Amino-activated Agarose Resin

A1492637

Storage: 2-8℃.

Introduction:

This product consists of agarose microspheres containing primary amine active groups. With the aid of a chemical crosslinking agent, this product can be covalently coupled to molecules containing carboxyl groups to prepare specific affinity media for the rapid and efficient one-step purification of target substances from complex systems. The Amino-activated affinity chromatography medium exhibits good pressure resistance and stable performance after protein coupling, making it suitable for large-scale industrial purification.

Aladdin Amino-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
Ion Capacity	>50 μmol Cl^- / mL medium
Particle Size Range	45~165 μm
Max Pressure	0.3 MPa，3 bar
Storage Buffer	1× PBS containing 20% Ethanol
Storage Temperature	4-30℃
Shelf Life	4 years

Instructions for Use:

1. Preparation of Buffers:

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

- Wash Solution: 1 mM HCl.
- Coupling Buffer: 0.1 M MES, pH 4.5.
- Wash solution1: 0.1 M Sodium acetate-acetic Acid, 0.5 M NaCl, pH 3.0.
- Wash solution 2: 0.1 M Tris-HCl, 0.5 M NaCl, pH 8.0.
- Storage solution: 1× PBS containing 20% Ethanol.

Note: Adding a certain concentration of salt ions to the buffer system helps reduce non-specific adsorption.

2. Sample Preparation:

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

If the sample is insoluble, 50% dioxane or ethylene glycol can be added, followed by adjusting

the pH to 4.5-6.0 using pH test paper.

3. Sample Coupling:

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

(1) Take an appropriate amount of Amino-Activated Affinity Chromatography Medium. Remove the storage solution. Wash three times by suction filtration using the Wash Solution (1 mM HCl), then once with Coupling Buffer.

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

(3) Directly add weighed EDC solid powder, or add a high-concentration EDC solution dropwise, to achieve a final concentration of 0.1 M. If using a stock solution, adjust its pH beforehand.

Note: Typically, the molar amount of EDC is about 10-100 times that of the ligand; 0.1 M is recommended. The EDC solution must be prepared fresh for immediate use.

(4) Mix and react with shaking at 4-25°C for 2-24 hours.

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

(5) After the reaction, collect the coupling solution to check coupling efficiency.

(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. Column Packing:

4.1 Gravity Column Packing:

(1) Take a gravity column of appropriate size. Insert the bottom frit, add a suitable amount of pure water to wet the column tube and frit, then close the bottom outlet.

(2) Mix the coupled medium suspension evenly, aspirate an appropriate amount with a pipette tip, and add it to the gravity column. Open the bottom outlet to drain the storage solution.

(3) Add an appropriate amount of pure water to wash the medium. After the liquid drains by gravity, close the bottom outlet.

(4) Insert the pre-wetted top frit, ensuring no gaps between the frit and the medium and that the frit is level.

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

4.2 Medium-pressure Chromatography Column Packing:

Coupled medium can also be used for large-scale sample purification, which involves packing various medium-pressure chromatography columns. The method for packing such columns is described below.

Before packing, calculate the column base area based on the column diameter, and calculate the required medium volume based on the desired bed height using the following formula:

$V = 1.15 \times \pi \times r^2 \times h$.

- V: Required medium volume (mL).

- 1.15: Compression factor.
- r: Column radius (cm).
- h: Packed bed height (cm).

Note: The volume of suspension taken should be twice the medium volume, as the medium occupies only half the total suspension volume, the other half being the storage solution.

(1) Rinse the column bottom frit and adapter with deionized water to ensure no air bubbles are trapped. Close the column outlet and leave 1–2 cm of deionized water at the column bottom.

(2) Resuspend the medium and carefully pour the slurry continuously into the column. Pouring along the column wall using a glass rod can reduce bubble formation.

(3) If using a reservoir, immediately fill both the column and reservoir with water. Place the packing adapter on the slurry surface and connect it to the pump, avoiding air bubbles in the adapter or tubing.

(4) Open the column outlet and start the pump at the set flow rate. Initially, allow the buffer to flow slowly through the column, then gradually increase to the final packing flow rate. This avoids hydraulic shock to the forming bed and prevents uneven packing. If the recommended pressure/flow cannot be reached, use the pump's maximum flow rate; this can also yield good packing results.

(Note: In subsequent chromatography runs, do not exceed 75% of the maximum packing flow rate.) Once the bed height stabilizes, run at least 3 CV of deionized water at the final packing flow rate. Mark the final bed height.

(5) Stop the pump and close the column outlet.

(6) If a reservoir was used, remove it and place the top flow adapter into the column.

(7) Lower the adapter until it touches the marked bed height. Allow packing liquid to enter the adapter, then tighten the adapter lock nut.

(8) Connect the packed column to the pump or chromatography system and begin equilibration. Readjust the adapter if necessary.

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.

5.4 Medium-Pressure Column Purification:

Once packed, the medium-pressure column can be used with conventional medium/low-pressure chromatography systems.

(1) Prime the pump tubing with deionized water. Remove the top cap, connect the column to the chromatography system, open the bottom outlet, attach the pre-packed column to the system, and tighten.

(2) Flush out the storage buffer with 3-5 CV of deionized water.

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

(4) Load the sample using a pump or sample loop.

Note: Increased sample viscosity can cause high backpressure even with small sample volumes. Do not exceed the binding capacity of the column. Large sample volumes can also cause high backpressure, making the injector harder to use.

(5) Wash the column with Wash Buffer until the UV absorbance reaches a stable baseline (typically at least 10-15 CV).

(6) Elute with 5-10 CV of Elution Buffer. Collect the eluate, which contains the target protein. Neutralize the eluted fractions immediately by adding a volume of Neutralization Buffer equal to 1/10th of the elution fraction volume.

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

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.