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Epoxy-activated Agarose Resin 6B

E1492640

Storage: 2-8℃. Do not freeze.

Introduction:

This product is an epoxy-activated agarose microsphere medium that can be directly used for coupling proteins and samples containing amino, thiol, and hydroxyl groups. The pre-activated medium can be customized to prepare specific affinity media for the rapid and efficient one-step purification of target substances from complex systems.

Aladdin Epoxy-activated Agarose Resin 6B is stored in 100% 1,4-dioxane, with a settled gel to storage solution ratio of 1:1. The product specification refers to the actual volume of the settled gel.

Parameter	Specification
Matrix	6% Agarose Microspheres
Active Group Density	30-40 μmol/mL medium
Particle Size Range	45~165 μm
Max Pressure	0.1 MPa, 1 bar
Storage Buffer	100% 1,4-Dioxane
Storage Temperature	4-30℃
Shelf Life	2 years

Instructions for Use:

1. Buffer Preparation:

It is recommended to filter all water and buffers through a 0.22 μm or 0.45 μm membrane before use.

- Coupling Buffer: 0.1 M Na_2CO_3, pH 8.5-10.0.
- Blocking Buffer: 1 M Ethanolamine, pH 8.0.
- Wash Buffer 1: 0.1 M Sodium Acetate-Acetic Acid, 0.5 M NaCl, pH 3.0.
- Wash Buffer 2: 0.1 M Tris-HCl, 0.5 M NaCl, pH 8.0.
- Storage Buffer: 1× PBS containing 20% Ethanol.

2. Sample Preparation:

Dissolve or dialyze the sample in Coupling Buffer to a concentration of approximately 5-10 mg/mL.

3. Sample Coupling:

The following procedure uses antibody coupling for antigen purification as an example to describe the coupling and subsequent purification steps.

3.1 Take an appropriate amount of Epoxy-activated Agarose Resin 6B. Remove the storage solution. Do not let the medium dry out. Wash three times with deionized water and once with Coupling Buffer.

3.2 Transfer the dissolved sample to the washed medium. The recommended medium-to-sample solution volume ratio is approximately 1:1-2 (V/V).

3.3 Mix and react with shaking at 25-40°C for 24 hours.

Note: Ensure the medium is suspended, otherwise the coupling efficiency will be significantly affected.

3.4 After the reaction, collect the coupling solution to check coupling efficiency. Wash the medium once with Coupling Buffer.

3.5 Add an equal volume of Blocking Buffer. Mix and react with shaking at 37°C for 1 hour.

3.6 Drain the solution from the reaction system. Wash the medium with 3 column volumes (CV) of deionized water. Rinse alternately with Wash Buffer 1, deionized water, Wash Buffer 2, and deionized water, repeating this cycle twice. Finally, store the medium in an equal volume of Storage Buffer at 2-8°C.

4. Gravity Column Packing:

4.1 Take a gravity column of appropriate size. Insert the bottom frit. Add some purified water to wet the column tube and frit. Close the bottom outlet.

4.2 Resuspend the coupled medium. Use a pipette tip to aspirate and transfer an appropriate amount of the slurry into the gravity column. Open the bottom outlet to drain the storage solution.

4.3 Add purified water to wash the medium. After the liquid has drained by gravity, close the bottom outlet.

4.4 Insert the pre-wetted top frit, ensuring no gaps between the frit and the medium and that it is level.

4.5 The packed gravity column can be directly equilibrated with equilibration buffer.

5. Sample Purification:

5.1 Buffer Preparation:

It is recommended to filter all water and buffers through a 0.22 μm or 0.45 μm membrane before use.

- Equilibration/Wash Buffer: 0.15 M NaCl, 20 mM Na_2HPO_4, pH 7.0.
- Elution Buffer: 0.1 M Glycine, pH 3.0.
- Neutralization Buffer: 1 M Tris-HCl, pH 8.5.

5.2 Batch Purification (Incubation):

(1) Based on the sample volume, add an appropriate amount of coupled medium to a column. Drain the storage solution by gravity.

(2) Wash the medium with 5 CV of Equilibration Buffer. Drain by gravity.

(3) Add the sample. Seal both ends of the column and incubate with shaking at 4°C for 2-4 hours or at 37°C for 30 minutes - 2 hours.

(4) After incubation, collect the medium by centrifugation or filtration. Retain the supernatant as the flow-through for SDS-PAGE analysis.

(5) Wash the medium with 5 CV of Wash Buffer. Remove the supernatant by centrifugation or filtration. Repeat this wash step 3-5 times. It is recommended to use a new tube during the process.

(6) Add 3-5 CV of Elution Buffer for elution. Incubate for 10-15 minutes. Collect the eluate by centrifugation or filtration. This elution step can be repeated 2-3 times. The eluted fractions must be neutralized immediately. It is generally recommended to add a volume of Neutralization Buffer equal to 1/10th of the elution fraction volume.

5.3 Gravity Column Purification:

(1) Equilibrate the packed gravity column with 5 CV of Equilibration Buffer to bring the medium into the same buffer system as the target protein.

(2) Apply the sample to the equilibrated column. Collect the flow-through. The sample can be reloaded to increase binding efficiency.

(3) Wash with 10 CV of Wash Buffer to remove non-specifically adsorbed impurities. Collect the wash fractions.

(4) Elute with 5 CV of Elution Buffer. Collect fractions separately. Neutralize the eluted fractions immediately by adding a volume of Neutralization Buffer equal to 1/10th of the elution fraction volume.

6. SDS-PAGE Analysis:

Analyze the samples obtained from the purification process (including flow-through, wash, and elution fractions) as well as the original sample using SDS-PAGE to evaluate the purification efficiency.

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