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Anti-HA Magnetic Agarose Beads

1 Packing list

Components	HY-K0237-1 mL	HY-K0237-5 mL	HY-K0237-10 mL
Anti-Flag Magnetic Agarose Beads	1 mL	5 mL	10 mL

2 Introduction

The HA tag corresponds to the 98-106 amino acid sequence of human influenza haemagglutinin, denoted by the sequence YPYDVPDYA.
MCE Anti-HA Magnetic Agarose Beads is produced through the covalent coupling of high-quality Flag antibody with Magnetic Agarose Beads. It boasts a high loading capacity, exceptional specificity, and stability. It can be used for the detection and purification of HA fusion-expressed proteins. Moreover, it can be utilized for IP assays.

3 Characteristics

Composition	Magnetic Agarose Beads
Ligand	Anti-HA Antibody (mouse)
Binding Capacity	≥ 1 mg HA tagged protein/mL of beads
Bead Diameter	30-100 μm
Bead Volume	20% of Suspension Volume
Storage Solution	1× PBS，0.02% NaN_3

4 General Protocol

Buffer Preparation

Equilibrium/Washing Buffer	50 mM Tris, 0.15 M NaCl, pH 7.4
Elution Buffer I	0.1 M Glycine, pH 3.0
Elution Buffer II	50 mM Tris, 0.15 M NaCl, 100-500 μg/mL HA Peptide, pH 7.4
Neutralization buffer	1 M Tris-HCl, pH 8.0
Storage Buffer	PBS，0.02% NaN_3

Note: 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.

Protein Purfication

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 Anti-HA 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. Sample Binding
   Add the sample and incubate at 4°C for 1-2 h or at room temperature for 0.5-1 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 discard the supernatant. Repeat 3-5 times.
4. Elution
   Two recommended elution methods depend on protein characteristics or further usage.
   1) Acidic condition Elution with Elution Buffer Ⅰ: This method maintains the original biological activity of the eluted samples and is suitable for subsequent functional analyses.
      Add 3-5× of Elution Buffer Ⅰ to the tube. Mix well and incubate for 5-10 min at room temperature. Perform magnetic separation for 1 min and collect the supernatant. The collected supernatant contains the target protein. The final collected eluate is acidic, Neutralization Buffer should be added immediately to adjust the pH (1/10 volume of total eluent volume), then the samples can be used for functional analysis. Repeat 2-3 times and collect the supernatant separately.
   Note: a. The magnetic agarose beads should be balanced with Washing Buffer immediately after acidic elution, and the beads should not be kept in the Elution Buffer Ⅰ for more than 20 min.
   b. The eluates can be used immediately if stored at 4ºC, or stored at -20ºC for long term storage.
   2) Competitive Elution with Elution Buffer Ⅱ: This method maintains the original biological activity of the eluted samples and is suitable for subsequent functional analyses.
      Add 3-5× of Elution Buffer Ⅱ to the tube. Mix well and incubate for 5-10 min at room temperature. Perform magnetic separation for 1 min and collect the supernatant. The collected supernatant contains the target protein.
      Note: The eluates can be used immediately if stored at 4ºC, or stored at -20ºC for long term storage.

IP/Co-IP

1. Preparation of Magnetic Agarose Beads
   1) Transfer an appropriate volume of Anti-HA magnetic agarose beads suspension to a tube, perform magnetic separation for 1 min and discard the supernatant.
   2) Add an equal volume of Equilibrium Buffer and mix well, perform magnetic separation for 1 min and discard the supernatant. Repeat 2-3 times.
2. Sample Binding
   Add samples of target proteins containing Flag tags to the magnetic beads, and incubate at 4°C for 1-2 h or at room temperature for 0.5-1 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 discard the supernatant. Repeat 3-5 times. For IP, proceed directly to step 6 for elution. For co-IP, steps 4 and 5 are required.
   Note: The purpose of multiple washes is to ensure the removal of non-specific adsorption.
4. Co-precipitation
   Add the sample containing the target protein to the protein-magnetic beads complex, and incubate at 4°C for 1-2 h or at room temperature for 0.5-1 h (the specific incubation time can be adjusted based on the binding effect).

5. Washing

   After incubation, perform magnetic separation for 1 min and remove the supernatant (the supernatant can be retained for electrophoretic analysis). Wash the beads with 5× the volume of Washing Buffer, perform magnetic separation for 1 min and discard the supernatant. Repeat 3-5 times.

6. Elution

   Three recommended elution methods depend on protein characteristics or further usage.

   1) Acidic condition Elution with Elution Buffer Ⅰ: This method maintains the original biological activity of the eluted samples and is suitable for subsequent functional analyses.

      Add 3-5× of Elution Buffer Ⅰ to the tube. Mix well and incubate for 5-10 min at room temperature. Perform magnetic separation for 1 min and collect the supernatant. The collected supernatant contains the target protein. The final collected eluate is acidic, Neutralization Buffer should be added immediately to adjust the pH (1/10 volume of total eluent volume), then the samples can be used for functional analysis. Repeat 2-3 times and collect the supernatant separately.

   Note: a. The magnetic agarose beads should be balanced with Washing Buffer immediately after acidic elution, and the beads should not be kept in the Elution Buffer Ⅰ for more than 20 min.

   b. The eluates can be used immediately if stored at 4ºC, or stored at -20ºC for long term storage.

   2) Competitive Elution with Elution Buffer Ⅱ: This method maintains the original biological activity of the eluted samples and is suitable for subsequent functional analyses.

      Add 3-5× of Elution Buffer Ⅱ to the tube. Mix well and incubate for 5-10 min at room temperature. Perform magnetic separation for 1 min and collect the supernatant. The collected supernatant contains the target protein.

      Note: The eluates can be used immediately if stored at 4ºC, or stored at -20ºC for long term storage.

   3) Gel Electrophoresis and Immunoblotting Elution with SDS-PAGE Loading Buffer.

      Add an equal volume of 2× SDS-PAGE Loading Buffer to the magnetic agarose beads, Mix well and heat for 5 min at 95 ºC, perform magnetic separation for 1 min, reserve the supernatant for SDS-PAGE analysis.

      Note: As regular SDS-PAGE Loading Buffer contains β-mercaptoethanol and DTT, which can break the light and heavy chains of the antibody in the packing. And the SDS-containing Loading Buffer can denature the medium ligand. Anti-ha Magnetic Agarose Beads cannot be reused after denaturing elution.

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. Before using this product for IP (Immunoprecipitation) experiments, it is necessary to confirm the expression status of HA tagged proteins in the sample.
3. To minimize protein degradation, protease inhibitor cocktails (MCE Cat. No. HY-K0010, HY-K0011) are highly recommended.
4. Do not use cell lysate samples containing DTT. DTT may cause the HA antibodies on the bead to detach.
5. This product is for R&D use only, not for drug, household, or other uses.
6. For your safety and health, please wear a lab coat and disposable gloves to operate.