**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.

**Column data**
- **Medium:** IMAC Sepharose 6 Fast Flow
- **Bead structure:** Highly cross-linked 6 % agarose
- **Mean particle size:** 90 μm
- **Dynamic binding capacity:**
  - *: Samples: Histidine-tagged proteins. Capacity data were obtained for a protein IM, 29,000 bound from an E. coli extract, and a pure protein IM, 45,000, applied at 1 mg/ml in binding buffer; capacity at 10 % breakthrough for human apotransferrin applied at 1 mg/ml in binding buffer.
  - **: For untagged target proteins, the imidazole concentrations that should be used are usually lower than the above, for binding sometimes no imidazole used and elution.
- **Metalation capacity:**
  - **: Approx. 15 μmol Ni²⁺/ml medium
  - **: 20 ml
- **Bed diameter × height:** 160 × 300 mm
- **Column hardware:** Polyethylene
- **Recommended flow rate:**
  - **: 1–10 ml/min (130–300 cm/h)
- **Maximum flow rate:**
  - **: 15 ml/min (300 cm/h)

**Column pressure**
- **: 0.15 MPa, 1.5 bar, 22 psi
- **: 0.5 MPa, 5 bar, 73 psi

**Compatibility during use**
See Table in Buffers and compatibility section.

**Dynamic binding capacity**
- **: metal-ion- and protein-dependent.

**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 pre-column pressure.
3. Disconnect the tubing and run at the same flow rate used in step 2. Note this backpressure as pre-column pre-column pressure.
4. Calculate the post-column pressure as total pressure minus pre-column pressure. If the post-column pressure is higher than 3.5 bar, take steps to reduce it (shortening 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.

**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.

**Try these conditions first**
- **Binding buffer for histidine-tagged proteins:** 20 mM sodium phosphate, 500 mM NaCl, 20–40 mM imidazole, pH 7.4
- **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 (130–300 cm/h)

**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 Ni²⁺ is compatible with:
- **Reducing agents:** DTT
- **Denaturing agents:** 6 M Gua-HCl
- **Detergents:** 2% Triton™ X-100 (nonionic)
- **Other additives:** 500 mM imidazole

**Sample preparation**
Centrifuge at 10,000 × g or higher for 10 min and/or filter the sample through a 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.

**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
- **: Wash with at least 100–200 ml stripping buffer
- **: Wash with at least 100–200 ml distilled water
- **: 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.

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

**Action**
- **Increase the imidazole concentration in the sample and binding buffer.**
- **Decreases the amount of contaminants binding to the medium.**

**Effect**
- **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.
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 ionicly 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 protein may be lost).

• 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 a-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 suitable for SDS-PAGE. Urea or Gua-HCl can be added to the sample to minimize elution.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Remedy</th>
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<tbody>
<tr>
<td>Column has clogged</td>
<td>Cell debris in the sample may clog the column. Clean the column using Cleaning-in-Place. It is important to filter and/or centrifuge the sample before loading.</td>
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<tr>
<td>Sample is too viscous</td>
<td>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 to 5 μg/ml, Mg²⁺ to 1 mM and recirculate on ice for 15–15 min. Alternatively, draw the lysate through a syringe needle several times.</td>
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<tr>
<td>Protein is difficult to dissolve or precipitates during purification</td>
<td>The following additivies may help: 2 % Triton X-100 and 2 % NaCl, 0.5 M Tris-HCl, pH 7.4, 50 % glycerol, 20 mM imidacarbopentanoic acid, 1-M histidine, 50 mM imidazole, or 10 mM EDTA.</td>
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