Anti-His(HHHHHH) Affinity Agarose

Catalog Number: EA-IP-008

Note: *Do not centrifuge and use after mixing gently.*

Performance metrics

Scope of application	Affinity purification and immunoprecipitation of His-tagged fusion proteins. His tags can be located at the N-terminus, C-terminus, or middle of the protein, such as the N-terminus His fusion protein (His-Protein), C-terminus His fusion protein (Protein-His), and Met modified N-terminus His fusion protein (Met-His-Protein). Suitable for secreted proteins.
Binding properties	His-Tag Mouse mAb：Mouse IgG.
Magnetic beads properties	Agarose coated superparamagnetic beads with an average particle size of 3 μm.
Binding capacity	1 mL of Sepharose 4B agarose particles, covalently conjugated to 6 mg of mouse-derived IgG. 1 mL affinity gel can purify or precipitate at least 1.2 mg of His fusion protein.
Components	1 mL Anti-His affinity gel, stored in 1 mL of PBS with preservatives and 50% glycerol.

Matters Needing Attention

1. This product is limited to scientific research by professionals and cannot be used for clinical diagnosis or treatment.
2. For your safety and health, please wear laboratory clothes and disposable gloves for operation.
3. This product is in the form of gel suspension, and the content of affinity gel is 50%. Before use, gently re-suspend the gel suspension, and then use it as required.
4. It is best to prepare and use the IP-WB sample on site to avoid affecting the experimental results.
5. Do not dry the gel, do not sonicate the gel, and do not allow the acid treatment of gel to exceed 10 minutes.
6. The amount of gel mentioned in the method is the demonstration amount prepared in small quantities, and the specific amount should be adjusted according to the actual situation.

Method of Application

1. Sample Preparation of Target Proteins

1) Sample processing serum and recombinant proteins

Collect serum or culture medium supernatant to detect target protein concentration. If the target protein concentration is high, it is recommended to dilute it with 1× PBS to a final protein concentration of 10~100μg/mL for subsequent experiments.

2) Sample processing of target protein for intracellular expression

a. Blow off in case of adherent cells or take suspension cells from the cell culture flask and transfer them to a centrifuge tube, centrifuge at 1000 rpm for 5 min, and discard the supernatant.

b. Re-suspend cells in 1× PBS pre-cooled at 4 °C, centrifuge at 1,000 rpm for 3 min, and discard the supernatant. Repeat once.

c. Add the corresponding volume of cell lysate according to the amount of cells, and place on ice for 10~20 min after repeated pipetting.
Note: Generally, 1mL of cell lysate can process about 0.5~1×10^7 cells. To avoid degradation of the target protein, you can add a protease inhibitor.

2. Column Installation and Incubation

1) Anti-His Affinity Agarose preparation

a. Gently re-suspend the Anti-His Affinity Agarose, mix well, and take 40 μL of the gel suspension (containing approximately 20 μL of gel) into a centrifuge tube.

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b. Add 1 × PBS 10 times the gel volume (approximately 200 μL) to gently re-suspend to wash the gel, centrifuge at 5,000 rpm for 30 sec, discard the supernatant, and repeat this step 3 times.
Note: When multiple samples are used, the gel can be re-suspended and aliquoted into several reaction tubes for separate reactions.

2) Binding of target protein to Anti-His Affinity Agarose

a. Incubation: Add 200 μL of the prepared sample to the washed gel, and incubate on a shaker at room temperature for 2 hours. It can also be incubated at 4°C overnight or longer.

b. Washing: After incubation, centrifuge at 5000rpm for 30 seconds and discard the supernatant. Add 200 μL 1×PBST, mix gently, wash the gel, centrifuge at 5000 rpm for 30 seconds, discard the supernatant, and repeat this step 4 times.

3) Target protein elution

This instruction manual provides the following two target protein elution schemes. Please choose target protein elution methods according to the needs of later detection.

Denaturing elution method

This method is only suitable for SDS-PAGE detection.

a. Add 16 μL 1×PBS and 4 μL 5× loading buffer, boil the sample for 5 minutes, cool to room temperature and centrifuge.

b. Take the supernatant and conduct SDS-PAGE experiment in preparation for subsequent Western Blotting detection.

Acid elution method

Acidic elution method has low cost, short operational time, generally does not cause protein denaturation, and facilitates subsequent analysis and detection of proteins.

a. Add pre-cooled acidic eluent pH 3.0, 10 times of the gel volume (approximately 200 μL), to the above precipitate, suspend the affinity gel, and incubate at room temperature for 5 minutes.
Note: An acidic environment will shorten the service life of the gel. The contact time between the gel and the acidic eluent should be shortened as much as possible. It is recommended not to exceed 10 minutes.

b. After the incubation, centrifuge at 5000 rpm for 30 seconds at 4°C, transfer the supernatant to a new centrifuge tube, and immediately add 1/10 volume of neutralizing solution pH 8.0 and mix well. The supernatant is the eluted His-tagged protein.

c. Process and store proteins according to subsequent experimental needs.

Background

Anti-His(HHHHHH) Affinity Agarose, made of high-quality His-tagged antibody covalently conjugated to agarose gel, has the characteristics of high loading capacity, high specificity, stable properties, are reusable, and can be used for immunoprecipitation related experiments such as affinity purification, immunoprecipitation (IP), and co-immunoprecipitation (Co-IP).

Storage

4°C for 12 months.

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