Instruction Manual

Protein A/G Agarose

Introduction

TargetMol's Protein A/G Agarose couples Protein A/G at high density and in a defined orientation to the surface of highly cross-linked agarose beads, exhibits excellent antibody-binding capacity and low nonspecific protein adsorption. TargetMol Protein A/G Agarose is suitable for immunoprecipitation (IP) and co-immunoprecipitation (Co-IP) of proteins and protein complexes, as well as for antibody purification. It is compatible with immunoprecipitation of antigens from samples such as cell lysates, cell culture supernatants, serum, and ascites.

Product Features

• Good physicochemical stability
• Low ligand leakage
• High durability
• Easy to use

Product Information

Protein A/G Agarose	Specification
Matrix	Highly cross-linked 4% agarose beads
Particle Size	30-100 μm
Ligand	Recombinant Protein A/G protein
Binding Capacity	≥ 25 mg hIgG/mL resin
Concentration	25% (v/v)

Product Applications

• Immunoprecipitation (IP), co-immunoprecipitation (Co-IP), chromatin immunoprecipitation (ChIP), and RNA immunoprecipitation (RIP) of proteins and protein complexes.
• Antibody purification

Instructions

I. Buffer Preparation

The following are commonly used buffer compositions. It is recommended to filter them with a 0.22 μm or 0.45 μm membrane filter before use:

1) Equilibration/Wash buffer: 0.15 M NaCl, 20 mM Na2HPO4, pH7.0
2) Acidic elution buffer: 0.1 M glycine, pH3.0

Neutralization buffer: 1 M Tris-HCl, pH8.0

II. Protein purification

1. Antibody Binding

1) Add an appropriate amount of Protein A/G Agarose to a 2 mL centrifuge tube, centrifuge at 1000 rpm for 5 min, and discard the supernatant.

2) Add 0.5 mL of equilibration buffer to resuspend the resin (ensure the resin and target protein are in the same buffer system to protect the protein), centrifuge at 1000 rpm for 5 min, and discard the supernatant. Repeat this step twice.

3) Add the antibody solution to the equilibrated resin, mix by gentle inversion or on a rotator at room temperature for 30 min. Centrifuge at 1000 rpm for 5 min and collect the supernatant for later analysis.

4) Add 0.5 mL of wash buffer to resuspend and wash the resin to remove nonspecifically bound proteins. Centrifuge at 1000 rpm for 5 min and discard the supernatant. Repeat this step twice.

2. Antibody Crosslinking (Optional)

If co-elution of the antibody and antigen complex is desired, this step can be skipped and proceed directly to the next step. For 50 µL to 1 mL of resin, the volume of crosslinking buffer does not need to be increased.

1) Add 1 mL of crosslinking buffer (0.2 M triethanolamine, pH 8.2) to the washed resin, centrifuge at 1000 rpm for 5 min, and discard the supernatant.

2) Add 1 mL of freshly prepared crosslinking buffer containing 20 mM DMP (dimethyl pimelimidate dihydrochloride), resuspend the resin, and gently mix by inversion at room temperature for 30 min. Centrifuge at 1000 rpm for 5 min and discard the supernatant.

3) Add 1 mL of quenching buffer (50 mM Tris, pH 7.5), resuspend the resin, and gently mix by inversion at room temperature for 15 min. Centrifuge at 1000 rpm for 5 min and discard the supernatant to terminate the crosslinking reaction.

4) Add 0.5 mL of wash buffer, resuspend and wash the resin, centrifuge at 1000 rpm for 5 min, and discard the supernatant. Repeat this step twice.

3. Antigen Precipitation Reaction

1) Antigen Binding: Add the antigen-containing sample to the antibody–resin complex and gently mix with a pipette. Incubate with gentle inversion at room temperature for 10 min to allow sufficient binding between antigen and antibody. For weaker interactions, extend incubation to 1 hour at room temperature or overnight at 4 °C.

