Epoxy-Activated Beads

Cat. 1004-5/10/25

Multi purpose activated support

ver. 1.2 03.07

Introduction

The **Epoxy-Activated Beads** is a preactivated support that contains high density of epoxy groups used for immobilization of various ligands. The preactivated support can be used to immobilize proteins, carbohydrates and various ligands via stable linkage to amino, thiol and hydroxyl groups. Coupling to hydroxyls is favored at a higher pH of 11-13, while Thiol-containing ligands are best coupled at pH 7.5-9.0.

Epoxy-Activated Beads characteristics

Matrix: Sepharose CL-4B
Active group: Oxiran.
Active group density: 10–25 µmole/ml
Bead size: 45-165 µm
Bead structure: Highly cross-linked spherical agarose, 6%
Max back pressure: 0.3 MPa, 3 bar
Max. flow rates: 4 ml/min/cm^2
Recommended flow rate: 1-2 ml/min/cm^2
Stability of the matrix: pH 2-11.

Protocol: Immobilization of ligands to Epoxy-Activated Beads

A. Buffers required

In general coupling is performed in carbonate, borate or phosphate buffers. Sodium hydroxide may be used for raising buffers pH. Avoid using Tris or free-amine containing buffers and other nucleophiles during conjugation, as these groups may couple to the epoxy groups. If organic solvents are required to dissolve the ligand, Dimethylformamide and Dioxane may be used to up to 50% of the final volume. The same concentration of organic solvents should be included in the coupling buffer in order to avoid ligand sedimentation during coupling.

Buffers required for conjugation of ligand to 10ml of **Epoxy-Activated Beads**.

1. Double distilled water (100 ml).

2. Coupling buffer (50 ml): Sodium bicarbonate or carbonate buffer buffer pH-8-10. Neutral conjugation buffer my also be used, although basic coupling buffer is preferred: Use ddH2O, or phosphate buffer such as PBS.
3. Blocking solution (100 ml): 1 M ethanolamine pH 8.0.
4. Wash buffer (200 ml): PBS pH 7.4.

B. Protocol

1. Gently mix the beads to form homogenous mix and remove by pipette the desired amount of beads. Wash beads 3 times with double distilled water. Washing could be done either using a suitable filter funnel or by centrifugations cycles of 1 minute long each one, done at approximately 600-1,000*xg*. Do not exceed 1,000*xg* as beads may deform.
2. Dissolve the ligand in the Coupling buffer. Organic solvent may be used. For large ligands (i.e. proteins with mw >10,000) prepare solution of 2-10 mg/ml use and for small ligands such as small peptides- prepare solution of at least 200 µmoles ligand per ml of coupling buffer.
3. Add two volumes of ligand solution to one volume of washed **Epoxy-Activated Beads** in polypropylene tube and mix gently. Sample 100ul upper supernatant (without beads) at time zero as reference sample and store in the refrigerator.
4. Mix slowly overnight at 4°C to 45°C (stability of the ligand limits the maximum temperature) preferably with the use of a rocker. **Do not use magnetic stirrer for mixing.**
5. Sample 100ul upper supernatant (without beads) for conjugation efficiency determination and wash away excess ligand using Coupling buffer in order to remove unbound biomolecules.
6. Add approximately two gel volumes of Blocking solution. Let it stand for at least 4 hours or overnight at 4°C.
7. Wash gel three times with 20 volumes of Wash buffer at room temperature, in order to remove unbound ethanolamine.

C. Storage

Store gel refrigerated in phosphate buffered saline (PBS) or other ligand-compatible buffer added with 0.1% azide (w/v) as a preservative until use.