CREATIVE BIOMART®
Chromatography

HiTrap IMAC Sepharose FF

Product Information

Cat#No#	Hi-077P

Product Overview

HiTrap IMAC FF is prepacked with IMAC Sepharose 6 Fast Flow. The resin is charged with the metalion of your choice for Immobilized Metal ion Affinity Chromatography (IMAC) and subsequent purification of polyhistidine tagged proteins.

Description

IMAC Sepharose FF is supplied free of metal ions. It is charged by the user with the transition metal ion of choice (e.g. Cu2+, Zn2+, Ni2+, or Co2+); these metal ions will bind to the covalently immobilized chelating ligand on the Sepharose.

Characteristic

For optimizing purification of histidine-tagged proteins when Ni2+ is not the best choice of metal ion.
Conveniently charge with your metal of choice.
Optimal bead size for scaling up.
High binding capacity.

Maximum operating pressure

5 bar [0.5 MPa] (70 psi)

Sample preparation

Adjust the sample to the composition and pH of the binding buffer by adding buffer, NaCl, imidazole, and additives (as required) from concentrated stock solutions, by diluting the sample with binding buffer, or by buffer exchange. Do not use strong bases or acids for pHadjustment (precipitation risk). Shortly before applying the sample to the column, centrifuge it and/or filter it through 0.45 or 0.22 µm filters.

Metal ion capacity

Approx. 15 µmol Ni2+/ml medium.

Matrix

Highly cross-linked spherical agarose, 6%

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Average particle size
90 µm
Dynamic binding capacity
Approx. 40 mg (histidine)6-tagged protein/ml medium (Ni2+ charged). Untagged protein: Approx. 25 mg/ml medium (Cu2+ charged), or approx. 15 mg/ml medium (Zn2+ or Ni2+ charged).
Recommended flow rate
1 ml/min and 5 ml/min for 1 ml and 5 ml column, respectively.
Chemical stability
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 minutes
pH working range
2–14
CIP stability
3–12
Storage
4 to 30°C, 20% Ethanol
Binding buffer
20 mM sodium phosphate, 0.5 M NaCl, 5 mM imidazole (1 mM for untagged protein), pH 7.4.
Elution buffer
20 mM sodium phosphate, 0.5 M NaCl, 0.5 mM imidazole (50 mM for untagged protein), pH 7.4.
Cleaning-in-place
Remove ionically bound proteins by washing with several CV of 1.5 M NaCl. Then wash with at least 3 CV of distilled water. Remove precipitated proteins, hydrophobically-bound proteins and lipoproteins by washing with 1 M NaOH, contact time usually 1–2 h (longer time may be required to inactivate endotoxins). Then wash with 3–10 CV of

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binding buffer, followed by 5–10 CV of distilled water.
Remove hydrophobically bound proteins, lipoproteins, and lipids by washing the column with 5–10 CV 30% isopropanol for at least 15–20 min. Then wash with approx. 10 CV of distilled water.

Purification procedures

1. After the column preparation, equilibrate with at least 5 column volumes (CV) of binding buffer. Recommended flow rates are 1 ml/min or 5 ml/min for the 1 ml and 5 ml columns, respectively.
2. Apply the pretreated sample using a syringe or pump.
3. Wash with binding buffer until the absorbance reaches a steady baseline (generally, at least 10–15 CV).
4. Elute the bound proteins with elution buffer, stepwise or with a linear gradient. Five CV are usually sufficient if the protein of interest is eluted with one step. A shallow gradient, e.g. a linear gradient over 20 CV or more, may separate proteins with similar binding strengths.

Pack size	
5 × 1 mL	
Maximum flow velocity	
4 ml/min and 20 ml/min for 1 ml and 5 ml column, respectively.	
Column volume	
1 mL	
Column i.d.	
7 mm	
Column hardware pressure limit	
5 bar (0.5 MPa, 70 psi)	

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