Anti-HA Magnetic Beads

OM625712

Product Description

Anti-HA magnetic beads are based on carboxyl magnetic beads, with 200 nm particle size, covalently coupling with high quality mouse IgG_1 monoclonal antibody that recognizes the HA-epitope tag (YPYDVPDYA) derived from the human influenza hemagglutinin (HA) protein.

Product Features

Composition	mouse IgG_1 monoclonal Ab
Magnetization	Superparamagnetic
Particle size	200 nm
Concentration	10 mg/mL
Binding Capacity	≥ 0.4 mg HA-tagged fusion protein /mL of bead
Application	IP , CoIP
Storage Condition	Store at 4°C for 2 years.

Protocol

1. Cell lysis

Cells may be lysed using any standard cell lysis protocol compatible with your starting material. We recommend the use of Cell Extraction Buffer or NP-40 Cell Lysis Buffer.

2. Preparation of Magnetic Beads

2.1 Resuspend the Magnetic Beads in the vial (tilt and rotate for 2 minutes or gently pipette for 10 times).

2.2 Transfer 10-20 µL of Anti-HA Magnetic Beads into a 1.5 mL tube (Transfer amount may be adjusted as required).

2.3 Add 500 µL of binding/wash buffer to the beads and gently pipette to mix. Place the tube into a magnetic stand to collect the beads against the side of the tube (Hereinafter referred to as magnetic separation). Remove and discard the supernatant. Repeat this step for 2 times.

3. Immunoprecipitation

3.1 Remove the tubes from the magnetic separator and add your sample containing HA-tagged protein to the pre-washed magnetic beads and incubate at room temperature for 30 minutes or 2 hours at 4°C with mixing.

3.2 Collect the beads with a magnetic stand, remove the unbound sample and save for analysis.

3.3 Add 300µL of TBS-T to the tube and gently mix. Collect the beads and discard the supernatant. Repeat this wash twice.

3.4 Add 300µL of ultrapure water to the tube and gently mix. Collect the beads on a magnetic stand and discard the supernatant.

4. Elution

Note: Select one of the elution protocols below. If the eluted HA-tagged protein will be used for function applications or is sensitive to pH extremes, then elute the protein with HA Peptide.

Gentle Elution Protocol

1. Prepare HA Peptide at 2mg/mL in TBS.
2. Add 100 uL of 2mg/mL HA Peptide to the beads, gently vortex to mix and incubate the sample at 37°C on a rotator for 5-10 minutes. Elution may be performed at reduced temperatures, but lower yields may result.
3. Separate the beads on a magnetic stand and save the supernatant containing the target antigen.
4. Repeat elution step once for higher recovery.

Chemical Elution Protocols

• Basic Elution

1. Add 100µL of 50mM NaOH to the tube.
2. Gently vortex to mix and incubate the sample at room temperature on a rotator for 5 minutes.
3. Magnetically separate the beads and save the supernatant containing the target antigen.
4. Neutralize the sample by adding 50µL of Neutralization Buffer for each 100µL of eluate.

• Acidic Elution

1. Add 100µL of IgG Elution Buffer, pH 2.0 or 0.1M glycine, pH 2.0.
2. Gently vortex to mix and incubate the sample at room temperature on a rotator for 5-10 minutes.

3. Magnetically separate the beads and save the supernatant containing the target antigen.
4. To neutralize the low pH, add 15μL of Neutralization Buffer for each 100μL of eluate.

The final solution can be used as samples for denaturing SDS-PAGE. Or the elution can be adjusted to neutral pH with neutralization buffer immediately and used for further analysis.

Troubleshooting

Problem	Possible Cause	Solution
High background band	Non-specific binding of proteins to antibodies, insufficient washing on magnetic beads or EP tubes	The lysate was pretreated to remove non-specific proteins; before the last wash, the entire sample was transferred to a new EP tube and centrifuged.
	Not enough washing	Increase the time and number of washes
No protein band	No or minimal tagged protein was expressed	Verify protein expression ; Prepare fresh lysate ; Use the appropriate protease inhibitor.
	Insufficient incubation time	Increase incubation time
	Interfering substances in the sample	High concentrations of DTT, 2-mercaptoethanol or other reducing agents are present in the lysate.