Anti-DYKDDDDK Affinity Agarose

Cat. No: EA-IP-001 **Size: 2 mL**

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

Performance metrics

Scope of application	Affinity purification and immune (co) precipitation of DYKDDDDK tag fusion protein. DYKDDDDK tag can be located at the N-terminal, C-terminal or middle of the protein, such as N-terminal DYKDDDDK fusion protein (DYKDDDDK-Protein), C-terminal DYKDDDDK fusion protein (Protein-DYKDDDDK) and Met modified N-terminal DYKDDDDK fusion protein (Met-DYKDDDDK-Protein).
Antibody properties	Mouse monoclonal antibody, IgG2a subtype.
Gel properties	Agarose gel granules, average size 50 μm.
Binding capacity	1mL Sepharose 4B agarose granules, covalently coupled with 8 mg Anti-DYKDDDDK mouse monoclonal antibody. 1mL affinity gel can purify or precipitate at least 1.2mg DYKDDDDK fusion protein.
Repeatability	It can be used repeatedly for more than 5 times.
Components	1mL Anti-DYKDDDDK affinity gel, stored in 1mL PBS containing preservatives 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 DYKDDDDK Tagged Proteins by Immuno (Co) Precipitation Method

1) Gently re-suspend Anti-DYKDDDDK affinity agarose, mix evenly, and aspirate 40 μL of 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 (approximately 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 and 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 DYKDDDDK Tagged Protein by Affinity Purification

1) Gel pretreatment and sample incubation

a) Gently re-suspend Anti-DYKDDDDK 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 100μL) ,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 (Cat. No.: EA-TP-K001).

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 acid 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 3xDYKDDDDK polypeptide solution with twice the gel volume (about 40μL) and a concentration of 100μg/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 3xDYKDDDDK polypeptide solution to 2mg/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 DYKDDDDK 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 DYKDDDDK labeled 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-DYKDDDDK affinity agarose is made by covalent coupling of high-quality DYKDDDDK mouse monoclonal antibody and agarose

gel. It has the characteristics of high binding capacity of protein, high specificity, stability and repeatability. It can be used for affinity purification and immuno (co) precipitation of DYKDDDDK tagged fusion proteins.

Storage

-20°C for 12 months.