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Ni-IDA Agarose

1 Packing list

Components	HY-K0238-5 mL	HY-K0238-10 mL	HY-K0238-50 mL
Ni-IDA Agarose	5 mL	10 mL	10 mL × 5

2 Introduction

MCE Ni-IDA Agarose is a highly cross-linked 4% agarose gel, synthesized through the chemically directed, high-density attachment of iminodiacetic acid (IDA). When chelated with Ni^{2+}, it forms an extremely stable planar tetragonal structure, providing three ionic bonding sites. This configuration allows for the higher affinity purification of His-tag proteins from various expression sources such as *E.coli*, yeast, insect cells, and mammalian cells.

3 Characteristics

Composition	4% cross-linked agarose
Ligand	IDA
Binding Capacity	> 40 mg 6× His protein/mL
Bead Diameter	45-135 μm
Pressure	0.3 MPa, 3 bar
Flow Rate	80-150 cm/h
Gel Concentration	50% slurry
Storage Solution	1× PBS containing 20% ethanol

4 General Protocol

Buffer Preparation

Lysis/Equilibration Buffer	50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8.0
Washing Buffer	50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, pH 8.0
Elution Buffer	50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, pH 8.0
Storage Buffer	1× PBS, 20% ethanol

Note: a. It is recommended to prepare all buffers with ultrapure water and. After preparation, filter them through a 0.45 μm or 0.22 μm membrane for sterilization.

b. The above buffer formulation is for reference only and can be adjusted according to the experiment.

Protocol

1. Sample Preparation

   For protein expressed in *E. coli* or yeast cytoplasm

   1) Collect cells by centrifugation at 4°C.

   2) Resuspend the cell pellet in an appropriate volume of lysis buffer, and add protease inhibitor according to the experimental requirements.

      Note: The buffers and inhibitors must have no effect on the ability of the Ni2+ resin (such as EDTA, EGTA, DTT, β-ME).

   3) Choose appropriate sonication or homogenization conditions to lyse the cells, keeping the samples at a low temperature to prevent denaturation and precipitation.

      Note: If the lysate is too viscous, add RNase A (10 μg/mL) and DNase I (5 μg/mL) and incubate on ice for 10-15 min.

   4) Centrifuge the lysate at 12,000 rpm for 30 min at 4°C to pellet the cellular debris, collect the supernatant and filter it using a 0.22 μm or 0.45 μm filter to prepare the sample for purification.

   For proteins secreted into culture medium by yeast, insect, or mammalian expression systems

   1) Collect the supernatant by centrifugation at 4°C.

   2) If the supernatant from the culture medium does not contain components such as EDTA, EGTA, DTT, β-ME, or His, it can be directly filtered using a 0.22 μm or 0.45 μm filter to prepare the sample for purification. However, if these components are present, the sample should be dialyzed after filtration.

      Note: For larger volumes of culture medium supernatant, the proteins can be concentrated using ammonium sulfate precipitation or enriched through ion exchange before affinity chromatography.

2. Column Preparation

   1) Load the column: Load Ni-IDA Agarose in an appropriate chromatography column and connect it to the chromatography system.

   2) Equilibration: Equilibrate the column by washing it with 5× column volumes of Binding Buffer. Repeat 2-3 times until the absorbance at 280 nm stabilizes.

3. Purfication

   1) Loading: Loading sample using a pump or sample loops, collecting the effluent. Repeating this loading process can improve the binding efficiency.

      Note: a. Please choose a suitable gel volume according to the amount of protein to avoid exceeding the loading capacity of agarose.

      b. An increase in sample viscosity or volume may lead to column backpressure.

   2) Equilibration: Wash the column with approximately 5-10× column volumes of Equilibration Buffer to equilibrate the column.

   3) Washing: Wash the column with approximately 10-20× column volumes of Washing Buffer to remove nonspecifically adsorbed hetero-proteins and collect the wash effluent until the absorbance at 280 nm stabilizes.

   4) Elution: Elute the column with approximately 5-10× column volumes of Elution Buffer, and collect the eluate in separate tubes based on the absorbance at 280 nm.

   5) The eluted protein should be dialyzed into an appropriate storage solution (such as 1× PBS), according to the requirements of the experiment.

4. Regeneration

   1) 2× column volumes of 6 M GuHCl and 0.2 M acetic acid.

   2) 5× column volumes of deionized water.

   3) 3× column volumes of 2% SDS.

   4) 5× column volumes of deionized water.

   5) 5× column volumes of 100% EtOH.

   6) 5× column volumes of deionized water.

   7) 5× column volumes of 100 mM EDTA (pH 8.0).

   8) 5× column volumes of deionized water.

   9) 5× column volumes of 100 mM $NiSO_4$.

   10) 10× column volumes of deionized water.

   11) 5× column volumes of Storage Buffer, and store at 2-8°C.

5 Storage

4°C, 2 years

Do not dry or freeze

6 Precautions

1. Please resuspend the gel thoroughly before use.
2. To minimize protein degradation, protease inhibitor cocktails (MCE Cat. No. HY-K0010, HY-K0011) are highly recommended.
3. This product is for R&D use only, not for drug, household, or other uses.
4. For your safety and health, please wear a lab coat and disposable gloves to operate.