HiTrap IMAC HP

Product Information

Cat#No#	Hi-076P

Product Overview

HiTrap IMAC HP is prepacked with IMAC Sepharose High Performance. The resin is charged with the metalion of your choice for Immobilized Metal ion Affinity Chromatography (IMAC) and subsequent high-resolution purification of polyhistidine tagged proteins.

Description

Proteins and peptides that have an affinity for metal ions can be purified using immobilized metal ion affinity chromatography (IMAC), a method that has been growing in popularity and effectiveness.
IMAC Sepharose HP 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. The immobilized metal ions will interact with certain amino acid residues on protein surfaces (mainly histidine, but often also cysteine and tryptophan), if the amino acid side chains are sufficiently exposed. The bound protein can be eluted either with a competitive agent such as imidazole or by lowering the pH.

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.
Small bead size for high-performance purification.
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 um filters.

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CREATIVE BIOMART®
Chromatography

HiTrap IMAC HP

Metal ion capacity
~15 µmol Ni2+/mL medium
Matrix
Highly cross-linked spherical agarose, 6%
Average particle size
34 µm
Dynamic binding capacity
At least 40 mg (histidine)6-tagged protein/mL medium (Ni2+-charged)
Recommended flow rate
< 4 ml/min
Recommended column height
25 mm
Chemical stability
0.01 M HCl, 0.1 M NaOH. Tested for 1 week at 40°C 1 M NaOH, 70% HAc. Tested for 12 hours 2% SDS. Tested for 1 hour 30% 2-propanol. Tested for 30 min.
pH working range
2 to 14
CIP stability
3 to 12
Storage
4 to 30°C, 20% Ethanol
Binding buffer
20 mM sodium phosphate, 0.5 M NaCl, 5 mM imidazole, pH 7.4.
Elution buffer

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20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4.

Cleaning-in-place

Remove ionically bound proteins by washing with several column volumes (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 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. Alternatively, wash with 2 CV of detergent in a basic or acidic solution.

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 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 CVs 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 and 20 mL/min for 1 and 5 mL column, respectively.

Dimensions

7 × 25 mm

Column volume

1 mL

Column i.d.

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7 mm
Column hardware pressure limit
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