Antibody Crosslinking Kit

Immunology-Using Antibody

CATALOGUE NUMBER: IPK005

DESCRIPTION: To enable elution of protein with little antibody contamination (for cleaner protein preparation and cleaner western blots), it is recommended to cross link the antibody to the beads. An example procedure for this is shown below. The target protein should then be eluted with a mild eluent, such as glycine buffer.

KIT COMPONENTS:

A	Dilution Buffer	100 mL
B	Protein A or G Agarose	5 mL (50% flurry)
C	Cross Linking Reagent	350 mg
D	Wash Buffer	100 mL
E	Quenching Buffer	100 mL
F	Elution Buffer	100 mL

SIZE: Include enough reagents for 5 standard crosslinking reactions (up to 10 mg antibody per reaction)

STORAGE/STABILITY: 4°C

PROCEDURES:

1. Bind the antibody to protein A or protein G beads.

   Notes: For general-purpose columns, bind approximately 10 mg of antibody per millilter of 50% slurry. Immunoaffinity columns are normally prepared with monoclonal antibodies or a affinity-purified polyclonal antibodies. Antibodies can be added from any source including tissue-culture supernatant, ascites, or purified solutions, because the binding to the protein A/G beads will act as a purification step to remove other compounds in the storage buffer or to exchange buffers.

   - ✧ Transfer 1 mL 50% potein A or protein G slurry into 15-mL Falcon tube and wash beads with 10 mL PBS buffer by centrifuging (500 rpm, 1 min) into a pellet. Carefully remove the PBS supernatant without aspirating the beads.
   - ✧ Add 5 mL Dilution Buffer, mix gently and rotate for 10 minutes at room temperature. Centrifuge and discard the supernatant.
   - ✧ Prepare the antibody solution in 5 mL Dilution Buffer at the required concentration (the final concentration of antibody ≤ 1 mg/mL). Add diluted antibody to the beads. Mix gently and rotate 1 hr at room temperature.
   - ✧ Centrifuge and discard the supernatant.
   - ✧ Add 5 mL Dilution Buffer to beads. Rotate for 5 min at 4°C. Centrifuge and discard the supernatant.

- ✧ Add 10 mL PBS to beads. Centrifuge and discard the supernatant.

2. Wash the beads twice with 5 mL of Washing Buffer by centrifugation at 500 rpm for 5 minutes.
3. Resuspend the beads in 5 mL of Washing Buffer and remove the equivalent of 10ul of beads.
4. Add 65 mg Cross Linking Reagent (solid), shake till the powder is completely dissolved
5. Incubate for 30 minutes at room temperature with gentle mixing. Remove the equivalent of 10 ul of the coupled beads.
6. Stop the reaction by washing the beads once in 5 mL Quenching Buffer and then incubate for 2 hours at room temperature in 5 mL Quenching Buffer with gentle mixing.
7. Wash with 5 mL Elution Buffer to remove excess (unlinked) antibody. Repeat three times with 10 min rotation at RT.
8. After the final wash, resuspend the beads in PBS. Check the efficiency of coupling by boiling samples of beads taken before and after coupling in protein loading buffer (Cat.: MP006). Run the equivalent of 1 ul and 9 ul of both samples on a 10% SDS-polyacrylamide gel and stain with Coomassie blue. Good coupling is indicated by heavy-chain bands (55,000 MW) in the "before" but not in the "after" lanes. Beads can be stored at 4°C for a few days. Sodium azide can be added to prevent bacterial growth.

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