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MCE MedChemExpress

Anti-HA Magnetic Beads (1 μm)

1 Contents

Cat. No.	Product Name	Package
HY-K0201A-1 mL	Anti-HA Magnetic Beads (1 μm)	1 mL
HY-K0201A-5 mL	Anti-HA Magnetic Beads (1 μm)	1 mL × 5

2 General Information

MCE Anti-HA Magnetic Beads are used for immunoprecipitation (IP) of specific HA-tagged proteins expressed in bacterial and mammalian cells and *in vitro* expression systems. Anti-HA magnetic beads are based on hydroxyl magnetic beads, with 1 μm particle size, covalently coupling with high quality mouse IgG3a monoclonal antibody that recognizes the HA-epitope tag (YPYDVPDYA) derived from the human influenza hemagglutinin (HA) protein. Magnetic beads are removed from the solution manually by using a magnetic stand or automatically by using an instrument. With high loading of HA-tagged protein and high specificity, Anti-HA Magnetic Beads are also suitable for Co-immunoprecipitation and purification of HA-tagged protein.

3 Characteristics

Composition	Mouse IgG3a monoclonal antibody covalently coupled to a blocked magnetic bead surface
Antibody Purification	Purify antibodies using the Protein A ligand
Bead Diameter	1 μm
Binding Capacity	>0.6 mg protein/mL of beads
Application	IP, Protein Purification
Recommended Dose	10 μL for per 500 μL cell lysates

4 General Protocol

Wash Buffer	TBST: 50 mM Tris-HCl, 150 mM NaCl, 0.5% Tween-20, pH 7.4
Elution Buffer A	0.15 M Glycine, pH 2.5-3.1
Elution Buffer B	2 mg/mL HA peptide, 50 mM Tris, 150 mM NaCl, pH 7.4
Neutralization Buffer	1 M Tris-HCl, pH 8.0

1. Preparation of Magnetic Beads

1.1 Resuspend the Magnetic Beads in the vial (tilt and rotate for 2 minutes or gently pipette for 10 times, do not vortex). Transfer 10 μL of Anti-HA Magnetic Beads suspension into a new tube.

1.2 Add 500 μL of wash buffer to the beads and gently pipette to mix. Place the tube into a magnetic stand (MCE Cat. No.: HY-K0200) to collect the beads against the side of the tube. Remove and discard the supernatant. Repeat this step for 2 times.

2. Protein Binding

2.1 Add 500 μL of cell lysate (the sample containing HA-tagged protein) to the washed beads. For Ag binding, incubate for 2 hours at room temperature or overnight at 4°C while gently rotating the tube.

2.2 Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant.

Note: Occasional aggregation of magnetic beads during the binding process doesn't affect experimental results.

3. Washing

Add 500 μL of wash buffer to the Magbeads-Ag complex and mix gently. Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant. Repeat this step for 4 times.

4. Elution & Detection

Three elution methods are recommended according to protein characteristics or further usage:

1) Elution with sample buffer for gel electrophoresis and immuoblotting.

Add 50 μL of 1× SDS-PAGE loading buffer to each tube and boil for 5 minutes. Cool and place the tube into a magnetic stand to collect the beads and transfer the supernatant to a new tube. Keep the supernatant containing the target antigen for SDS-PAGE analysis.

2) Elution with Elution Buffer A under acidic condition.

Add 50 μL of Elution Buffer A to each tube. Incubate with gentle shaking or on a rotator for 10 minutes at room temperature. Place the tube into a magnetic stand to collect the beads and transfer the supernatant to a new tube. Adding 25 μL of Neutralization Buffer for each 50 μL of eluate to neutralize the low pH, which may help preserve bioactivity of target protein.

3) Elution with Elution Buffer B under native condition.

Add 3-5 (v/v) volume of Elution Buffer B to each tube. Incubate with gentle

shaking or on a rotator for 1 hour at room temperature or 2 hours at 4ºC. Place the tube into a magnetic stand to collect the beads and transfer the supernatant to a new tube. For immediate use, store the eluates at 4ºC, or store at -20ºC for long term storage.

5 Storage

Stored at 4°C, and is stable for up to 2 years.

6 Precautions

1 The pH of Anti-HA Magnetic Beads is 6-8.

2 Do not centrifuge, dry or freeze the magnetic beads. Centrifuging, drying or freezing will cause the beads to aggregate and lose binding affinity.

3 For best results, determine optimal conditions for expression of HAtagged fusion protein before attempting immunoprecipitation.

4 To minimize protein degradation, protease inhibitor cocktails (MCE Cat.No.: HY-K0010, HY-K0011) are highly recommended.

5 For the best experimental performance, it is recommended to use the MCE magnetic stand (Cat. No: HY-K0200).

6 Do not use cell lysate containing dithiothreitol (DTT). DTT may cause the HA antibody to leach from the beads.

7 This product is for R&D use only, and is not for drug, house hold, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

7 Troubleshooting

Problem	Possible Cause	Solution
High background	Nonspecific binding of protein to the antibody, magnetic beads or EP tubes	Pre-clear lysate toremove nonspecific binding proteins
		After suspending beads for the final wash, transfer the entiresample to a clear EP tube and then magnetic separation orcentrifugation
	Washing times were not sufficient	Increase the number and time of washes
Little or no HA-tagged protein is detected	No or minimal tagged protein was expressed	Verify protein expression by SDS-PAGE or Western Blot analysis of the lysate using an HA-tagged positive control as a reference
		Increase the amount of lysate used for IP
	Tagged protein degraded	Prepare freshlysate
		Use appropriate protease inhibitors (MCE Cat. No.: HY-K0010, HY-K0011)
	Incubation time wasinadequate	Prolong the incubation time
	Interfering substancewas contained	Do not use cell lysate containing dithiothreitol(DTT), 2-mercaptoethanol, or other reducing agents
		Excessive detergentconcentration may interfere with theantibody-antigen interaction