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ECH-Activated Beads 4FF

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1. Product Description

ECH-Activated Beads 4FF is a pre-activated agarose microsphere, which has a free carboxyl group at the end of a 10-atom hydrophilic spacer arm and can be used directly for the coupling of amino containing proteins and samples.The longer hydrophilic spacer arm makes it more suitable for coupling small molecules. **ECH-Activated Beads 4FF** can be used to prepare affinity adsorbents which can isolate specific substances from complex mixtures, often achieving very high purity in a single step.

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

Item	Description
Matrix	Highly cross-linked 4% agarose
Ligand density	＞15 μmol/ml medium
Particle	45-165 μm
Maxi pressure	0.3 MPa, 3 bar
Storage buffer	1×PBS containing 20% ethanol
Storage	2-8℃

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 0.22 μm or 0.45 μm filter before use.

Cleaning Buffer: 1mM HCl

Coupling Buffer: 0.1 M MES, pH 4.5-6.0

Blocking Buffer: 1.0 M ethanolamine,pH 8.0 or 0.1 M Tris, pH 8.5

Wash Buffer 1: 0.1 M acetic acid-sodium acetate ,0.5 M NaCl, pH 3.0

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

Storage Buffer: 1XPBS containing 20% ethanol

Note: 1) Do not contain amino, phosphate and carboxyl groups in the coupling buffer, otherwise it will affect the reaction.A certain concentration of salt ions can be added to buffer liquid system to reduce non-specific adsorption.

2) When the conjugate sample is antibody, 1XPBS, 0.02%NaN_3 or 1XPBS, 0.02%Proclin 300 can be selected as the storage buffer.

2.2 Sample Preparation

The samples are dissolved with Coupling Buffer at a concentration of 5-10 mg/ml.

2.3 Couple the ligands to ECH-Activated Beads 4FF

1) Resuspend and pack the **ECH-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 wash the resin and drain the buffer. Repeat the step for 2 times.

3) Add 3 column volumes Coupling Buffer onto the column to equilibrate the resin and drain the buffer.

4) Add the sample(dissolved in Coupling Buffer) .

5) Add the measured EDC solid powder directly, or add a high concentration of EDC drop by drop, so that its final concentration is 0.1M. If it is mother liquor, the pH should be adjusted in advance.

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

6) Mixed contact at least 2 hours at 28℃ or overnight at 4℃. To ensure that the resin is suspended, otherwise it will affect the coupling efficiency.

Note: The pH of the solution will decrease after the first hour of reaction, and the pH needs to be adjusted with 0.1 M NaOH to 4-6.

7) Collect the sample of coupling reaction and detect the coupling efficiency.

8) 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
ECH-Activated Beads 4FF	SA063005	5 ml
	SA063025	25 ml
	SA063100	100 ml
	SA063500	500 ml
	SA06301L	1 L