Instruction Manual

HA Tag Agarose

Introduction

TargetMol's HA Tag Agarose covalently couple a mouse-derived monoclonal antibody specific for HA to the surface of highly cross-linked agarose microspheres, enabling selective binding to HA-tagged proteins.

The TargetMol HA Tag Agarose are suitable for immunoprecipitation (IP) and co-immunoprecipitation (Co-IP) involving proteins and protein complexes. They can also be used for the purification of antibody. This product is compatible with antigen samples derived from cell lysates, culture supernatants, serum, ascitic fluid, and other biological sources.

Product Features

- Excellent physicochemical stability
- Minimal ligand leakage
- Superior durability
- User-friendly operation

Product Components

HA Tag Agarose	Specification
Matrix	Highly Cross-linked 4% Agarose
Particle Size	30-100 μm
Ligand	Mouse-derived anti-HA monoclonal antibody (G2)
Binding Capacity	≥ 1.5 mg HA-tagged protein/mL gel
Bead Concentration	50% (v/v)

Product Applications

- Immunoprecipitation (IP) and co-immunoprecipitation (Co-IP) for proteins and protein complexes.
- Purification of HA-tagged proteins

Instruction

User-Supplied Reagents

Reagent	Suggested Composition
Washing Buffer (1×)	TBST: 50 mM Tris-HCl，150 mM NaCl, 0.1%(v/v) Tween-20，pH7.4
HA Peptide Elution Buffer	PBS，1 mg/mL HA peptide (TP1276)，pH 7.4
Acidity Elution Buffer	0.1 M Glycine，0.1% (v/v) Tween-20，pH2.5
Neutralization Buffer	1 M Tris-HCl, pH 9.0

1. Cell Lysate Preparation

Select an appropriate lysis buffer to process cell samples. Prepare cell lysates following standard procedures, keep the lysates on ice for immediate use, or store at –20 °C for long-term storage.

2. Agarose Gel Pretreatment

1) Vortex the agarose gel for 1 minute to fully resuspend. Transfer 25~50 μL of gel suspension into a 1.5 mL EP tube.
2) Add 500 μL of Washing Buffer into EP tube to wash the gel. Gently invert the tube several times to resuspend the gel.

3) Centrifuge the EP tube at 1,000 rpm for 5 minutes to pellet the beads at the bottom. Carefully discard the supernatant, then repeat the washing procedure once.

3. Immunoprecipitation Procedure

1) Add the prepared protein sample containing HA tag to the EP tube, then place the tube on a rotator and incubate at room temperature for 1-2 hours, or at 4 °C for 2-4 hours.
2) After incubation, centrifuge at 1000 rpm for 5 minutes. Collect the gel and either discard or save the supernatant for further analysis.
3) Add 1000 µL of Washing Buffer to the EP tube and mix gently for 5-10 minutes. Centrifuge at 1000 rpm for 5 minutes, collect the gel and discard the supernatant. Repeat this washing procedure two more times.

4. Antigen Elution

Three elution methods are provided below. Choose the appropriate method based on your downstream analysis.

1) Denaturing Elution: Suitable for SDS-PAGE analysis. Add 100 µL SDS-PAGE Loading Buffer (user-supplied) to the tube, mix well, and heat at 95 °C for 5 minutes. Then centrifuge and collect the supernatant for SDS-PAGE.
2) Neutral Elution Method: Add 50 µL of HA Peptide Elution Buffer to the EP tube. Incubate on a rotator at 37 °C for 5–10 minutes (extend the incubation time if the temperature is below 37 °C). Then centrifuge and collect the supernatant. To improve antigen recovery, the elution step can be repeated.
3) Acidic Elution: Add 100 µL of Acidity Elution Buffer to the tube. Incubate on a rotator at 37 °C for 5–10 minutes. Then centrifuge and collect the supernatant. To neutralize the eluate, add 50 µL of Neutralization Buffer to 100 µL of eluate to adjust the pH to neutral.

Storage

Store at 4 °C for 2 years.

Precautions

1. The gel should be stored in storage solution to prevent drying.
2. Before removing agarose gel from the storage tube, vortex thoroughly to ensure homogeneous suspension. Avoid bubble formation during operation.
3. Operators may analyze the binding efficiency of antibodies/antigens to the gel using supernatants collected from either the antibody-binding or antigen-binding reaction steps, according to experimental needs.
4. In IP experiments, binding affinity between different antibodies and antigens may vary. If the buffer system provided in this kit does not yield satisfactory results, users are encouraged to optimize or formulate alternative buffers.
5. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
6. Please wear a lab coat and disposable gloves.