Epoxy-001A Use and Applications

Epoxy-activated agarose 6B is a pre-activated medium for immobilization of various ligands including sugars through coupling of hydroxy, amino or thiol groups on the ligand to agarose 6B via a 12-atom hydrophilic spacer arm. Epoxy-activated agarose 6B can be used to couple sugars and other carbohydrates via stable ether linkages to hydroxyl groups.

1. Coupling method

1.1 Buffer preparation

Coupling Solution: 0.1M Na_2CO_3, pH 8.5~10.0.

Blocking Solution: 1M ethanolamine, pH 8.0.

Washing Solution 1: 0.1 M acetic acid-sodium acetate, 0.5 M NaCl, pH 3.0.

Washing Solution 2: 0.1 M Tris-HCl, 0.5 M NaCl, pH 8.0.

Protective Solution: 1 X PBS with 20% ethanol.

1.2 Ligand preparation

Dissolve the ligand to be coupled in Coupling Solution with a concentration of 5-10 mg/ml.

1.3 Ligand coupling

The following takes conjugated antibodies as an example:

1) Washing: Weigh out the required amount of Epoxy-activated agarose 6B, remove the protective solution, do not drain it, wash 3 to 5 times with distilled water, then wash once with Coupling Solution.

2) Coupling: Add the dissolved ligand to the medium, coupling ratio (medium: Coupling Solution) = 1:2 (V: V). Use a shaker in a water bath for 24 hours at 25°C to 40°C. Other gentle stirring methods may be employed. Do not use a magnetic stirrer. After the reaction is completed, the coupled ligand is collected to detect the coupling efficiency. Wash away excess ligand using coupling buffer.

3) Blocking: Add equal volume of Blocking Solution. Use a shaker in a water bath for 1 hour at 37°C.

4) Washing: Take out the blocked ligand, drain the solution in it, and wash the coupled ligand with 3 bed volumes of distilled water. Repeat washing with Washing Solution 1, distilled water, Washing Solution 2 and distilled water twice.

5) Storage: Store in an equal volume of Protective Solution at 2~8°C.

2. Regeneration

Conditions for regeneration depend on which ligand has been coupled. Literature references and textbooks may give good guidelines.

A general regeneration method is described below:

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An affinity medium may be regenerated for re-use by washing it with 2-3 bed volumes of alternating high pH (0.1 M Tris-HCl, 0.5 M NaCl, pH 8.5) and low pH (0.1 M sodium acetate, 0.5 M NaCl, pH 4.5) buffers. This cycle should be repeated 3 times followed by re-equilibration in binding buffer.

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