged proteins*:	20 mM sodium phosphate, 500 mM NaCl, 20–40 mM imidazole, pH 7.4 (The imidazole concentration for optimal purity/yield is protein-dependent, see Optimization).
Elution buffer for histidine-tagged proteins*:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4
Flow rate:	1–10 ml/min (30–300 cm/h)

* *For untagged target proteins, the imidazole concentrations that should be used are usually lower than the above, both for binding (sometimes no imiadzole used) and elution.*

Note: Especially for untagged target proteins, low-pH elution is an alternative to competitive elution with imidazole, e.g. a linear gradient from pH 7.4 to pH 4.

De-gas and filter all solutions through a 0.45-µm filter to increase column lifetime. High purity imidazole gives very low or no absorbance at 280 nm.



Buffers and compatibility

IMAC Sepharose 6 Fast Flow charged with Ni^{2+} is compatible with:

Reducing agents (See Blank run below)	5 mM DTE 5 mM DTT 20 mM ß-mercaptoethanol 5 mM TCEP (Tris[2-carboxyethyl] phosphine) 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 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*

* Tested for one 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 buffer). Any metal-ion stripping may be counteracted by adding a small excess of $MgCl_2$ before centrifugation/filtration of the sample. Note that stripping effects may vary with the applied sample volume.

Optional: 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.

1. If the column has been stored in 20% ethanol after metal ion charging, wash it with 5 column volumes of distilled water.
2. Wash with 5 column volumes of the buffer that has been chosen for protein elution, e.g., imidazole elution buffer or low-pH elution buffer.
3. Equilibrate with 5–10 column volumes of binding buffer. Imidazole equilibration can be monitored by absorbance, e.g. at 220 nm.

Charging with metal ions

- Charge the water-washed column by loading 10 ml 0.1 M metal-ion solution in distilled water. Chlorides, sulfates, etc. can be used. For choice of metal ion, see Optimization.
- Wash with 100 ml distilled water and 100 ml binding buffer (washing with binding buffer – to adjust pH – should be done even if the metal-charged column is only to be stored in 20 % ethanol)
- In some cases, a blank run may be needed for optimal performance, see Blank run.

Note: The column does not have to be stripped and recharged between each purification if the same protein is going to be purified; it may be sufficient to strip and recharge it after approx. five purifications, depending on the sample properties, sample volumes, metal ion, etc.

Metal ion stripping

Stripping buffer: 20 mM sodium phosphate, 500 mM NaCl, 50 mM EDTA, pH 7.4

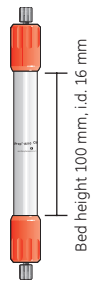
1. Wash with at least 100–200 ml stripping buffer
2. Wash with at least 100–200 ml binding buffer
3. Wash with at least 100–200 ml distilled water
4. Clean the column, see "Cleaning-in-Place (CIP)" and/or recharge with metal ions.

Avoid

Chelating agents in buffers, e.g. EDTA, EGTA, citrate (see Table 2). Unfiltered solutions.

Sample preparation

Centrifuge at 10 000 × g (or higher) for 10 min and/or filter the sample through 0.45-µm filter.
If possible, dilute the sample in binding buffer.
The sample should contain imidazole at the same concentration as in the binding buffer.



Delivery/storage

The column is supplied in 20 % ethanol.

If the column is to be stored for a longer period, clean it according to the procedure described under "Cleaning-in-Place (CIP)". Then equilibrate with at least 50 ml 20 % ethanol.

DO NOT OPEN THE COLUMN

Optimization

Perform your first run according to "First-time use" and "Try these conditions first". If the results are unsatisfactory, consider the following:

Action	Effect
Increase the imidazole concentration in the sample and binding buffer.	Decreases the amount of contaminants binding to the medium.
Increase the imidazole concentration in the binding/wash buffer.	Washes out contaminants bound to the medium more effectively.

Elute with a stepwise or 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 the highest possible concentration of imidazole that does not cause elution of the target protein.

Note: There is an optimal imidazole concentration at binding and wash that will balance high purity and high yield. This optimal concentration is different for different histidine-tagged proteins.

Note: The possibility of reusing HiPrep IMAC FF 16/10 depends on the properties of the samples and on the metal ion used. Beware of cross-contamination if using the same column for purification of more than one target protein.

Ni^{2+} is usually the first-choice metal ion for purifying most histidine-tagged proteins.

For purification of untagged proteins, Cu^{2+} ions have frequently been used. When the binding characteristics of an untagged target protein are not known, it is advisable to test other metal ions (e.g. Zn^{2+}, Ni^{2+}, Co^{2+}) to establish the most suitable metal ion to use. In some instances, a weak binding to a metal ion can be exploited to achieve selective elution (higher purity) of a target protein. In some special applications, Fe^{3+} and Ca^{2+} have also been used.

