Mouse IgG Affinity Agarose

Cat. No: EA-IP-100 **Size: 2 mL**

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

Performance metrics

Scope of application	Affinity purification of antibodies, elimination of non-specific adsorption, and negative control of mouse-derived antibody agarose gel in immunoprecipitation-related experiments such as IP, CO-IP, ChIP, etc.
Binding Properties:	Mouse-derived IgG.
Gel properties	Agarose gel granules, average size 100~200 μm.
Loading Capacity:	1mL Sepharose 4B agarose particles, covalently conjugated to 6mg mouse-derived IgG.
Components	1 mL of mouse IgG agarose gel in 2 mL of PBS with preservatives and 50% glycerol.

Matters Needing Attention

1. This product is only for scientific research by professionals and may not be used for clinical diagnosis or treatment.
2. For your safety and health, please wear a lab coat and disposable gloves.
3. This product is in the form of gel suspension, and the content of affinity gel is 50%. Gently re-suspend the gel suspension before use, and then use it as needed.
4. IP-WB samples are best prepared and used immediately to avoid affecting the experimental results.
5. Do not dry the gel, do not sonicate the gel, and do not allow acid treatment of the gel for more than 10 minutes.
6. The gel dosage in the usage method is a demonstration dosage prepared in a small amount. Please adjust the specific dosage according to the actual situation.

Method of Application

1. Immuno(co)precipitation

a. Gently re-suspend the Mouse IgG Affinity Agarose, mix evenly, and use a pipette tip with the end cut off to draw 40 μL of the gel suspension (containing approximately 20 μL of affinity gel) into a centrifuge tube. Add 10 times the gel volume (about 200 μL) of 1×PBS to wash the affinity gel, centrifuge at 5000 rpm for 30 seconds, discard the supernatant, and repeat this step three times.

b. Add 50-200 μL of cell lysis buffer containing target protein, and incubate on a shaking table at room temperature for 2 hours or overnight at 4°C.

c. Add 10 times the gel volume (about 200 μL) of 1×PBS to wash the affinity gel, centrifuge at 5000 rpm for 30 seconds, discard the supernatant, and repeat this step three times.

d. Add 5 times the gel volume (approximately 100 μL) of PBST pre-wash solution pre-cooled to 4°C to wash the affinity gel to remove non-specific binding proteins. Centrifuge at 5000 rpm for 30 seconds and discard the supernatant.

e. Depending on the nature of the antibody and subsequent experimental requirements, you can choose competitive elution or acidic elution.

Taking Anti-DYKDDDDK affinity gel (product No.: EA-IP-001) as an example, when anti-DYKDDDDK affinity gel uses a certain elution method, Mouse IgG Affinity Agarose is used as the negative control, and the same elution method needs to be used.

1) Competitive elution

Competitive elution method has high elution efficiency, strong specificity, does not cause protein denaturation, and is convenient for subsequent analysis and detection of proteins.

a) Add 2 times the gel volume (approximately 40 μL) of DYKDDDDK peptide solution with a concentration of 2 mg/mL to the

above precipitate, suspend the affinity gel, and incubate on a shaker at 4°C for 2 hours. To improve elution efficiency, the incubation time can be extended or elution can be repeated.

Note: Depending on the ease of protein elution, adjust the concentration of DYKDDDDK peptide solution up to a maximum of 5mg/mL.

b) After the incubation, centrifuge at 5000 rpm for 30 seconds at 4°C and transfer the supernatant to a new centrifuge tube. The supernatant is the eluted DYKDDDDK -tagged protein.

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

2) Acid elution

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 DYKDDDDK-tagged protein.

Gel washing and regeneration

If the Affinity Agarose needs to be reused, it must be washed and regenerated immediately after elution.

a. Wash once with 10 times the gel volume of acidic eluent, 10 times the gel volume of neutralizing solution, and 10 times the gel volume of 1×PBS.

b. Wash once more with 3 times the volume of PBS containing preservative and 50% glycerin.

c. Store in an equal volume of gel containing PBS, preservatives and 50% glycerol, and store sealed at -20°C.

Background

Mouse IgG Affinity Agarose is made of high-quality mouse IgG covalently conjugated to agarose gel. It has the characteristics of high loading capacity, high specificity, stable properties, and can be used repeatedly. It can be used for negative control of mouse antibody agarose gel in immunoprecipitation related experiments, such as immunoprecipitation (IP), co immunoprecipitation (Co IP), chromatin immunoprecipitation (ChIP), and to remove non-specific adsorption.

Storage

-20°C for 12 months.