NHS-activated Agarose Resin (LA)

N1492642

Storage: -20℃.

Introduction:

This product is a type of pre-activated agarose microsphere, which can be directly used for the conjugation of proteins or peptides containing amino groups. Compared with conventional NHS-activated affinity chromatography media, the NHS-Activated Affinity Chromatography Media (LA) features an extended spacer arm length between the microspheres and ligands. This design reduces steric hindrance between ligands, facilitating the stability of the ligand's own structure and the preservation of its activity.Pre-activated media can be prepared into specialized affinity media as needed, enabling rapid and efficient one-step purification of the target substances from complex systems. The NHS-Activated Affinity Chromatography Media (LA) exhibits excellent pressure resistance, maintains stable performance after protein conjugation, and is suitable for large-scale industrial purification.

Aladdin NHS-activated Agarose Resin (LA) is stored in 100% isopropanol, 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
Coupling Capacity	>10 mg IgG / mL medium
Particle Size Range	45~165 μm
Max Pressure	0.3 MPa, 3 bar
Storage Buffer	100% Isopropanol
Storage Temperature	-20℃
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.

- Wash Solution: 1 mM HCl.
- Coupling Buffer: 0.2 M $NaHCO_3$, 0.5 M NaCl, pH 8.0.
- Blocking Buffer: 0.5 M Ethanolamine, 0.5 M NaCl, pH 8.3 *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 20% Ethanol.

Notes:

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

(2) When the coupled sample is an antibody, 1× PBS with 0.02% NaN_3 or 1× PBS with 0.02% ProClin 300 can be used as the Storage Buffer.

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) Take an appropriate amount of NHS-activated Agarose Resin (LA). Wash three times by suction filtration using the Wash Solution (1 mM HCl), then once with Coupling Buffer.

Note: Do not let the medium dry out completely to avoid clumping. If clumping occurs, resuspend by vortexing or pipetting. Using pre-chilled solutions for rapid washing can reduce hydrolysis of the pre-activated medium.

(4) Add the dissolved sample to the washed NHS-activated Agarose Resin (LA). The recommended medium-to-sample solution volume ratio is approximately 1:1-2 (V/V).

(5) React with shaking at 28°C for 2-4 hours or at 4°C overnight.

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

(6) After the reaction, collect the coupling solution to check coupling efficiency. Wash the medium with deionized water. Add 2 CV of Blocking Buffer and react with shaking at 28°C for 1 hour.

Note: UV absorbance cannot be used to determine the protein concentration in the supernatant after coupling. It is recommended to use electrophoresis or a BCA assay to determine the coupling efficiency.

(7) 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 gravity column of appropriate size. Insert the bottom frit. Add some purified water to wet the column tube and frit. Close the bottom outlet.

(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.

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

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

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

4.2 Medium-Pressure Chromatography Column Packing:

Coupled medium can also be used for large-volume sample purification, requiring packing into medium-pressure columns. The method is described below:

Before packing, calculate the column base area based on its diameter and the required medium volume based on the desired bed height using the 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: Packing height (cm).

Note: The slurry volume taken should be twice the medium volume, as the medium only occupies half the total slurry volume; the other half is storage solution.

(1) Flush the column bottom screen and adaptor with deionized water to ensure no air bubbles are trapped. Close the bottom outlet, leaving 1-2 cm of deionized water in the column bottom.

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

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

(4) Open the column bottom outlet and start the pump at the set flow rate. Initially, allow buffer to flow slowly through the column, then gradually increase to the final flow rate. This avoids disturbing the formed bed and ensures uniform packing. If the recommended pressure/flow cannot be reached, use the pump's maximum flow rate for acceptable results. (Note: Do not exceed 75% of the maximum packing flow rate in subsequent chromatographic runs.) After the bed height stabilizes, continue pumping at least 3 CV of deionized water at the final packing flow rate. Mark the bed height.

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

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

(7) Lower the adaptor down to the marked bed height. Allow packing buffer to enter the adaptor and tighten the adaptor fitting.

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