Cleaning-in-Place (CIP)

Decreased binding capacity and/or increased backpressure may be due to an accumulation of debris or of precipitated, denatured, or non-specifically bound proteins. These problems can be solved using the procedures described below. For difficult cases, use reversed flow direction.

Note: Before cleaning, strip off the metal ions by using the recommended procedure. Stripping, without any additional CIP procedures, may sometimes give a satisfactory cleaning effect.

- **Removal of ionically bound substances:** Wash with several column volumes of 1.5 M NaCl. Then wash the column with approx. 10 column volumes of distilled water.
- **Removal of precipitated and/or hydrophobically-bound substances and lipoproteins:** Wash with 1 M NaOH, contact time usually 1–2 h (longer time may be required to inactivate endotoxins); then wash with approx. 10 column volumes of binding buffer, followed by 5–10 column volumes of distilled water.
- **Removal of hydrophobically-bound proteins, lipoproteins, and lipids:** Wash with 5–10 column volumes 30 % isopropanol for at least 15–20 min; then wash with approx. 10 column volumes of distilled water. Alternatively, wash with 2 column volumes of detergent in a basic or acidic solution. Use, for example, 0.1–0.5 % nonionic detergent in 0.1 M acetic acid, contact time 1–2 h. After treatment, always remove residual detergent by washing with at least 5 column volumes of 70 % ethanol. Then wash with approx. 10 column volumes of distilled water.

DO NOT OPEN THE COLUMN

Troubleshooting

The following tips serve as a guide.

Note: Proteins generally unfold when using high concentrations of urea or Gua-HCl (as described below). 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 to a buffer with urea before SDS-PAGE. Solid urea or Gua-HCl can be added to the sample to minimize dilution.

Symptom	Remedy
Column has clogged	Cell debris in the sample may clog the column. Clean the column according to Cleaning-in-Place. It is important to filter and/or centrifuge the sample before loading, see Sample preparation.
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 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 help: 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 possible but is protein-dependent), 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 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 or decreasing pH gradient to determine the optimal elution conditions. **Protein has precipitated on the column:** For the next experiment, decrease the amount of sample, or decrease protein concentration by eluting with a linear imidazole gradient instead of steps. Elute under denaturing (unfolding) conditions (use 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 change the NaCl concentration. **Concentration of imidazole in the binding buffer is too high:** The protein is found in the flowthrough. Decrease the imidazole concentration in the binding buffer. **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, e.g., anti-His antibodies in Western blotting. **Histidine-tag may be insufficiently exposed:** The protein is found in the flowthrough; 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; 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 sample, is not too high.
SDS-PAGE of samples collected during preparation of the bacterial lysate indicates that most histidine-tagged protein is located in the centrifugation pellet	**Sonication may be insufficient:** Check cell disruption by microscopic examination or monitor by measuring the release of nucleic acids at 260 nm. Adding 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 foaming as this may denature the tagged protein. Over-sonication 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 4–6 M Gua-HCl, 4–8 M urea, or strong detergents. Prepare buffers containing 20 mM sodium phosphate, NaCl, 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 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 Compatibility). **Contaminants have high affinity for the metal ion used:** See Optimization instructions. 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 (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, or shortly afterwards if foaming is a problem. Increase 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. **Change metal ion:** The metal ion used may not be the most suitable, see Optimization.

Ordering information

Product	No. per pack	Code No.
HiPrep IMAC FF 16/10	1 (20 ml)	17-0921-06

Companion products

HiTrap IMAC FF	5 × 1 ml	17-0921-02
HiTrap IMAC FF	5 × 5 ml	17-0921-04
IMAC Sepharose 6 Fast Flow	25 ml	17-0921-07
IMAC Sepharose 6 Fast Flow	100 ml	17-0921-08
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
HisPrep™ FF 16/10	1 (20 ml)	17-5256-01
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
HiPrep 26/10 Desalting	1 (53 ml)	17-5087-01
HiPrep 26/10 Desalting	4 (53 ml)	17-5087-02
HiTrap Desalting	5 × 5 ml	17-1408-01
HiTrap Desalting	100 × 5 ml*	11-0003-29

* Pack size available by special order.

Accessories

To connect columns with 1/16" connectors to FPLC™ System:		
Union M6 female/1/16" male*	5	18-3858-01
HiTrap/HiPrep 1/16" male connector for ÄKTAdesign	8	28-4010-81

* Two units (in red polypropylene) are included in HiPrep IMAC FF 16/10 package

Related printed literature

The Recombinant Protein Handbook	18-1142-75
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
Affinity Chromatography Columns and Media, Product profile	18-1121-86

Further information

For more information, please visit *www.chromatography.amershambiosciences.com*. Refer also to the handbooks above, contact our technical support team, or your local representative.

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