

# Anti-HA Magnetic Beads

## 1 Contents

Cat. No.	Product Name	Package
HY-K0201-1 mL	Anti-HA Magnetic Beads	1 mL
HY-K0201-5 mL	Anti-HA Magnetic Beads	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 2  $\mu$ m 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	2 $\mu$ m
Binding Capacity	>0.6 mg protein/mL of beads
Application	IP, Protein Purification
Recommended Dose	10 $\mu$ L for per 500 $\mu$ L 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  $\mu$ L of Anti-HA Magnetic Beads suspension into a new tube.

1.2 Add 500  $\mu$ L 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  $\mu$ L 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  $\mu$ L 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 immunoblotting.

Add 50  $\mu$ L 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  $\mu$ L 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  $\mu$ L of Neutralization Buffer for each 50  $\mu$ L 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 4°C. 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 4°C, or store at -20°C 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 HA-tagged 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 to remove nonspecific binding proteins
		After suspending beads for the final wash, transfer the entire sample to a clear EP tube and then magnetic separation or centrifugation
	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 fresh lysate
		Use appropriate protease inhibitors (MCE Cat. No.: HY-K0010, HY-K0011)
	Incubation time was inadequate	Prolong the incubation time
	Interfering substance was contained	Do not use cell lysate containing dithiothreitol (DTT), 2-mercaptoethanol, or other reducing agents
		Excessive detergent concentration may interfere with the antibody-antigen interaction