



# NHS-Activated Beads 4FF

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## 1. Product Description

**NHS-Activated Beads 4FF** is a pre-activated agarose beads that increases the choice of coupling chemistries available. NHS coupling forms a chemically stable amide bond with ligands containing primary amino groups. **NHS-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. **NHS-Activated Beads 4FF** fulfills industrial demands for security of supply and robust performance.

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

| Item           | Description                    |
|----------------|--------------------------------|
| Matrix         | Highly cross-linked 4% agarose |
| Capacity       | > 10 mg IgG/ml medium          |
| Particle       | 45-165 μm                      |
| Maxi pressure  | 0.3 MPa, 3 bar                 |
| Storage buffer | 100% isopropanol               |
| Storage        | -20°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 0.22 μm or 0.45 μm filter before use.

**Cleaning Buffer:** 1mM HCl

**Coupling Buffer:** 0.2 M NaHCO<sub>3</sub>, 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, 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) Coupling should be performed in bicarbonate or borate buffers. Tris and other buffer salts containing amino groups or other nucleophilic components should not be used since these will couple to the medium.

2) When the conjugate sample is antibody, 1XPBS, 0.02%NaN<sub>3</sub> 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 NHS-Activated Beads 4FF

1) Resuspend and pack the **NHS-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.

**Note:** The Cleaning Buffer and Coupling Buffer can be pre-cooled and quickly cleaned to reduce hydrolysis of the pre-activated medium.

4) Add the sample(dissolved in Coupling Buffer) ,shaking at least 2 hours at 28°C or overnight at 4°C. To ensure that the resin is suspended, otherwise it will affect the coupling efficiency.





5) Collect the sample of coupling reaction and detect the coupling efficiency. Add 2 column volumes Blocking Buffer onto the column, shaking 1hours at 28°C. Drain the buffer.

**Note:** The concentration of supernatant protein cannot be determined by UV absorption after coupling. SDS-PAGE or BCA protein assay is recommended to detect the coupling efficiency.

6) 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°C-8°C.

## 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<sub>2</sub>HPO<sub>4</sub>, 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°C.

### 2.4.4 Analysis

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

## 3. Related Products

| Product                    | Cat. No. | Size   |
|----------------------------|----------|--------|
| NHS-Activated Beads 4FF    | SA039005 | 5 ml   |
|                            | SA039025 | 25 ml  |
|                            | SA039100 | 100 ml |
|                            | SA039500 | 500 ml |
|                            | SA03901L | 1 L    |
| NHS-Activated LA Beads 4FF | SA083005 | 5 ml   |
|                            | SA083025 | 25 ml  |
|                            | SA083100 | 100 ml |
|                            | SA083500 | 500 ml |
|                            | SA08301L | 1 L    |

