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Protein A Plus Magnetic Agarose Beads

1 Packing list

Components	HY-K0242-1 mL	HY-K0242-5 mL	HY-K0242-10 mL
Protein A Plus Magnetic Agarose Beads	1 mL	5 mL	10 mL

2 Introduction

Protein A is a bacterial cell wall protein isolated from Staphylococcus aureus and binds to most mammalian IgGs mainly through Fc regions. Native Protein A contains 5 IgG binding domains and many other domains with unknown functions. The latter have been eliminated from recombinant Protein A to reduce nonspecific binding.

MCE Protein A Plus Magnetic Agarose Beads is produced through the covalent coupling of recombinant protein A with magnetic agarose beads. It has high loading capacity, exceptional specificity, and stability, can be used for the detection and purification of IgG from serum, ascites fluid, cell culture supernatant and other antibody samples.

3 Characteristics

Composition	Magnetic agarose
Ligand	Protein A
Binding Capacity	> 20 mg Human IgG/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	20 mM Na_2HPO_4, 500 mM NaCl, pH 7.2 or 1× PBS
Elution Buffer	0.1 M Glycine, pH 3.0
Neutralization buffer	1 M Tris-HCl, 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

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 Protein A Plus 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 3-5× the volume of Elution Buffer to the magnetic agarose beads, and mix thoroughly. Perform magnetic separation for 5-10 min and collect the eluate. The final collected eluate is acidic, Neutralization Buffer should be added immediately to adjust the pH (1/10 volume of total eluent volume), and the samples can be used for functional analysis. Repeat 2-3 times and collect the supernatant separately.
5. Regeneration
   1) Add 5× the volume of Elution Buffer, perform magnetic separation for 2 min and discard the supernatant. Repeat 2-3 times.
   2) Add 5× the volume of Washing Buffer, perform magnetic separation for 2 min and discard the supernatant. Repeat 3-5 times.
   2) Add 5× the volume of deionized water, perform magnetic separation for 2 min and discard the supernatant. Repeat 3-5 times.
   3) Add 5× the volume of Storage Buffer, perform magnetic separation for 5-10 min and discard the supernatant. Repeat 2-3 times.
   4) Add 5× the volume of Storage Buffer, and store at 2-8°C.

5 Storage

4ºC, 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.