Protein A/G Agarose Gel, 30~100 µm
PRODUCT DATA SHEET

Protein A/G Agarose Gel, 30~100 µm

Cat No: AG-Protein A/G-3000

Description

Protein A/G agarose gel was composed of homemade agarose gel and recombinant Protein A/G protein as ligand, which has high physical and chemical stability, and the ligand is not easy to fall off. It has long life and is easy to use. The recombinant Protein A/G Protein contains 5 immunoglobulin binding regions of Protein A and 2 binding regions of Protein G, which greatly improves the binding capacity compared with the single Protein A and Protein G. This product is widely used for immunoprecipitation (IP) or immunoco-precipitation (Co-IP) of antigens in cell lysate, cell secreted supernatant, serum, ascites and other samples, and can also be used for antibody purification.

For custom sizes, formulations or bulk quantities please contact our customer service department.

Website: www.abvigen.com Phone: +1 929-202-3014 Email: info@abvigenus.com

Characteristics

Matrix	4% highly cross-linked agarose gel
Ligand	Recombinant Protein A/G protein
Particle size	30~100 µm
Concentration	The gel volume accounts for 25% of the suspension volume
Binding ability	≥ 25 mg hIgG/mL gel
Scope of application	IP, Co-IP, ChIP, RIP, antibody purification, etc
Shelf life	Stable storage at 2 ~ 8°C for 2 years

Operation process

Note: Prepare your own buffer or purchase our IP kit directly.

Adherent cell sample:

1. Remove the medium and wash the cells twice with PBS.

2. Cells were collected into 1.5 mL EP tube and IP Lysis/Wash Buffer was added proportioned, and add corresponding inhibitor such as PMSF. After mixing, the cells were placed on ice for 5 ~ 20 min (mixed several times).

3. At 4°C, 12000 ~ 16000 xg, centrifuge for 10 min to collect the supernatant and place it on ice for subsequent experiments (or place it at -80°C for long-term storage).

Petri dish IP Lysis/Wash Buffer Recommended volume:

Petri dish size/surface area	Volume of IP Lysis/Wash Buffer
100 mm x 100 mm	500 ~ 1000 µL
100 mm x 60 mm	100 ~ 300 µL
6-well plate	100 ~ 200 µL

Suspended cell sample:

1. Cells were collected at 4°C, 500 ~ 1000 xg, 10 min, and the supernatant was discarded.

2. Wash the cells with PBS once, that is, suspend the cell mass with PBS at 4°C, 500 ~ 1000 xg, 5 min, collect the cells, discard the supernatant.

3. Resuspend cells with pre-cooled IP Lysis/Wash Buffer. The 500 µL IP Lysis/Wash Buffer was used for every 50 mg cells. At the same time, add the corresponding inhibitors such as PMSF, mix well and put on the ice for 5 ~ 20 min (mix several times during the period).

4. At 4°C,12000 ~ 16000 xg, centrifuge for 10 min to collect the supernatant and place it on ice for subsequent experiments (or place it at -80°C for long-term storage).

Serum sample:

It is recommended to dilute a serum sample with IP Lysis/Wash Buffer to a final concentration of target protein of 50 to 150 µg/mL and place it on ice for future use (or place it at -20°C for long-term storage).

Preparation of immune complex

Note: The amount and incubation time required for the sample are dependent on each specific antibody antigen system and may need to be optimized for maximum yield.

The following protocol is for 2 ~ 10 µg affinity purified antibodies, which can be scaled up as required.

1. In a centrifuge tube, the cell lysate of each sample was combined with 2 ~ 10µg immunoprecipitation antibody. The recommended total protein amount for each immunoprecipitation reaction is 500 ~ 1500 µg.

