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Ni-NTA Magnetic Agarose Beads

1 Packing list

Components	HY-K0241-1 mL	HY-K0241-5 mL	HY-K0241-10 mL
Ni-NTA Magnetic Agarose Beads	1 mL	5 mL	10 mL

2 Introduction

MCE Ni-NTA Magnetic Agarose Beads is a highly cross-linked 4% magnetic agarose, synthesized through the chemically directed, high-density attachment ofnitrilotriacetic acid (NTA). When chelated with Ni^{2+}, it forms an extremely stable planar tetragonal structure, providing three ionic bonding sites. It has high loading capacity, exceptional specificity, and stability, can be used for the detection and 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 magnetic agarose
Ligand	NTA
Binding Capacity	> 20 mg 6× His protein/mL
Bead Diameter	30-100 μm
Bead Volume	50% of Suspension Volume
Storage Solution	1× PBS containing 20% ethanol

4 General Protocol

Buffer Preparation

Binding/Washing Buffer	50 mM Tris, 500 mM NaCl, pH 7.4
Elution Buffer	50 mM Tris, 500 mM NaCl, 250 mM imidazole, pH 7.4
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

It is recommended to filter the sample with a 0.22 μm or 0.45 μm filter before purification.

1. Preparation of Magnetic Agarose Beads
   1) Thoroughly mix the magnetic agarose beads. Choose an appropriate volume of Ni-NTA magnetic agarose beads suspension according to the amount of samples and transfer it into a tube. Place the tube onto a magnetic separator, perform the magnetic separation for 1 min, and then discard the supernatant.
   2) Add an equal volume of the equilibrium buffer to the suspension, and mix thoroughly. Perform magnetic separation for 1 min, and discard the supernatant. Repeat this process 2-3 times.
2. Binding
   Add the sample and incubate at 4°C for 2-4 h or at room temperature for 1-2 h (the specific incubation time can be adjusted based on the binding effect).
3. Washing
   After incubation, perform magnetic separation for 1 min and remove the supernatant (the supernatant can be retained as flow-through for electrophoretic analysis). Wash the beads with 5× the volume of Washing Buffer, perform magnetic separation for 1 min and collect the supernatant. Repeat 3-5 times.
4. Elution
   Add an equal volume of Elution Buffer to the magnetic agarose beads, and mix thoroughly. Perform magnetic separation for 5-10 min and collect the eluate. Repeat 5-10 times and collect the eluate separately.
   Note: Gradient elution with different concentrations of imidazole can also be used and the eluates collected separately.
5. Regeneration
   1) Add 2× the volume of Elution Buffer, perform magnetic separation for 5-10 min and discard the supernatant. Repeat 2-3 times.
   2) Add 2× the volume of deionized water, perform magnetic separation for 5-10 min and discard the supernatant. Repeat 2-3 times.
   3) Add 2× the volume of Storage Buffer, perform magnetic separation for 5-10 min and discard the supernatant. Repeat 2-3 times.
   4) Add 2× the volume of Storage Buffer, and store at 2-8°C.

5 Storage

4°C, 2 years
Do not dry or freeze

6 Precautions

1. Do not centrifuge, dry or freeze the magnetic beads, which will cause the beads to aggregate and lose binding affinity.
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.