U.S. Biotech Sources, LLC, Richmond, CA 94820
Tel. (510) 488-3671; (510) 932-8735; (510) 734-3371; Fax (510) 758-9488
info@usbiotechsources.com; www.usbiotechsources.com

BTS Provides the Best tools for Protein Purification!

ENZYME & ANTIBODY IMMOBILIZATION

AMINOETHYL AGAROSE BEADS PROCEDURE FOR USE

COUPLING LIGAND

Ligand: enzyme, protein or biomolecule.

COUPLING REACTION SCHEME:

PROCEDURE

1. Wash the Aminoethyl 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. Prepare a solution of 8.85 ml distilled water, 0.19 g 1-(3-dymethylaminopropyl)-3-ethylcarbodiimide hydrochloride (CDI) and add the ligand. To find an appropriate concentration of ligand, albumin may be used as indicator, since it binds in similar proportions.
   If the ligand is not stable at room temperature, run the following steps in a cold room.
4. Add 0.7 g Aminoethyl Agarose Beads to the previous solution.
5. Stir gently, withdraw aliquots of suspension and test the activity and/or absorbance at 280 nm.
6. Continue gentle stirring for several hours (1-3) or until the activity measurements remain constant, which indicates complete immobilization. Avoid magnetic stirring.

   Note: Do not stir more than 3 hours because CDI will decompose. However, if the immobilization has to be performed in a cold room, because of the low stability of the ligand, stirring time may be longer.
7. Wash the suspension with distilled water to eliminate excessive reagents, then with 1.0 M NaCl, and finally with distilled water. After this stage, the ligand is bound to the aminoethyl matrix and can be stored in 0.03% sodium azide solution (4-10 ºC).

BIBLIOGRAPHY

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