2. Dilute the antibody and prepared sample to 300 ~ 500 µL from IP Lysis/Wash Buffer.

3. Incubate at room temperature for 1 ~ 2 h, or 4°C for 2 ~ 4 h to form immune complexes.

Immunoprecipitation:

Note: In order to ensure the uniform distribution of gel, mix the gel in the bottle by repeatedly reversing or slightly vortexing before use.

1. Add 20 to 50 µL of Protein A/G agarose gel into 1.5 mL centrifuge tube.
2. Add 500 µL pre-cooled PBS to the gel and mix gently.
3. Put the centrifuge tube into the centrifuge at 1000 rpm for 5 min to collect the gel to the bottom of the centrifuge tube and remove the supernatant.
4. Add 200 ~ 500 µL IP Lysis/Wash Buffer to the centrifuge tube. Reverse the centrifugal tube several times or mix with a slight vortex for 1 min. Put the centrifuge tube into the centrifuge at 1000 rpm for 5 min to collect the gel to the bottom of the centrifuge tube and remove the supernatant.
5. The prepared antigen sample/antibody mixture sample was added to the centrifuge tube with gel and incubated at room temperature for 1 ~ 2 h, or 4°C 2 ~ 4 h.
6. Centrifuge at 1000 rpm for 5 min and collect gel , remove unbound sample, and store for analysis.
7. Add 1000 µL IP Lysis/Wash Buffer to the centrifuge tube and gently mix for 5 ~ 10 min. Collect the gel and discard the supernatant. Repeat twice.
8. **Denaturing elution:** Add 80 ~ 100 µL SDS-PAGE Sample Loading Buffer (1×) to the centrifuge tube and heat the sample in 100°C water bath or metal bath for 10 min. Gel are separated by centrifugation to retain the supernatant containing the target antigen.

Note: The following elution methods can also be used to maintain protein activity.

Low pH Elution: Add 100 µL Elution Buffer to centrifuge tube. Keep mixed and incubate centrifuge tube at room temperature for 5 ~ 10 min. The gel is separated by centrifugation to retain the supernatant containing the target antigen. Low pH is neutralized by adding 20 µL Neutralization buffers per 100 µL of wash out solution.

Notes

1. Before performing the experiment, please read this operation manual carefully.
2. The affinities between different types of antibodies and antigen Lysis in IP experiments are different. Lysis/Wash Buffer between antibodies and antigens is also affected by IP Lysis/Wash Buffer, so **do your own experiments by optimizing the operation details or screening and formulating buffers**.
3. Agarose gel should be fully oscillated and evenly before use. The gel should be kept in storage solution to prevent drying.

1378 US-206 Ste 6-126, Skillman, NJ USA
Email: info@abvigenus.com
Tel: 1-816-388- 0112 Fax: 1- 888-616-0161

4. This product is for scientific research only.

Problem solving

1. No immunoprecipitation of antigen

① The sample contains too little antigen to be detected

Recommendation: To verify the expression and/or cleavage efficiency of proteins in the lysate by SDS-PAGE or protein immunoblotting; If necessary, increase the sample size

② Antibodies cannot bind to antigens

Recommendation: Choose another specific antibody, or choose another antibody that recognizes a different epitope.

③ Ingredients in IP Lysis/Wash Buffer interfere with the binding of antigens to antibodies

Recommendation: Immunoprecipitation and rinsing with other buffers (e.g., TBS containing 0.5% CHAPS)

2. Low protein intake

① The protein is degraded

Recommendation: Add protease inhibitor

② The amount of gel used is not enough

Recommendation: Increase the amount of gel used to capture immune complexes

③ The amount of target protein in the sample is not enough

Recommendation: Increase the amount of antigen sample

3. Multiple non-specific bands

There are non-specific proteins that bind to the gel

Recommendation: Add 50 ~ 350 mM NaCl to IP Lysis/Wash Buffer. Increase elution intensity and frequency.

4. Gel sticks easily to the tube wall

Recommendation: Use consumables with low adsorption rates for gel manipulation.

Ordering Information

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