Anti-HA Agarose Magnetic Particles, 30~100 µm
PRODUCT DATA SHEET

Anti-HA Agarose Magnetic Particles, 30~100 µm

Cat No: AM-Anti-HA-3000

Description

Anti-HA agarose beads are agarose beads with an average particle size of about 70 µm that are covalently bound to a large number of high-quality murine anti-HA monoclonal antibodies on the surface. Agarose magnetic bead series is a new type of functional magnetic microsphere formed by the combination of natural polymer material agarose and superparamagnetic material, which has the characteristics of faster magnetic response while maintaining good dispersion of the microsphere, extremely low non-specific adsorption and more abundant binding sites. This product is widely used in the immunoprecipitation (IP), immunoco-precipitation (Co-IP) or Pull-down test with HA label fusion protein in cell lysate, cell secreted supernatant, serum, ascites and other samples, and can also be used for the purification of HA label fusion protein.

For custom sizes, formulations or bulk quantities please contact our customer service department.

Website: www.abvigen.com Phone: +1 929-202-3014 Email: info@abvigenus.com

Characteristics

Matrix	Agarose magnetic beads
Ligand	Murine monoclonal antibody against HA (G2)
Particle size	30~100 µm
Concentration	The magnetic particles volume accounts for 20% of the suspension volume
Binding ability	≥ 1.0 mg HA tag fusion protein/mL magnetic particles
Scope of application	IP, Co-IP, purification of HA tag fusion protein
Shelf life	Stable storage at 2 ~ 8°C for 2 years

Operation process

Note: Prepare your own buffer or purchase our IP kit directly.

Adherent cell sample:

1. Remove the medium and wash the cells twice with PBS.

2. Cells were collected into 1.5 mL EP tube and IP Lysis/Wash Buffer was added proportioned, and add corresponding inhibitor such as PMSF. After mixing, the cells were placed on ice for 5 ~ 20 min (mixed several times).

3. At 4°C, 12000 ~ 16000 xg, centrifuge for 10 min to collect the supernatant and place it on ice for subsequent experiments (or place it at -80°C for long-term storage).

Petri dish IP Lysis/Wash Buffer Recommended volume:

Petri dish size/surface area	Volume of IP Lysis/Wash Buffer
100 mm x 100 mm	500 ~ 1000 µL
100 mm x 60 mm	100 ~ 300 µL
6-well plate	100 ~ 200 µL

Suspended cell sample:

1. Cells were collected at 4°C, 500 ~ 1000 xg, 10 min, and the supernatant was discarded.

2. Wash the cells with PBS once, that is, suspend the cell mass with PBS at 4°C, 500 ~ 1000 xg, 5 min, collect the cells, discard the supernatant.

3. Resuspend cells with pre-cooled IP Lysis/Wash Buffer. The 500 µL IP Lysis/Wash Buffer was used for every 50 mg cells. At the same time, add the corresponding inhibitors such as PMSF, mix well and put on the ice for 5 ~ 20 min (mix several times during the period).

4. At 4°C,12000 ~ 16000 xg, centrifuge for 10 min to collect the supernatant and place it on ice for subsequent experiments (or place it at -80°C for long-term storage).

Serum sample:

It is recommended to dilute a serum sample with IP Lysis/Wash Buffer to a final concentration of target protein of 50 to 150 µg/mL and place it on ice for future use (or place it at -20°C for long-term storage).

Immunoprecipitation:

Note: In order to ensure the uniform distribution of magnetic beads, mix the magnetic beads in the bottle by repeatedly reversing or slightly vortexing before use.

1. Add 20 to 50 µL of Anti-HA agarose magnetic bead into 1.5 mL centrifuge tube.

2. Add 500 µL pre-cooled PBS to the magnetic bead and mix gently.

3. Put the centrifugal tube into the magnetic rack and collect magnetic beads to one side of the centrifugal tube. Remove the supernatant.

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4. Add 200 ~ 500 µL IP Lysis/Wash Buffer to the centrifuge tube. Reverse the centrifugal tube several times or mix with a slight vortex for 1 min. Use a magnetic rack to collect magnetic beads. Remove the supernatant.

5. Add the protein samples containing HA labels into the centrifuge tube fitted with magnetic beads and incubate at room temperature for 1 ~ 2 h, or 4°C 2 ~ 4 h.

6. Collect magnetic beads with a magnetic rack, remove uncombined samples, and store them for analysis.

7. Add 1000 µL IP Lysis/Wash Buffer to the centrifuge tube and gently mix the magnetic ball for 5 ~ 10 min. Collect the beads and discard the supernatant. Repeat twice.

8. **Denaturing elution:** Add 80 ~ 100 µL SDS-PAGE Sample Loading Buffer (1×) to the centrifuge tube and heat the sample in 100°C water bath or metal bath for 10 min. Magnetic beads are separated by magnetic rack to retain the supernatant containing the target antigen.

Note: The following elution methods can also be used to maintain protein activity.

Low pH Elution: Add 100 µL Elution Buffer to centrifuge tube. Keep mixed and incubate centrifuge tube at room temperature for 5 ~ 10 min. Magnetic beads are magnetically separated to retain the supernatant containing the target antigen. Low pH is neutralized by adding 20 µL Neutralization buffers per 100 µL of wash out solution.

Notes

1. Before performing the experiment, please read this operation manual carefully.

2. Do not centrifuge, dry or freeze magnetic beads at high speed, these operations will cause magnetic beads to gather and reduce the binding ability.

3. The affinities between different types of antibodies and antigen Lysis are different in IP experiments. The binding of antibodies to antigens is also affected by IP Lysis/Wash Buffer, so **you can do your own experiments by optimizing the operation details or screening and formulating buffers**.

4. Magnetic beads should be fully oscillated and uniform before use. Magnetic beads should be kept in storage solution to prevent drying.

5. This product is for scientific research only.

Problem solving

1. No immunoprecipitation of antigen

① The sample contains too little antigen to be detected

Recommendation: To verify the expression and/or cleavage efficiency of proteins in the lysate by SDS-PAGE or protein immunoblotting; If necessary, increase the sample size

② Ingredients in IP Lysis/Wash Buffer interfere with the binding of antigens to antibodies

Recommendation: Immunoprecipitation and rinsing with other buffers (e.g., TBS containing 0.5% CHAPS)

2. Low protein intake

① The protein is degraded

Recommendation: Add protease inhibitor

② The amount of magnetic beads used is not enough

Recommendation: Increase the amount of magnetic beads used to capture immune complexes

③ The amount of target protein in the sample is not enough

Recommendation: Increase the amount of antigen sample

3. Multiple non-specific bands

There are non-specific proteins that bind to the magnetic beads

Recommendation: Add 50 ~ 350 mM NaCl to IP Lysis/Wash Buffer. Increase elution intensity and frequency.

4. Magnetic beads gather

The aggregation of magnetic beads in the low pH Elution Buffer is a normal phenomenon and does not affect the normal use of magnetic beads.

Recommendation: The magnetic beads can be restored to uniform state by using IP Lysis/Wash Buffer to neutral and then oscillating with Tris buffer (pH 7.5) containing 0.1% (v/v) Tweene-20 and treated with ultrasonic water bath. None of the above treatments affect the antibody binding efficiency of the magnetic beads. A non-ionic detergent with a final concentration of 0.1% (v/v), such as Tween-20 or Triton X-100, can also be added to effectively prevent the accumulation of magnetic beads.

Note: Ultrasonic treatment will also remove antibodies captured by magnetic beads in the sample solution, so this method should not be used before elution of magnetic beads after sample addition.

Ordering Information

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