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Epoxy-Activated Beads 6B

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1. Product Description

Epoxy-Activated Beads 6B is a pre-activated resin which can be used in the coupling of proteins or samples containing amino, sulfhydryl and hydroxyl groups. The preactivation media can be prepared into special affinity media according to the needs, and the corresponding substances can be quickly and effectively purified in one step from the complex system.

Table 1. Characteristics of **Epoxy-Activated Beads 6B**

Item	Description
Matrix	6% agarose
Ligand Density	30-40 μmol/ml
Particle	45-165 μm
Maxi pressure	0.1 MPa, 1 bar
Storage buffer	100% 1,4-Dioxane
Storage	Room Temperature

2. Coupling Procedure

2.1 Buffer Preparation

Water and chemicals used for buffer preparation should be high purity. It is recommended filtering the buffers by passing them through a 0.22 μm or 0.45 μm filter 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 NaAc, 0.5 M NaCl, pH 3.0

Wash Buffer 2: 0.1M Tris-HCl, 0.5 M NaCl, pH 8.0

Storage Buffer: 20 mM sodium phosphate, 20% ethanol, pH 8.0

2.2 Sample Preparation

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

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

2.3 Couple to Epoxy-Activated Beads 6B

1) Resuspend and pack the **Epoxy-Activated Beads 6B** 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 distilled water 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) Apply the sample onto the column, shaking 24 hours at 25-40℃.

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

5) Add equal column volume Blocking Buffer onto the column, shaking 1 hour at 37℃. Drain the 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
Epoxy-Activated Beads 4FF	SA040005	5 ml
	SA040025	25 ml
	SA040100	100 ml
	SA040500	500 ml
	SA04001L	1 L
Epoxy-Activated Beads 6B	SA071005	5 ml
	SA071025	25 ml
	SA071100	100 ml
	SA071500	500 ml
	SA07101L	1 L