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Amino-Activated Beads 4FF

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1. Product Description

Amino-Activated Beads 4FF is an agarose beads containing the active group of primary amine. With the help of chemical cross-linking agent, this product can be covalently coupled with molecules containing carboxyl groups to prepare special affinity media, which can quickly and effectively purify the corresponding substances in one step from the complex system. **Amino-Activated Beads 4FF** have good pressure resistance and the conjugated proteins have stable properties. They can be used in industrial purification on a large scale.

Table 1. Characteristics of **Amino-Activated Beads 4FF**

Item	Description
Matrix	Highly cross-linked 4% agarose
Ligand Density	>12μmol amino groups/ml
Particle (μm)	45-165
Maxi pressure	0.3 MPa, 3 bar
Storage buffer	1XPBS containing 20% ethanol
Storage	4-30°C

2. Coupling Procedure

2.1 Buffer Preparation

Water and chemicals used for buffer preparation should be high purity. It is recommended to filter the buffers by passing them through a 0.22μm or 0.45 μm filter before use.

Cleaning Bufffer: 1mM HCl

Coupling Buffer: 0.1M MES，pH 4.5

Wash Buffer 1: 0.1M NaAc, 0.5M NaCl, pH3.0

Wash Buffer 2: 0.1M Tris-HCl，0.5M NaCl，pH8.0

Storage Buffer: 20mM sodium phosphate, 20% ethanol，pH8.0

2.2 Sample Preparation

It is recommended to filter the sample solution by passing them through a 0.22μm or 0.45 μm filter before use.

The sample should be dissolved in **Coupling Buffer** with a concentration of about 5-10mg/ ml.

If the sample is insoluble, add 50% dioxane or ethylene glycol and adjust pH to 4.5-6.0 using a pH test paper.

2.3 Couple to Amino-Activated Beads 4FF

1) Resuspend and pack the **Amino-Activated Beads 4FF** into the desired affinity column system. Allow the resin to settle down and the buffer to drain from the column.

2) Add 3 column volumes Cleaning Buffer onto the column to equilibrate the resin and drain the buffer. Repeat the above test for 2 times. Equilibrate with 3 column volumes of Coupling Buffer once.

3) Directly add the measured EDC solid powder, or add the high concentration of EDC solution drop by drop to make the final concentration 0.1M. If it is mother solution, pH should be adjusted in advance.

Note: Generally, the number of EDC is about 10-100 times of the number of ligand, and the recommended concentration is 0.1M. The EDC solution needs to be ready to use.

4) Apply the sample onto the column, shaking 2-24 hours at 4-25℃.

5) After coupling, allow the buffer to drain from the column. Wash the medium with 3 column volumes Coupling Buffer.

6) Add 3 column volumes distilled water onto the column to wash the resin and drain the buffer. Repeat the above test for 2 times. Wash the medium with 3 column volumes Wash Buffer 1 , deionized water, Wash Buffer 2 and deionized water. Repeat this step one more time.

7) Store the medium with Storage Buffer at 2℃-8℃.

2. 4 Purification Procedure

2.4.1 Buffer Preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter the buffers by passing them through a 0.22 or 0.45 μm filter before use.

Binding/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

2.4.2 Sample Preparation

To insure that proper ionic strength and pH are maintained for optimal binding, it is necessary to dilute serum samples, ascite fluid or cell culture supernatant at least 1:1 with Binding/Wash Buffer. Alternatively, the sample may be dialyzed overnight with Binding/Wash Buffer.

2.4.3 Column Purification

1) Mix the slurry by gently inverting the bottle several times to completely suspend the rProtein G Beads. Close the column outlet leaving the net covered with packing buffer. Transfer the slurry to the column.

2) Allow the resin to settle down and the buffer to drain from the column. Add 5 column volumes Binding Buffer onto the column to equilibrate the beads.

3) Apply the sample to the column and drain the flow-through. Collect the flow-through for measuring the binding efficiency to the beads, i.e. by SDS-PAGE.

4) Wash the column with 10-15 column volumes Wash Buffer or until the absorbance of the effluent at 280 nm is stable.

5) Elute the sample with 5-10 column volumes Elution Buffer. Collect the eluate containing the target immunoglobulin and immediately neutralize to pH 7.4 with Neutralization Buffer (1/10 volume of total eluate).

6) Equilibrate the column with 3 column volumes of Binding Buffer , 5 column volumes of distilled water and 5 column volumes of 1XPBS containing 20% ethanol. Finally store the resin with 1XPBS containing 20% ethanol at 4℃.

2.4.4 Analysis

Identify the fractions using UV absorbance, SDS-PAGE, or western blot.

3. Related Products

Product	Cat. No.	Size
Amino-Activated Beads 4FF	SA043005	5 ml
	SA043025	25 ml
	SA043100	100 ml
	SA043500	500 ml
	SA04301L	1 L