Protein A Agarose gel

Catalog Number: EA-IP-015

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

Performance metrics

Scope of application	Immuno(co)precipitation of IgG proteins from multiple species derived from cell lysate, cell secretion supernatant, serum, animal ascites and other samples, covering most IgG subtypes.
Binding Properties:	Highly pure recombinant Protein A.
Gel properties	Agarose gel granules, average size 100~200 μm.
Loading Capacity:	1mL Sepharose 4B agarose particles, covalently conjugated to 20mg recombinant Protein A.
Components	0.5mL Protein A agarose gel in 1.5mL PBS containing preservative and 50% glycerol.

Matters Needing Attention

1. This product can be stored at -20℃ for 1 year and transported under refrigerated conditions.
2. This product is only for scientific research by professionals and may not be used for clinical diagnosis or treatment.
3. For your safety and health, please wear a lab coat and disposable gloves.
4. This product provides affinity gel in the form of gel suspension. The content of affinity gel in the gel suspension is 25%. Gently re-suspend the gel suspension before use, and then use it as needed.
5. Antibodies (IgG, IgM, IgA, IgD) of various species have different binding affinities to Protein A. Please read the attachment of this instruction manual carefully before use.
6. Do not dry the gel, do not sonicate the gel, and do not allow acid treatment of the gel for more than 10 minutes.
7. The relevant reagents used must be prepared by the laboratory.

Method of Application

NOTE: All steps should be performed on ice whenever possible to avoid degradation of the target protein. For the following steps, use 40 μL of gel suspension (including 10 μL of gel). You can combine 20 μg of IgG from 15 μL of serum or 100 μL of cell supernatant. Please adjust the amount of gel according to the amount of antibody to be bound.

1. Sample Preparation of Target Proteins

1) Sample processing serum and recombinant proteins

Collect serum or culture medium supernatant and detect the 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 lysis solution can process about 0.5~1×10^7 cells. To avoid degradation of your target protein, you can add protease inhibitors.

d. Use a sonicator to treat the cell lysate until the cell lysate is transparent and no longer viscous. After placing on ice for 30 minutes, centrifuge at 12,000 rpm and 4°C for 10 minutes. Take the supernatant for subsequent experiments.

3) Binding of gel and antibody

a. Preparation of Protein A gel: Suspend the gel fully, use a pipette tip with the end cut off to mix 40 μl of gel suspension (containing 10 μl of gel), place it in a centrifuge tube, add 250 μl of 1×PBS, and mix thoroughly. Re-suspend, centrifuge at 5000 rpm for 30 seconds, and discard the supernatant; repeat this washing step 2 times.

b. Antibody preparation: According to the IP dilution ratio recommended in the antibody instruction manual, dilute the antibody in 1×PBS to prepare an antibody working solution. Adjust the total volume to 500 μl and place it on ice for later use.

c. Add the diluted antibody to the pre-washed gel, mix gently, and incubate on a shaker at room temperature for 30 minutes.

d. Centrifuge at 5000 rpm for 30 seconds and transfer the supernatant to a new centrifuge tube for subsequent use.

e. Add 250 μl 1×PBS to the gel, mix gently, wash the gel, centrifuge at 5000 rpm for 30 seconds, and discard the supernatant. Repeat this step 4 times. Obtain antibody-gel complex.

2. Binding of target protein to antibody-gel complex

a. Incubation: Add 200 μl of the prepared sample to the antibody-gel complex, and incubate on a shaker at room temperature for 30 minutes. It can also be incubated at 4°C for 2 hours or longer.

b. Centrifugal separation: After incubation, centrifuge at 5000 rpm for 30 seconds and discard the supernatant. Add 250 μl 1×PBST, mix gently, wash the gel, centrifuge at 5000 rpm for 30 seconds, and discard the supernatant. Repeat 4 times.

3. Target protein elution

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

Denaturing elution method

This method is only suitable for SDS-PAGE detection.

a. Transfer the gel to a 1.5 ml centrifuge tube, centrifuge, discard the supernatant, add 20 μl 1×PBS and 5 μl 5× loading buffer to the gel, mix evenly, and heat the sample at 95°C for 5 minutes.

b. Centrifuge the gel, collect the supernatant, and run SDS-PAGE.

Acid elution method

The target protein eluted by this method can be used for later functional analysis.

a. Add 100~200μl acidic eluent to the gel and incubate at room temperature for 10 minutes;

b. Centrifuge at 5000 rpm for 30 seconds, collect the supernatant into a new centrifuge tube, and immediately add 1/10 of the total volume of 10×PBST Buffer to neutralize, adjust the pH of the elution product to neutral, and the sample can be used for later functional analysis.

Background

This product is made of high-quality Protein A covalently conjugated to agarose gel. It can be used for immunoprecipitation (IP) and co-immunoprecipitation (Co-IP). It has high loading capacity, fast and convenient operation, and strong specificity.

Storage

-20°C for 12 months.

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