Agarose, Anti-HA Tag Mouse Mab Antibody (4F6)

Cat #: D-AKE2043

Size: 1 mL / 5 mL

Storage: Store at 4°C for 12 months. Avoid freeze-thaw or centrifugation

Product Information

Applications: IP

Reactivity: Mammals, Bacteria

Formulation: 1 mg of Antibody coupled to 1 mL of packed Agarose, per mL of product contains 1 mL Agarose and 1 mL Storage Buffer

Storage: Store at 4°C for 12 months. Avoid freeze-thaw or centrifugation

Storage Buffer: 50% gel slurry suspended in PBS, pH 7.4, containing 0.02% Sodium Azide.

Beads Size: 45-165 µm

Capacity: ≥0.6 mg HA-Tag protein/mL Agarose

Assay Principle

Anti-HA Tag Agarose are prepared by covalently coupling Anti-HA Tag Mouse Monoclonal Antibody to Agarose, useful for detection and capture of fusion proteins containing a HA peptide sequence by commonly used immunoprecipitation procedures. The coupling technique is optimized to give a high binding capacity for HA-Tag protein.

Reagent Required but Not Supplied

Elution Buffer: 0.1 M Glycine-HCl pH 3.0.

Neutralization Buffer: 1 M Tris-HCl, pH 8.5.

Assay Procedure

A.Preparation of Agarose

Note: Per 500 µL of protein sample add 20 µL (total 40 µL suspension) Agarose.

(1)Add Agarose to a 1.5 mL centrifuge tube. Centrifuge at 800 rpm for 2 min at 4°C, remove the supernatant.

(2)Add 1 mL 1×TBS to re-suspend Agarose, centrifuge at 800 rpm for 2 min at 4°C, remove the supernatant, repeat 3 times. Add 20 µL1×TBS to re-suspend Agarose.

B.Immunoprecipitation

(1)Add 500 µL protein samples to the processed Agarose, and incubate at room temperature for 1-2 h or overnight at 4° C (It is recommended to use vertical rotating mixer with Low-speed rotation).

(2)Centrifuge at 800 rpm for 2 min at 4°C, remove the supernatant.

(3)Add 1mL 1×TBS, and re-suspend Agarose, centrifuge at 800 rpm for 2 min at 4°C, remove the supernatant, repeat 3-5 times, until OD280 of the supernatant is lesser than 0.05.

(4) Elution

a） Denatured elution: This method is suitable for SDS-PAGE and Western Blotting analysis of elution samples. Add 100 µL (5 times volume of Agarose) 1×SDS-PAGE Loading Buffer to the tube and mix well, incubate at 100°C for 5 min, then Centrifuge at 800 rpm for 1 min, and collect the supernatant to a new tube for SDS-PAGE and Western Blotting analysis.

Competitive elution of peptide: This method can maintain their original biological activity, elution can be used for functional analysis. Add 100 µL (5 times volume of Agarose) HA Peptide (0.1-0.2 µg/mL) to the tube and mix well, incubate at 4°C for 1-2 h (It is recommended to use vertical rotating mixer with Low-speed rotation), then centrifuge at 800 rpm for 2 min at 4°C, and collect the supernatant which is HA-Tag protein and its complex to a new tube. In order to improve the elution efficiency, the incubation time can be Increased or repeat elution. Place HA-Tag protein and its complex on ice to be used, or store at -20°C/-80°C for long-term. **It is recommended to add 100 µL 1×SDS-PAGE Loading Buffer to Agarose precipitation to test the effect of immunoprecipitation and elution.**

b） Acid elution: This method can maintain their original biological activity, elution can be used for functional analysis. Add 100 µL (5 times volume of Agarose) Elution Buffer to the tube and mix well, incubate at room temperature for 5-10 min (It is recommended to use vertical rotating mixer with Low-speed rotation), then centrifuge at 800 rpm for 2 min at 4°C, and collect the supernatant which is HA-Tag protein and its complex to a new tube, and immediately add 10 µL Neutralization Buffer to adjust the pH to 7.0-8.0. In order to improve the elution efficiency, elution can be repeated, and combine the same samples. Place HA-Tag protein and its complex on ice to be used, or store at -20°C/-80°C for

long-term. **It is recommended to add 100 µL 1×SDS-PAGE Loading Buffer to Agarose precipitation to test the effect of immunoprecipitation and elution.**

Note: a) For a few samples, due to differences in target proteins, the binding of HA-Tag and Anti-HA antibody is very strong, and the effect of Acid elution and Competitive elution of peptide may be poor. Therefore, SDS-PAGE Loading Buffer denaturation elution method is recommended as a priority; b) Due to the difference of target protein, the elution efficiency of acid elution method also varies to some extent. If the requirement of elution efficiency is high, the pH value of acidic eluent can be adjusted appropriately between 2.5-3.1, and the pH value or quantity of corresponding neutralizing solution should be adjusted appropriately. For example, 100 µL Acid Elution Buffer (0.1 M Glycine-HCl, pH 2.8) and 15 µL Neutralizing Buffer (1 M Tris-HCl, pH 8.5).

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.