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Anti-Flag Magnetic Beads

1 Contents

Cat. No.	Product Name	Package
HY-K0207-1 mL	Anti-Flag Magnetic Beads	1mL
HY-K0207-2 mL	Anti-Flag Magnetic Beads	1mL × 2
HY-K0207-5 mL	Anti-Flag Magnetic Beads	1 mL × 5

2 General Information

MCE Anti-Flag magnetic beads are used for immunoprecipitation (IP) of specific Flag-tagged proteins expressed in bacterial and mammalian cells and in vitro expression systems. Anti-Flag magnetic beads are based on amino magnetic beads, with 200 nm particle size, covalently coupled with high quality mouse IgG_1 monoclonal antibody that recognizes the Flag octapeptide sequence (DYKDDDDK). Magnetic beads are removed from the solution manually by using a magnetic stand or automatically by using an instrument. With high loading of Flag-tagged protein and high specificity, Anti-Flag Magnetic Beads are also suitable for Co-immunoprecipitation and purification of Flag-tagged protein.

3 Characteristics

Composition	Mouse IgG_1 monoclonal antibody covalently coupled to a blocked magnetic bead surface
Bead Diameter	200 nm
Bead Concentration	10 mg/mL
Binding Capacity	>0.6 mg protein/mL of beads
Application	IP, Co-IP, Protein Purification
Recommended Dose	10 μL for per 500 μL cell lysates

4 General Protocol

Recommended Buffer

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	1 mg/mL 3× Flag peptide, 50 mM Tris, 0.15 M NaCl, pH 7.4
Neutralization Buffer	1 M Tris-HCl, pH 8.0

1. Preparation of Magnetic Beads

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-Flag Magnetic Beads suspension into a new tube.

2) Add 500 μL of 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. Remove and discard the supernatant. Repeat this step for 2 times.

2. Protein Binding

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

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

Store at 4ºC, and is stable for up to 2 years.

6 Precautions

1. The pH of Anti-Flag 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 the best results, determine optimal conditions for expression of Flag-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 Anti-Flag antibody to leach from the beads.
7. This product is for R&D use only, not for drug, house hold, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

7 Trouble Shooting

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 use magnetic separation or centrifugation
	Washing times were not sufficient	Increase the number and time of washes
Little or no Flag-tagged protein is detected	No or minimal tagged protein was expressed	Verify protein expression by SDS-PAGE or Western blot by using an Flag-tagged positive control
		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