Aldehyde-activated Agarose Resin

A1492636

Storage: 2-8℃.

Introduction:

This product consists of pre-activated agarose microspheres that can be directly used for coupling samples such as polysaccharides, proteins, or peptides containing amino and thiol 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. The Aldehyde-activated affinity chromatography medium exhibits good pressure resistance and stable performance after protein coupling, making it suitable for large-scale industrial purification.

Aladdin Aldehyde-activated Agarose Resin is stored in 1× PBS containing 20% ethanol, 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	Highly Cross-linked 4% Agarose Microspheres
Binding Capacity	10-15 mg Protein A / mL medium
Particle Size Range	45~165 μm
Max Pressure	0.3 MPa, 3 bar
Storage Buffer	1× PBS containing 20% Ethanol
Storage Temperature	2-8℃
Shelf Life	1 years

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, 0.5 M NaCl, pH 9.0.
- Blocking Buffer: 1.0 M Ethanolamine, pH 8.0 *or* 0.1 M Tris-HCl, pH 8.5.
- 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 0.02% Sodium Azide (NaN_3).

Note: Coupling Buffer can be carbonate, phosphate, or other buffers lacking primary amino groups. Adding salt to the buffer system helps reduce non-specific adsorption.

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.

(1) Take an appropriate amount of Aldehyde-Activated Affinity Chromatography Medium. Wash once with purified water, then three times with Coupling Buffer.

(2) Add the dissolved sample to the washed Aldehyde-activated medium. Add sodium cyanoborohydride ($NaCNBH_3$) to a final concentration of 1 mg/mL. The recommended medium-to-sample solution volume ratio is approximately 1:1-2 (V/V). React with shaking at 28°C overnight.

Note: Ensure the medium is suspended, otherwise coupling efficiency will be significantly affected. If the protein is unstable, the reaction can be performed at 4°C overnight. After the reaction, collect the coupling solution to check coupling efficiency.

(3) Wash the medium with deionized water. Add 2 column volumes (CV) of Blocking Buffer and react with shaking at 28°C for 1 hour.

(4) Drain the blocking solution from the reaction system. Wash the medium with 3 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. Column Packing:

4.1 Gravity Column Packing:

(1) Take a suitable gravity column. Insert the bottom frit. Add purified water to wet the column tube and frit. Close the bottom outlet.

(2) Resuspend the coupled medium. Transfer an appropriate amount of the slurry into the column using a pipette tip. Open the outlet to drain the storage solution.

(3) Add purified water to wash the medium. After gravity flow, close the outlet.

(4) Insert the pre-wetted top frit, ensuring no gaps and a level surface.

(5) The packed column can be equilibrated directly with equilibration buffer.

4.2 Medium-Pressure Column Packing:

For large-scale purification, the coupled medium can be packed into medium-pressure columns. Calculate the required medium volume using: $V = 1.15 \times \pi \times r^2 \times h$.

- V: Medium volume (mL).
- 1.15: Compression factor.
- r: Column radius (cm).
- h: Packing height (cm).

Note: The slurry volume should be twice the medium volume.

(1) Flush the column bottom screen/adaptor with deionized water to remove bubbles. Close the outlet, leaving 1-2 cm of water.

(2) Resuspend the medium and carefully pour the slurry into the column (use a glass rod to minimize bubbles).

(3) If using a reservoir, fill it and the column with water. Place the inlet adaptor on the slurry surface and connect to the pump, avoiding bubbles.

(4) Open the column outlet. Start the pump at a low flow rate, gradually increasing to the final rate. If the recommended pressure/flow isn't achievable, use the pump's maximum rate. (Note: In subsequent runs, do not exceed 75% of this maximum packing flow rate.) After the bed stabilizes, pump at least 3 CV of deionized water at the final rate. Mark the bed height.

(5) Stop the pump and close the outlet.

(6) If a reservoir was used, remove it and place the adaptor inside the column.

(7) Lower the adaptor to the marked bed height. Tighten the fitting.

(8) Connect the column to the chromatography system and begin equilibration. Readjust the adaptor if needed.

5. Sample Purification:

5.1 Buffer Preparation:

Filter all solutions 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) Add coupled medium to a column. Drain the storage solution by gravity.

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

(3) Add the sample. Seal the column ends. Incubate with shaking at 4°C for 2-4h or 37°C for 30min-2h.

(4) Collect the medium by centrifugation/filtration. Keep the supernatant (flow-through).

(5) Wash the medium with 5 CV of Wash Buffer. Remove supernatant. Repeat 3-5 times, changing tubes midway.

(6) Elute with 3-5 CV of Elution Buffer. Incubate 10-15 min. Collect eluate. Repeat 2-3 times. Neutralize eluted fractions immediately using a volume of Neutralization Buffer equal to 1/10th of the eluate volume.

5.3 Gravity Column Purification:

(1) Equilibrate the column with 5 CV of Equilibration Buffer.

(2) Apply the sample. Collect the flow-through. Reload to increase binding.

(3) Wash with 10 CV of Wash Buffer. Collect wash fractions.

(4) Elute with 5 CV of Elution Buffer. Collect fractions. Neutralize immediately as above.

5.4 Medium-Pressure Column Purification:

(1) Prime the pump with deionized water. Connect the column to the system and tighten.

(2) Flush with 3-5 CV of deionized water to remove storage buffer.

(3) Equilibrate with at least 5 CV of Equilibration Buffer.

(4) Load the sample via pump or sample loop. Note: High viscosity can cause backpressure. Do not exceed column capacity.

(5) Wash with Wash Buffer until UV baseline is stable (≥10-15 CV).

(6) Elute with 5-10 CV of Elution Buffer. Collect the target protein fraction. Neutralize immediately as above.

After elution, wash the medium with 5-10 CV of Equilibration Buffer, then 5-10 CV of purified water, and finally 2 CV of 20% ethanol. Store at 2-8°C.

6. SDS-PAGE Analysis:

Analyze the original sample, flow-through, wash, and elution fractions using SDS-PAGE to evaluate purification efficiency.