

GLYOXAL AGAROSE BEADS PROCEDURE FOR USE

COUPLING LIGAND

Ligand: enzyme, protein or biomolecule.

COUPLING REACTION SCHEME:



PROCEDURE

1. Wash the Glyoxal Agarose Beads with distilled water using a glass filter.
2. Prepare the ligand solution and test the activity and/or absorbance at 280 nm.
3. Add 1 ml Glyoxal Agarose Beads to 9 ml ligand solution in a buffer at pH 10.05. If the ligand is not stable at room temperature, run the following steps in a cold room.
4. Stir gently and check pH frequently. Withdraw aliquots of suspension and assay for activity or absorbance at 280 nm.
5. Continue gentle stirring for several hours or until the activity measurements remain constant, which indicates complete immobilization. Avoid magnetic stirring.
Note: A longer immobilization time favors a strong biomolecule/bead reaction and stability, but may result in unfavorable distortions.
6. When the activity/absorbance is constant, add 10 mg solid sodium borohydride to the suspension and stir for 30 minutes at room temperature in an open container to allow hydrogen to escape. Do not perform this step near an open flame. Run near an extractor fan if possible.
7. Wash the suspension with 25 mM phosphate buffer pH 7.0 using a vacuum filter to eliminate the excess borohydride. Subsequently, wash the suspension thoroughly with distilled water, and filter to dryness.
8. The ligand-coupled Glyoxal Agarose Beads should be stored at 4-10°C in a preservative containing a buffer which is suitable for the ligand.

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