UltraBio™ Anti-HA Magnetic Beads

U1508191

Storage: 2-8℃. Do not freeze.

Shipping: Shipped with ice packs. Please store under the specified storage conditions immediately upon receipt.

Size: 0.5 mL/1 mL.

Introduction

This product is prepared by covalently coupling high-quality mouse monoclonal Antibody against HA to micron-sized carboxyl magnetic beads. It features rapid magnetic responsiveness, superparamagnetism, good dispersibility, uniform particle size, extremely low non-specific binding, and abundant binding sites. It can be used for immunoprecipitation (IP), co-immunoprecipitation (Co-IP), or purification of Flag-tagged proteins.

Product information is detailed in the table below:

Parameter	Specification
Bead Matrix	Polymer magnetic
Ligand	HA tag Mouse mAb
Isotype	Mouse IgG2b
Particle Size	1 μm
Bead Concentration	10 mg/mL
Storage Buffer	0.01M sodium phosphate (pH 7.2)and 0.02% ProClin 300

Product Features

1. Low non-specific adsorption: Compared with most similar products domestically and abroad, the magnetic beads in this product are smaller in particle size and less prone to non-specific adsorption. Each milliliter of the magnetic bead suspension contains approximately 10 mg of magnetic beads and no less than 0.5 mg of Anti-HA Antibodies. These magnetic beads essentially do not recognize any Antigens. The recommended usage volume follows that of normal Antibody-conjugated magnetic beads.
2. Convenient to use: This product is stored in a special protective solution, free of glycerol, and enables rapid and efficient separation through magnetic adsorption without the need for centrifugation.

Instruction for use

1. Sample Preparation

1.1 Select an appropriate lysis buffer for preparing cell or tissue lysates. Ensure that the pH of

the lysis buffer is between 6 and 8.

1.2 For specific steps on preparing cell or tissue lysates, refer to the instructions provided with the lysis buffer. The supernatant of the prepared lysate should be stored on ice or at 4ºC and can then be used for operations such as immunoprecipitation (IP), co-immunoprecipitation (Co-IP), or purification of tagged proteins. For newly prepared samples, it is recommended to complete subsequent operations such as IP on the same day if possible. If the samples cannot be used on the same day, they can be appropriately aliquoted and stored at -80ºC.

2. Preparation of Anti-HA Magnetic Beads

Since Anti-HA magnetic beads are stored in a special protective solution, they need to be appropriately washed before being added to the sample.

2.1 Gently resuspend the Anti-HA magnetic beads by pipetting. According to the ratio of 10 µl or 20 µl of magnetic bead suspension per 500 µl of sample, transfer an appropriate amount of Anti-HA magnetic beads to a clean centrifuge tube and add 1×TBS to a final volume of approximately 0.5 ml.

2.2 Gently resuspend the Anti-HA magnetic beads by pipetting. Perform magnetic separation and remove the supernatant. Repeat this step twice.

2.3 Resuspend the Anti-HA magnetic beads in 1×TBS to the original volume.

3. Immunoprecipitation (IP)

3.1 Add magnetic beads and incubate. Add Anti-HA magnetic beads at a ratio of 10 µl or 20 µl of magnetic bead suspension per 500 µl of protein sample. Place on a rocking platform or rotary mixer and incubate at room temperature for 2 hours or at 4ºC overnight. Note: During incubation, if the magnetic beads aggregate or form a sheet, it is normal and will not affect the experimental results.

3.2 Magnetic separation. After incubation, perform magnetic separation and remove the supernatant. Note: A portion of the supernatant can be retained for detecting the efficiency of immunoprecipitation.

3.3 Washing. Add 500 µl of 1×TBS and gently resuspend the Anti-HA magnetic beads by pipetting. Perform magnetic separation and remove the supernatant. Repeat the washing step three times. Note: The completeness of washing can also be judged by detecting the OD_{280} of the washing supernatant. If OD_{280} > 0.05, the number of washes should be appropriately increased.

4. Elution

This kit provides two elution protocols. The operator can choose the appropriate method based on downstream application requirements. Denaturing elution is suitable for Western blot analysis, while non-denaturing elution—after neutralization—is applicable for enzymatic assays or functional studies.

4.1 Denaturing Elution: Samples eluted using this method are suitable for SDS-PAGE analysis. Add 20 µL of 1×SDS-PAGE loading buffer (prepared by diluting 5×loading buffer, if applicable) to the washed sample after. Mix thoroughly and heat at 100°C for 10 minutes.

After cooling, place the tube on a magnetic rack for 1 minute. Collect the supernatant for SDS-PAGE analysis.

4.2 Non-denaturing Elution: This method preserves the native biological activity of the eluted samples for subsequent functional analysis. Add 50 μL of elution buffer to the washed sample after. Mix and incubate on a rotator at room temperature for 5 minutes. After incubation, place the tube on a magnetic rack for 1 minute. Transfer the supernatant to a new microcentrifuge tube and immediately add 5 μL of neutralization buffer. Mix well to adjust the pH of the eluate to neutral for subsequent functional analysis.

Notes:

To maximize elution efficiency, repeat the elution step and pool the eluates.

Eluted and neutralized proteins/complexes can be stored at 4°C for immediate use or at -20 °C/-80°C for long-term storage.

Although acidic elution is efficient, it may be less effective than denaturing elution.

Elution efficiency of the acidic method may vary depending on the target protein. If high elution efficiency is critical, optimize the pH of the elution buffer (with corresponding adjustments to the neutralization buffer). Alternatively, consider using potentially more efficient methods such as peptide competition elution or the highly efficient denaturing elution. Note that denaturing conditions may impact subsequent experiments requiring protein activity.

Storage

Store at 4℃ for up to 12 months. Do not store at -20℃ or lower temperatures.

Matters needing attention

1. This product should be maintained at a pH of 6-8, and high-speed centrifugation and drying should be avoided. Do not leave the magnetic beads in a magnetic field for an extended period, as this may cause them to aggregate.
2. Prior to using this product, it should be adequately and gently resuspended. Mixing operations should be gentle and not involve vigorous vortexing or shaking to prevent antibody denaturation.
3. It is recommended to set up positive and negative control groups during immunoprecipitation or purification procedures.
4. After collecting protein samples, it is advisable to complete the purification process as soon as possible and keep them at 4ºC or on ice at all times to slow down protein degradation or denaturation. To effectively inhibit protein degradation, an appropriate amount of protease inhibitor cocktail can be added to the protein samples.
5. When using instruments such as a vacuum pump to aspirate the supernatant, pay attention to the suction strength of the vacuum pump to avoid aspirating aggregated magnetic beads due to excessive suction.
6. Aggregation of magnetic beads may occur during elution with acidic solutions, which is a normal phenomenon and does not affect their normal use. A 0.1% non-ionic detergent

(such as Triton X-100, Tween-20, or NP-40) can effectively prevent magnetic bead aggregation without affecting their antibody-binding efficiency.

7. High concentrations of DTT, mercaptoethanol, guanidine hydrochloride, etc., may have certain effects on the binding of this product to tagged proteins. However, Western and IP cell lysis buffers, RIPA lysis buffer, or NP-40 lysis buffer are all fully suitable.
8. This product is intended solely for research use by professionals. For your safety and health, please wear a lab coat and disposable gloves during operation.