Anti-Flag Affinity Gel

Components

Contents	Cat#: B23101	Cat#: B23102
Anti-Flag Affinity gel	1 mL	5mL

Storage

Anti-Flag Affinity Gel should be stored in 50% glycerol at -20°C for maximum stability. The unopened product is stable for 2 years when stored as indicated. After use, the gel should be cleaned and stored in 50% glycerol with 10 mM sodium phosphate, 150 mM sodium chloride, pH 7.4, containing 0.02% sodium azide to protect the product. Do not freeze in the absence of glycerol.

Protocol

This method is recommended for immunoprecipitation of Flag-tagged proteins.

Affinity gel Preparation

1. Thoroughly suspend the Anti-Flag Affinity gel in the vial, in order to make a uniform suspension of the gel. Quickly transfer 10 µL of the gel suspension (about 5 µL pure gel) to a fresh tube.
2. Add 500 µL TBS buffer (50 mM Tris HCl, 150 mM NaCl, pH 7.4). Thoroughly suspend the Anti-Flag Affinity gel. Centrifuge the gel at 5000 rpm for 30 sec. Remove the supernatant carefully. Be sure that most of the wash buffer is removed and no gel is discarded. This step should be repeated for 3-4 times.

Sample Binding

3. Add 50-200 µL Cell lysate into the washed gel. Incubate all samples at room temperature for 2 h or overnight at 4°C.
4. Centrifuge at 5000 rpm for 30 sec. Remove the supernatant to a fresh tube to detect whether Flag-tag protein remains.

Beads Washing

5. Add 0.5 mL PBST buffer (NaCl 136.89 mM; KCl 2.67 mM; Na_2HPO_4 8.1 mM; KH_2PO_4 1.76 mM; 0.5% Tween20) into the precipitation of last step. Gently disperse the gel with a pipette, then flip the sample up and down for 5 min. Centrifuge for 30 sec at 5000 rpm and discard the supernatant.
6. Repeat 2 times until the OD280 of the supernatant liquid < 0.05. If the non-specific impurity proteins are left over, please extend the cleaning time, increase the cleaning times or properly enlarge the detergent content in the cleaning solution.

Elution

Choose different elution methods according to the downstream use. For IP, go to the step 7-8. For protein purification, go to step 9-10 for competitive polypeptide elution or step 11-12 for low pH elution.

Denaturing elution (suitable for IP experiments by Anti-Flag Affinity Gel):

7. For direct detection of target proteins, add 50 µL 1 × protein sample loading buffer in the precipitation mentioned above, boil for 5 min, chill to room temperature and then carry out centrifugation.
8. Detect the supernatant by SDS-PAGE.

Competitive poly FLAG polypeptide elution (Suitable for protein purifications by Anti-Flag Affinity Gel):

9. Add the TBS buffer with 200 µg-1 mg/mL Poly FLAG Peptide (B23111) into the product of step 6, and then incubate them at shaker (4°C) for 2 h. Generally, the volume of Poly FLAG Peptide is 5 times of the gel.
10. Centrifugation the products obtained from the above step at 5000 rpm for 30 sec. Transfer the supernatant containing target protein into a new EP tube. If needing reuse the gel, please clean the gel with 0.1 M glycine HCl (pH 3.0) and carry out recycling.

Low-pH elution (Suitable for protein purification by Anti-Flag Affinity Gel):

11. Add the 0.1 M glycine HCl (pH 3.0) elution buffer into the product of step 6, and incubate at shaker for 5 min (The elution time should be less than 20 min). Generally, the volume of elution buffer is 5 times of the gel.
12. Centrifugation the products obtained from the above step at 5000 rpm for 30 sec. Then transfer the elution product quickly into 1 M Tris (pH 8.0) for neutralization until the pH is near neutral.

Trouble Shooting

Q1: No signal in the experiment result?

(1) There was no Flag fusion protein in the sample.

A. Test whether there is Flag tag in the target protein by WB;
B. Use fresh protein samples;
C. Select appropriate protease inhibitors or increase the concentration of inhibitors to avoid the degradation of Flag tag protein.

(2) Excessive washing.

A. Decrease the washing time;
B. Avoid adding the NaCl of high concentration;
C. Use solutions with no detergent or low concentration detergent.

(3) Insufficient incubation time.

Prolong the incubation time from hours to overnight.

(4) Interfering substances in the sample.

In the sample, avoid the existence of high concentration of DTT, 2-mercaptoethanol or other reductive substances which can destroy the function of antibodies.

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(5) Insufficient sensitivity of detection system.
A. Set reasonable control to determine the binding characteristics of primary and secondary antibodies;
B. Detect the efficiency of protein transfer by ponceau red staining;
C. Use fresh substrates or other test methods.

Q2: High background in the experiment results?
(1) Nonspecific binding of protein impurity with anti-FLAG antibodies, agarose matrix or centrifugal tube walls.
A. Removal of non-specific binding proteins by mouse IgG-Agrose;
B. After the last washing, suspend and transfer to new centrifugal tube for centrifugation;
C. Avoid the non-specific binding of proteins to agarose matrix by low-speed centrifugation.

(2) Insufficient washing.
A. Increase the washing time;
B. Prolong the washing time (at least 15 min for every washing);
C. Increase the concentration of NaCl or detergent.