Product Information

Product Name:	NHS-Activated Beads 4FF
Product Code:	SA039005/SA039100
Size(s):	5ml & 100ml

Product Description:

NHS-Activated Beads 4FF is a pre-activated agarose matrix that provides a wide selection for the coupling of amino-containing ligands, such as small proteins and peptides, to the spacer arm on the matrix. Simple and efficient affinity purification procedures can be performed with NHS-Activated Beads 4FF to attain target protein isolates of high purity from complex matrixes. NHS-Activated Beads 4FF fulfils the industrial requirements for the supply security and the robust performance.

NHS-Activated Beads 4FF is composed of highly cross-linked 4% agarose resins, containing N-hydroxysuccinimide (NHS) functional groups. The NHS coupling form stable amine linkages to primary amine groups on protein ligands.

Product Specification:

Matrix:	Highly cross-linked 4% agarose beads
Binding Capacity:	>10mg IgG/ml medium
Particle Size:	45 - 165μm
Maximum Pressure:	3bar (0.3MPa)
Storage Conditions	2 – 8°C, 100% Isopropanol

Test Items	Specifications
Appearance	White to off-white slurry
Volume	50% suspension
Binding Capacity	>10mg IgG/ml medium

Operation Protocol

1. **Buffer Preparation**
   Water and chemicals of high purity should be used. It is recommended to filter all buffers through a 0.22μm or 0.45μm filter before usage.

Cleaning Buffer: 1mM HCl
Coupling Buffer: 0.2M $NaHCO_3$, 0.5M NaCl, pH8.0
Blocking Buffer: 0.5M ethcholamine, 0.5M NaCl (pH8.3) or 0.1M Tris (pH8.5)
Wash Buffer I: 0.1M acetic acid-sodium acetate, 0.5M NaCl, pH4.0
Wash Buffer II: 0.1M Tris-HCl, 0.5M NaCl, pH8.0
Storage Buffer: 1XPBS containing 20% ethanol

Note: Coupling should only be performed in either bicarbonate or borate buffers. Tris and buffer salts containing amino groups or other nucleophilic components should not be used, as these buffers are reactive to NHS groups and will couple to the medium

2. Sample Preparation

Dissolve the ligand in the coupling buffer.

i. For ligands with low molecular weight, the recommended ratio of ligand to medium concentration lies within 75-150μmol ligand/mL
ii. For ligands with high molecular weight (i.e. proteins), the recommended ratio of ligand to medium concentration lies within 2 - 5mg ligand for each millilitres of medium

3. Coupling the Ligands

3.1. 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 out from the column
3.2. To equilibrate the resin, add 3 column volumes of Cleaning Buffer into the column and drain the buffer. Repeat this step twice
3.3. Apply the Coupling Buffer into the column, and agitate on a rocker shaker for at least 2hrs at room temperature or overnight at 4°C. Ensure that the resin is well suspended, to prevent affecting the coupling efficiency
3.4. Collect the supernatant from the coupling reaction to determine the coupling efficiency. Add 2 column volumes of Blocking Buffer into the column. Agitate for 1 hour at room temperature
3.5. Drain the buffer
3.6. Wash the ligand-conjugated resin with 3 column volumes of each of the solutions in the following order: Wash Buffer I → deionized water → Wash Buffer II → deionized water
3.7. Repeat the washing in step **3.6** in the same sequential order

4. Cleaning-in-Place

i. Removal of precipitated or denatured substances:
a. Wash the column with 2 column volumes of 6M guanidine hydrochloride
b. Wash immediately with at least 5 column volumes of sterile filtered Binding Buffer

ii. Removal of bounded hydrophobic substances:
a. Wash the column with either 3 - 4 column volumes of 70% ethanol
b. Wash immediately with at least 5 column volumes of sterile filtered Binding Buffer

Storage

Store the medium in Storage Buffer at 2 - 8°C

Troubleshooting Guide

Problems	Possible Causes	Solutions
The coupling efficiency is too low	Before coupling, the primary amine-containing buffer is not completely removed	To completely remove Tris or glycine, dialyze or desalt the sample
The protein is insoluble in the Coupling Buffer	The protein molecule is hydrophobic	Dissolve the protein molecule in Coupling Buffer containing up to 4M Guanidine-HCl or 20% DMSO