HiTrap Chelating HP columns

Product Information

Cat#No#	Hi-073P

Product Overview

HiTrap Chelating HP columns are prepacked with an uncharged IMAC purification resin for purification of proteins and peptides with affinity for metal ions.

Description

HiTrap Chelating HP is one of a range of prepacked 1 ml and 5 ml columns for metal chelate affinity chromatography. Fast, simple, and easy separations are provided by the combination of the prepacked column and a high-performance affinity medium. HiTrap Chelating HP is particularly suitable for the isolation and purification of proteins and peptides containing exposed histidine residues.

Characteristic

Ready to charge with the metal ion of choice for optimized selectivity.
Packed with Chelating Sepharose High Performance IMAC resin.
Convenient affinity purification of native and recombinant proteins, including proteins with a histidine-tag (his-tag).
Convenient prepacked columns provide fast, simple, and reproducible his-tag protein purification.
HiTrap format compatible with a syringe, pump or chromatography system such as ÄKTA system or other FPLC system.

Maximum operating pressure

5 bar [0.5 MPa] (70 psi)

Sample preparation

The sample should be adjusted to the composition of the binding buffer. This can be done by either diluting the sample with binding buffer or by buffer exchange using HiTrap Desalting, HiPrep 26/10 Desalting or PD-10 column. The sample should be filtered through a 0.45 µm filter or centrifuged immediately before it is applied to the column.

Metal ion capacity

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CREATIVE BIOMART®
Chromatography

HiTrap Chelating HP columns

~ 23 µmol Cu2+/ml medium
Matrix
Highly cross-linked, spherical agarose
Average particle size
34 µm
Ligand
Iminodiacetic acid
Dynamic binding capacity
Approx. 23 µmoles Cu2+/ml medium
Recommended flow rate
< 4 ml/min
Recommended column height
25 mm
Chemical stability
Stable in all commonly used buffers and denaturants such as 6 M guanidine hydrochloride, 8 M urea and chaotropic salts.
pH working range
4 to 12
CIP stability
3 to 13
Temperature stability
4°C to room temp
Storage
4 to 30°C, 20% Ethanol

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Purification procedures
1. After column preparation equilibrate the column with binding buffer by washing with 5–10 column volumes. Recommended flow rates are 1 ml/min or 5 ml/min for 1 ml and 5 ml column respectively. 2. Apply the sample, using a syringe or a pump. A partial displacement of chelated metal ions is often noted as the protein is adsorbed. This is visible, especially when using metal ions that are colored, such as Cu2+ and Ni2+, as a downward extension of the zone of chelated ions. 3. Wash with 5–10 column volumes binding buffer. To increase the purity of eluted protein a wash with binding buffer containing 5– 40 mM imidazole is often effective when working with recombinant (histidine)6-tagged proteins (3–5 column volumes). 4. Elute with elution buffer using a step or linear gradient. 2–5 column volumes is usually sufficient if the molecule of interest is rapidly eluted, e.g. a simple protein mixture eluted by a step gradient. Other volumes (or a different elution buffer) may be required if the interaction is difficult to break. A shallow gradient is used to separate proteins with similar binding strengths, e.g. a linear gradient of 10–20 column volumes.
Pack size
5 × 1 mL
Maximum flow velocity
4 ml/min (1 ml); 20 ml/min (5 ml)
Maximum operating backpressure
0.3 MPa, 3 bar
Dimensions
7 × 25 mm
Column volume
1 mL
Column i.d.
7 mm
Column hardware pressure limit

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