Ni Sepharose High Performance resin

Product Information

Cat#No#	Ni-402C

Product Overview

Ni Sepharose High Performance nickel-charged IMAC resin is for high-resolution his-tagged protein purification with high binding capacity for maximized recovery.

Description

The preparative purification of histidine-tagged recombinant proteins by immobilized metal affinity chromatography (IMAC) is both popular and highly effective. IMAC exploits the ability of the amino acid histidine to bind chelated transition metal ions. Histidine is globally the most used tag, often found as six histidine residues in series, but it is also present on the surface of many unmodified proteins. Of the metal ions used in this technique, nickel (Ni2+) has generally been proven to be the most successful. Ni Sepharose High Performance further increases the use and reliability of this valuable method of purification.

Characteristic

Negligible leakage of the Ni2+ ion.
Compatibility with a very wide range of reducing agents, detergents and other additives.
Very high protein binding capacities.
Convenient and time-saving prepacked HiTrap format in the form of HisTrap HP 1 ml and 5 ml columns.

Maximum operating pressure

0.3 MPa, 3 bar

Sample preparation

The sample should be fully dissolved. To avoid column clogging, we recommend centrifugation and filtration through a 0.45 µm or 0.22 µm filter to remove cell debris or other particulate material.
If the sample is dissolved in a buffer other than 20 mM phosphate buffer with 0.5 M NaCl pH 7.4, adjust its NaCl concentration to 0.5 M and pH to 7 to 8. This can be achieved by addition of concentrated stock solutions, by dilution with the binding buffer, or by buffer exchange (on HiTrap Desalting, PD-10 Desalting Column, or HiPrep 26/10 Desalting, depending on the sample volume). Do not use strong bases or acids for pH-adjustments (precipitation risk).

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To prevent binding of host cell proteins with exposed histidine, add the same concentration of imidazole to the sample as to the binding buffer.

Metal ion capacity

approx. 15 µmol Ni2+/ml medium

Packing Column

1. Assemble the column. 2. Remove air from the end-piece and adapter by flushing with water. Make sure no air has been trapped under the column bed support. Close the column outlet leaving the bed support covered with water.
3. Resuspend the medium and pour the slurry into the column in a single continuous motion. Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.
4. If using a packing reservoir, immediately fill the remainder of the column and reservoir with water. Mount the adapter or lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.
5. Open the bottom outlet of the column and set the pump to run at the desired flow rate.
6. Maintain packing flow rate for at least 3 bed volumes after a constant bed height is reached. Mark the bed height on the column. 7. Stop the pump and close the column outlet.
8. If using a packing reservoir, disconnect the reservoir and fit the adapter to the column.
9. With the adapter inlet disconnected, push the adapter down into the column until it reaches the mark. Allow the packing solution to flush the adapter inlet. Lock the adapter in position.
10. Connect the column to a pump or a chromatography system and start equilibration. Re-adjust the adapter if necessary.

Matrix

Highly cross-linked agarose, 6%

Average particle size

~34 µm

Dynamic binding capacity

At least 40 mg histidine-tagged protein/mL resin

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Recommended flow rate
< 150 cm/h
Chemical stability
For Ni2+-stripped medium 0.01 M HCl, 0.1 M NaOH. Tested for one week at 40°C 1 M NaOH, 70% HAc. Tested for 12 h 2% SDS. Tested for 1 h 30% 2-propanol. Tested for 30 min.
Chemical compatibility
Stable in all commonly used buffers, reducing agents, denaturants and detergents.
pH working range
3–12
CIP stability
2–14
Temperature stability
4°C to 30°C
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
20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4.
Regeneration
To remove residual Ni2+, wash with 5 column volumes 20 mM sodium phosphate, 0.5 M NaCl, 50 mM EDTA, pH 7.4. Remove residual EDTA by washing with at least 5 column volumes of binding buffer followed by 5 column volumes of distilled water before recharging the column. To recharge the water-washed column, load 0.5 column volumes of 0.1 M NiSO4 in distilled water. Salts of other metals, chlorides or sulfates, may also be used.

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Wash with 5 column volumes of distilled water followed by 5 column volumes of binding buffer (to adjust pH) before storage in 20% ethanol.
In some applications, substances such as denatured proteins or lipids cannot be eluted in the regeneration. These can be removed by Cleaning-in- Place.

Cleaning-in-place

1. Wash with several column volumes of 1.5 M NaCl. Then wash with several column volumes of distilled water.
2. Wash the column with 1 M NaOH, contact time usually 1 to 2 hours (12 hours or more to remove endotoxins). Then wash with approximately 10 column volumes of binding buffer, followed by 10 column volumes of distilled water.
3. Wash with 5 to 10 column volumes of 30% isopropanol for about 15 to 20 minutes. Then wash with approximately 10 column volumes of distilled water.
4. Alternatively, wash with 2 column volumes of 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 hours. After treatment, always remove residual detergent by washing with 5 to 10 column volumes of 70% ethanol1 . Then wash with approximately 10 column volumes of distilled water.

Purification procedures

1. If the column contains 20% ethanol, wash it with 5 column volumes of distilled water. Use a linear flow rate of 50 to 100 cm/h.
2. Equilibrate the column with 5 to 10 column volumes of binding buffer. Recommended linear flow rate: 150 cm/h.
3. Apply the pretreated sample.
4. Wash with binding buffer until the absorbance reaches the baseline.
5. Elute with elution buffer using a step or linear gradient.

Pack size

25 mL

Maximum flow velocity

300 cm/h

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CREATIVE BIOMART®
Chromatography

Ni Sepharose High Performance resin

Dimensions
1.6 × 2.5 cm (5 ml)
Column volume
5 ml
Column hardware pressure limit
5 bar (0.5 MPa, 70 psi)

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