Anti-HA（YPYDVPDYA）Affinity Agarose

Cat. No: EA-IP-002 **Size: 2 mL**

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

Performance metrics

Scope of application	Affinity purification and immune (co) precipitation of HA tag fusion protein. HA tag can be located at the N-terminal, C-terminal or middle of the protein, such as N-terminal HA fusion protein (HA‐Protein), C-terminal HA fusion protein (Protein-HA) and Met modified N-terminal HA fusion protein (Met HA‐Protein).
Antibody properties	Anti HA (YPYDVPDYA) mouse monoclonal antibody.
Gel properties	Agarose gel granules, average size 100~200 μm.
Binding capacity	1mL Sepharose 4B agarose granules are covalently coupled with 6 mg Anti-HA mouse monoclonal antibody. 1mL affinity gel can purify or precipitate at least 1.2mg HA fusion protein.
Repeatability	It can be used repeatedly for more than 5 times.
Components	1mL Anti-HA affinity gel, stored in 1mL PBS containing preservative and 50% glycerol.

Matters Needing Attention

1. This product is only used for scientific research by professionals, and shall not be used for clinical diagnosis or treatment.
2. For your safety and health, please wear lab clothes and disposable gloves.
3. This product provides affinity gel in the form of gel suspension. The content of affinity gel in gel suspension is 50%. Before use, gently re-suspend the gel suspension, and then use it as required.
4. Related reagents for supporting use shall be prepared by the laboratory itself.

Method of Application

1. Detection of HA Tagged Proteins by Immuno (Co) Precipitation Method

1) Gently re-suspend Anti-HA affinity gel, mix it evenly, and aspirate 40 μL gel suspension (containing about 20 μL affinity gel) into the centrifuge tube with the pipette (cut off the tip head). Wash the affinity gel with 10 times the gel volume of 1xPBS (approximately 200μL) ,centrifuge at 5000 rpm for 30 sec, discard the supernatant, and repeat the procedure three times.
2) Add 50-200 μL eukaryotic cell lysate containing target protein and incubate for 2h in a shaker at room temperature or overnight at 4°C.
3) Wash the affinity gel with 10 times the gel volume (approximately 200μL) of 1x PBS, centrifuge at 5000 rpm for 30 sec, discard the supernatant, and repeat the procedure three times.
4) Wash the affinity gel with 5 times the gel volume of PBST prewashing solution (about 100μL) precooled to 4°C to remove non-specific binding proteins. Centrifuge at 5000rpm for 30sec and discard the supernatant.
5) Add 20 μL 1x PBS 5μL 5x loading buffer, boil for 5 min, cool to room temperature and centrifuge.
6) Take the supernatant for SDS-PAGE test and for subsequent Western Blotting detection.

2. Purification of HA Tagged Protein by Affinity Purification

1) **Gel pretreatment and sample incubation**
   a) Gently re-suspend Anti-HA affinity gel, mix it evenly, and aspirate 40 μL gel suspension (containing about 20 μL affinity gel) into the centrifuge tube with the pipette (cut off the tip head). Wash the affinity gel with 10 times the gel volume of 1xPBS (approximately 200μL) of centrifuge at 5000 rpm for 30 sec, discard the supernatant, and repeat the procedure three times.
   b) Add 50-200 μL eukaryotic cell lysate containing target protein and incubate for 2h in a shaker at room temperature or overnight at 4°C.

Note: If large volume of cell lysate needs to be processed, it is recommended to use column purification (Product No.: EA-TP-K002).

c) Add 1xPBS, 10 times the volume of gel (about 200μL), and wash the gel three times by the above centrifugal method.

d) Wash the gel with 5 times the gel volume prewashing solution (about 100μL) precooled to 4°C to remove non-specific binding proteins. Centrifuge and discard the supernatant.

e) Competitive elution or acid elution can be selected according to protein properties and subsequent experimental requirements.

2) Competitive elution

Competitive elution method has high elution efficiency, strong specificity, no protein denaturation, convenient for subsequent analysis and detection of protein.

a) Add HA polypeptide solution with twice the gel volume (about 40μL) and a concentration of 2mg/mL to the precipitation. Suspend the affinity gel and incubate in a shaker at 4°C for 2h. In order to improve elution efficiency, the incubation time can be extended or elution can be repeated.

Note: Adjust HA polypeptide solution to 5mg/ml at most according to the difficulty of protein elution.

b) After incubation, centrifuge at 5000rpm for 30sec at 4°C and transfer the supernatant to a new centrifuge tube. The supernatant is the eluted HA labeled protein.

c) Treat and preserve proteins according to the requirements of subsequent experiments.

3) Acid elution

Acid elution is a low cost method, has short operation time, and generally does not cause protein denaturation, convenient for subsequent analysis and detection of proteins.

a) Add pre-cooled acidic eluent 10 times of the gel volume (about 200μL) and pH 3.0 to the precipitation. Suspend the affinity gel and incubate at room temperature for 5 min.

Note: The acidic environment will shorten the service life of the gel. The contact time between the gel and the acid eluent should be shortened as much as possible. It is recommended that the contact time should not exceed 10min.

b) After incubation, centrifuge at 5000rpm for 30sec at 4°C, transfer the supernatant to a new centrifuge tube, and immediately add one-tenth of the volume of neutralizing solution at pH 8.0, and mix. The supernatant is the eluted HA label protein

c) Treat and preserve proteins according to the requirements of subsequent experiments.

4) Cleaning and regeneration of gel

If the affinity gel needs to be reused, wash and regenerate immediately after elution.

a) Rinse with 10 times the gel volume of acid eluent, 10 times the gel volume of neutralization solution, 10 times the gel volume of 1x PBS successively.

b) Wash again with PBS containing 3 times the volume of preservatives and 50% glycerin.

c) Store the gel in PBS containing preservatives and 50% glycerin, at -20°C.

Background

Anti-HA (YPYDVPDYA) affinity gel is made by covalently coupling high-quality HA mouse monoclonal antibody with agarose gel. It has the characteristics of high binding capacity of protein, high specificity and stability, and repeatability. It can be used for affinity purification and immuno (co) precipitation of HA tagged fusion proteins.

Storage

-20°C for 12 months.