HisTrap excel

Product Information

Cat#No#	His-113P

Product Overview

HisTrap excel columns are prepacked with Ni Sepharose excel affinity resins for capture and purification of histidine-tagged proteins secreted into eukaryotic cell culture supernatants by immobilized metal ion affinity chromatography (IMAC).

Description

HisTrap excel 1 mL and 5 mL are ready-to-use IMAC columns prepacked with Ni Sepharose excel. The design of the columns in combination with the specific properties of the resin enables fast and convenient purifications. The special type of filter in the top and bottom of the columns makes it possible to load large volumes of cell-free, unclarified samples directly on the columns without causing back pressure problems. This time-saving property helps prevent degradation and loss of sensitive target proteins.

Characteristic

Load eukaryotic cell culture samples containing secreted histidine-tagged proteins directly with retained binding capacity.
Increase target protein yield and decrease degradation through reduced and simplified sample handling.
HisTrap excel columns allow direct purification of cell-free, unclarified samples.

Maximum operating pressure

5 bar [0.5 MPa] (70 psi)

Metal ion capacity

54 to 70 µmol Ni2+ /ml medium

Matrix

Highly cross-linked spherical agarose, 6%

Average particle size

90 µm

Dynamic binding capacity

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At least 10 mg histidine-tagged protein/ml sedimented medium.
Recommended flow rate
150 to 600 cm/h
Recommended column height
25 mm
Chemical stability
0.01 M HCl and 0.01 M NaOH. 10 mM EDTA, 5 mM DTT, 5 mM TCEP, 20 mM β-mercaptoethanol, 1 M NaOH, and 6 M guanidine-HCl. 500 mM imidazole and 100 mM EDTA. 30% 2-propanol.
Chemical compatibility
Stable in all buffers commonly used in IMAC.
pH working range
2 to 12
CIP stability
2 to 14
Storage
4 to 30°C, 20% ethanol
Elution buffer
20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4.
Elution
In general, imidazole is used for elution of histidine-tagged proteins. Alternatively, the proteins may be eluted by other methods or combinations of methods, for example by lowering pH within the range 2.5 to 5.0. With Ni Sepharose excel no recharging with nickel ions between each run is required when elution is performed using either low pH or imidazole. This is in contrast to many other IMAC media, which often have to be recharged with nickel ions after elution using low pH.
Cleaning-in-place

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Ionically bound proteins: 1. Wash with several column volumes (CV) of 1.5 M NaCl. 2. Wash with approximately 10 CV distilled water or equilibration buffer.
Precipitated proteins, hydrophobically bound proteins, and lipoproteins: 1. Wash the column with 1 M NaOH, contact time usually 1 to 2 h (12 to 24 h for endotoxin removal). 2. Wash with approximately 10 CV equilibration buffer.
Hydrophobically bound proteins, lipoproteins, and lipids: 1. Wash with 5 to 10 CV 30% isopropanol for about 15 to 20 min. 2. Wash with approximately 10 CV distilled water or equilibration buffer. OR 1. Wash with 2 CV detergent in a basic or acidic solution. Use, for example, 0.1 to 0.5% nonionic detergent in 0.1 M acetic acid, contact time 1 to 2 h. 2. Remove residual detergent by washing with at least 5 CV 70% ethanol. 3. Wash with approximately 10 CV equilibration buffer.

Pack size
5 × 1 mL
Maximum flow velocity
600 cm/h
Wash buffer
20 mM sodium phosphate, 0.5 M NaCl, 0 to 30 mM imidazole, pH 7.4.
Dimensions
7 × 25 mm
Column volume
1 ml
Column i.d.
7 mm
Column hardware pressure limit
5 bar (0.5 MPa)

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