2) Washing: Centrifuge at 1000 rpm for 5 min and collect the supernatant for analysis, keeping it on ice. Add 1 mL of wash buffer, gently resuspend, and centrifuge again at 1000 rpm for 5 min. Discard the supernatant. Repeat the wash step twice. Finally, transfer the antibody–antigen–resin complex to a new 1.5 mL centrifuge tube, centrifuge at 1000 rpm for 5 min, and discard the supernatant.

3) Antigen Elution: Two elution methods are provided, choose the appropriate one based on the downstream analysis.

A. Denaturing Elution: Suitable for SDS-PAGE analysis. Add 25 µL of 1× SDS-PAGE loading buffer, mix well, and heat at 95 °C for 5 min. Centrifuge at 1000 rpm for 5 min and collect the supernatant for SDS-PAGE and subsequent Western blot analysis.

B. Native Elution: Preserves antigen bioactivity and is suitable for functional assays. Add 5 column volumes of acidic elution buffer, gently pipette up and down 5 times to mix. Incubate with gentle inversion at room temperature for 10 min, centrifuge at 1000 rpm for 5 min, and collect the supernatant containing the target antigen. Transfer the eluate to a new tube and immediately add one-tenth volume of neutralization buffer to adjust the pH to 7.0–8.0 for downstream applications.

III. Immunoprecipitation

1. Antigen–Antibody Binding

Mix the antibody with the lysate containing the target protein and incubate with gentle shaking at room temperature for 30–60 minutes, or overnight at 2–8 °C. The incubation conditions should be optimized based on the binding efficiency of the antigen–antibody pair and the stability of the antigen, to promote the formation of a stable antigen–antibody complex.

Note: The amount of antibody used should be adjusted according to the volume of resin. Excess antibody may interfere with the binding of the complex to the resin. It is recommended that the amount of antibody not exceed 80% of the resin's maximum binding capacity.

2. Resin Preparation

1) Add an appropriate amount of Protein A/G Agarose to a 2 mL centrifuge tube, centrifuge at 1000 rpm for 5 min, and discard the supernatant.
2) Add 0.5 mL of equilibration buffer to resuspend the resin (ensuring that the resin and target protein are in the same buffer system to protect protein integrity), centrifuge at 1000 rpm for 5 min, and discard the supernatant. Repeat this step twice.

3. Binding of Antigen–Antibody Complex to Resin

Add the antigen–antibody complex prepared in Step 1 to the pre-equilibrated resin. Gently mix and incubate with rotation at room temperature for about 30 minutes to ensure sufficient contact and binding. Centrifuge at 1000 rpm for 5 minutes and collect the supernatant for subsequent analysis.

4. Washing

Add 0.5 mL of wash buffer and gently resuspend the resin to remove nonspecifically bound proteins. Centrifuge at 1000 rpm for 5 minutes and discard the supernatant. Repeat this step twice.

5. Antigen Elution

Two elution methods are provided, choose the appropriate one based on the downstream application.

1) Denaturing Elution: Suitable for SDS-PAGE analysis. Add 25 µL of 1× SDS-PAGE loading buffer, mix well, and heat at 95 °C for 5 minutes. Centrifuge at 1000 rpm for 5 minutes and collect the supernatant for SDS-PAGE and subsequent Western blot analysis.
2) Native Elution: Preserves the biological activity of the antigen, suitable for functional analysis. Add 5 column volumes of acidic elution buffer, gently pipette up and down 5 times to mix. Incubate with rotation at room temperature for 10 minutes, then centrifuge at 1000 rpm for 5 minutes. Collect the supernatant containing the target antigen, transfer it to a new tube, and immediately add one-tenth volume of neutralization buffer to adjust the pH to 7.0–8.0 for downstream applications.

Storage

Store at 4℃ for 2 years

Precautions

1. The gel should be stored in storage solution to prevent drying.
2. Before removing agarose gel from the storage tube, vortex thoroughly to ensure homogeneous suspension. Avoid bubble formation during operation.
3. To ensure optimal experimental performance, choose antibodies with high specificity for immunoprecipitation reactions.
4. In IP experiments, binding affinity between different antibodies and antigens may vary. If the buffer system provided in this kit does not yield satisfactory results, users are encouraged to optimize or formulate alternative buffers.
5. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
6. Please wear a lab coat and disposable gloves.