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Ni-NTA Agrose 6 Fast Flow

Product Number: S080203

Shipping and Storage

1. Packaging: PP material reagent bottle sealed packaging, with a specification of 25mL. Please consult for other packaging.
2. Storage: 2-8℃, 20% ethanol.
3. Shelf life: 24 months.

Component

Component	S080203
Ni-NTA Agrose 6 Fast Flow	25mL

Description

Ni NTA SweAgorase 6 Fast Flow is a metal ion chelating affinity chromatography separation medium formed by binding NTA ligands chelated with nickel ions onto agarose microspheres. Fixed metal ion affinity chromatography (NTA) utilizes the interaction between $Ni^{2+}$and the side chains of certain amino acids (mainly histidine, with small amounts of cysteine and tryptophan) on proteins for separation and purification.

Features: Wide range of use, simple operation, suitable for gravity columns and pre installed columns;

High flow rate, high loading capacity, low Ni^{2+}detachment, and wide compatibility of reagents;

Multiple selection can chelate various metal ions for use, usually Zn^{2+}, Ni^{2+}, Cu^{2+}or Co^{2+}.

Performance

Indicator	Description
Product Content	66% (v/v) agarose gel, stored in 20% ethanol
Gel type	6% cross-linked agarose gel
Ligands	-NTA-Ni^{2+}
Particle size range (μm)	45 - 165
Average particle size (μm)	90
Combined with load capacity	20-50 mg (His tagged protein)/mL medium
Ligand density (μmol/mL)	~ 25
Operating flow rate (cm/h)	≤600
Pressure resistance (MPa)	0.3
Chemical stability range	Commonly used aqueous buffer solutions; 0.01 M hydrochloric acid, 0.1 M sodium hydroxide (1 week); 1 M sodium hydroxide, 70% acetic acid (1 day); 2% SDS (1 hour); 30% 2-propanol (30 minutes)

Protocol

1. Buffer preparation

Prepare initial buffer (equilibrium solution) and elution buffer based on the properties of the separation target, such as:

Equilibrium solution: 20mM PB/Tris 500 mM NaCl (20-40mM Imidazole) pH 7.4-8.0

Eluent: 20mM PB/Tris 500mM NaCl 500mM Imidazole pH 7.4-8.0

Note: When purifying inclusion bodies, add 8 M Urea or 6 M Gua HCl to the solution.

2. Balance

Adjust the flow rate of the equilibrium buffer to balance the chromatography column, observe the changes in the detector until parameters such as conductivity and pH remain unchanged.

3. Sample preparation

Sample pretreatment: buffer replacement (consistent with the equilibrium solution);

Sample filtration: 0.22μm-0.8μm membrane filtration (different pore size membranes are selected based on the average particle size).

4. Purification process

4.1. Water washing: Rinse 2-3 column volumes at the operating flow rate;

4.2. Balance: Balance the volumes of 5 columns with the equilibrium buffer at the operating flow rate, observe the changes in the detector until parameters such as conductivity and pH remain unchanged;

4.3. Sample loading: Load the sample at the operating flow rate;

4.4. Rinsing: Rinse the chromatography column after loading with 5-10 column volumes of equilibrium buffer, observe the changes in the detector until the conductivity, pH, UV and other parameters remain unchanged;

4.5. Elution: Imidazole solution (containing 500 mM NaCl) in the range of 5-500 mM can be used for constant elution and gradient elution (linear gradient/stepwise). Gradient elution is generally recommended for better protein purification results.

5. Regeneration

5.1. Nickel removal process

5.1.1. Rinse 3-5 column volumes with nickel removal buffer (500 mM NaCl, 50-100 mM EDTA, pH 7.4);

5.1.2. Rinse 5-10 column volumes with deionized water;

5.1.3. Rinse the column with 5 volumes of 20% ethanol and store.

5.2. Re hang nickel

5.2.1. Rinse 5-10 column volumes with deionized water to remove 20% ethanol storage solution;

5.2.2. Add 3 column volumes of 100 mM NiSO4 and re coat with nickel;

5.2.3. Remove excess nickel using a 100 mM NaCl equilibration buffer or water and set aside for later use.

Note: When the color of the column material becomes lighter, there is obvious pollution, or the liquid discharge speed is slow due to repeated use of the filler, it is recommended to perform in-situ cleaning to reduce the binding efficiency. When problems such as protein precipitation occur during the purification process, in situ cleaning is required.

6. In place cleaning (CIP)

After long-term use, excessive pollutants in the medium can increase the column bed back pressure, thereby reducing column efficiency and medium adsorption capacity, and even damaging the medium's lifespan. Regular CIP can effectively protect the medium. If the medium is slightly contaminated, CIP can be performed every 1-5 cycles. But for media that are heavily contaminated after the experiment, CIP treatment must be carried out immediately. In the case of nickel removal, the medium can be subjected to CIP according to the following method:

6.1. Wash several column volumes with 1.5M NaCl to remove ion bound proteins, followed by rinsing approximately 10 column volumes with deionized water;

6.2. Wash the chromatography column with 1M NaOH to remove precipitated proteins, hydrophobic binding proteins, and lipoproteins. The contact time is usually 1-2 hours (endotoxin removal time is 12 hours or longer), followed by rinsing approximately 10 column volumes with deionized water;

6.3. Rinse 5-10 column volumes of the chromatography column with 70% ethanol or 30% isopropanol, followed by rinsing approximately 10 column volumes with deionized water.

Compatibility of reagents

Buffer solution	50mM sodium phosphate, pH 7.4 100mM Tris-HCl, pH 7.4 100mM Tris-acetate, pH 7.4 100mM HEPES, pH 7.4 100mM MOPS, pH 7.4 100mM sodium acetate, pH 4
Denaturant	8 M Urea

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	6 M Gua-HCl
Detergent	2% Triton X-100 2% Tween 20 2% NP-40 2% Cholate 1% CHAPS
Reducing agent	5mM DTE 2mM DTT 20mM β-mercaptoethanol 5mM TCEP 10mM reduced glutathione
other additives	500mM Imidazole 20% Ethanol 50% Glycerol 100mM Na2SO4 1.5 M NaCl 1mM EDTA 60mM Citrate