ENZYME & ANTIBODY IMMOBILIZATION

AMINOETHYL AGAROSE BEADS & AMINOETHYL RAPID RUN™ BEADS

PROCEDURE FOR USE

ABT Aminoethyl resins allow a covalent binding of agarose to carboxy groups of ligands. The amino groups (resin) react with exposed carboxy groups (biomolecule). The result of the biomolecule immobilization is a stable and reusable resin for affinity purification in batch, spin column or gravity procedures.

COUPLING LIGAND

Ligand: enzyme, protein, peptide, antibody or biomolecule.

COUPLING REACTION SCHEME:

$$CH_2 - CH_2 - NH_2 \; + \; LIGAND\text{-}CO_2H \xrightarrow{CDI} CH_2 - CH_2 - NH - CO - Ligand$$

PROCEDURE

The following summarized procedure is adapted for the Immobilization of Ligands in batch or column procedures.

1. Elimination of the Preservative:

   Determine the quantity of Aminoethyl Resin needed for your immobilization following the Recommendations below.

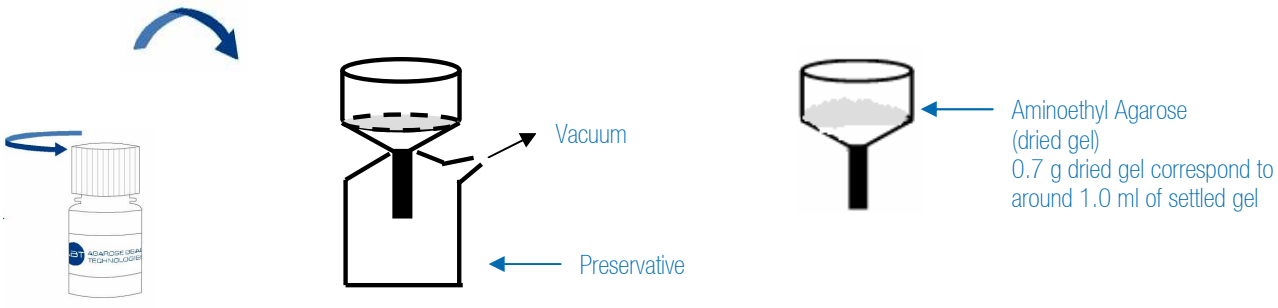
   The Resin is supplied as 50% slurry in preservative.

   Note: 1 ml gel corresponds to 2.0 ml of the supplied suspension.

   Wash the Aminoethyl Agarose Beads with distilled water using a medium porosity sintered glass funnel (for batch immobilization) or a gravity column (for column immobilization).

Batch Immobilization:

Manually shake the bottle of the resin to obtain a homogeneous suspension of beads and preservative. Invert the bottle of resin several times and then filter the resin and put it in a container.



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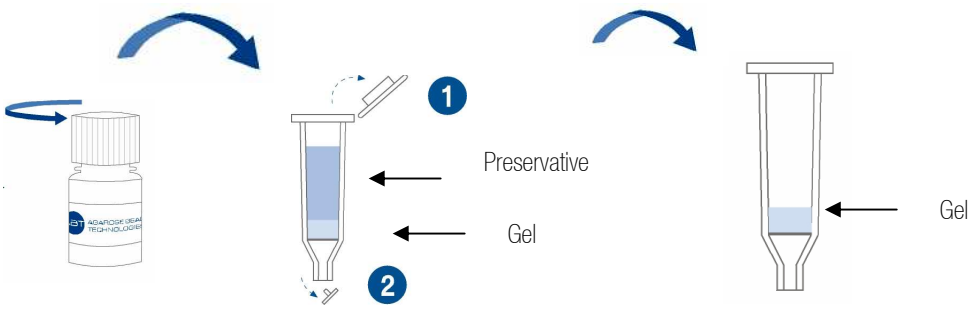
Gravity column immobilization: invert the bottle of the resin several times and then pipette[1] the desired volume into an empty gravity column (CAT. Nº: CXL-50) cutting pipette tip previously.



[1] Resin is supplied in an aqueous slurry containing preservative (50:50), so it is necessary to pipette double volume of liquid to get the desired amount of gel

2. Sample preparation:

Prepare the ligand solution and test the activity and/or absorbance at 280 nm.

Prepare a solution of 8.85 ml distilled water, 0.19 g 1-(3-dymethylaminopropyl)-3-ethylcarbodiimide hydrochloride (CDI) and add the ligand.

Note: To find an appropriate concentration of ligand, albumin may be used as indicator, since it binds in similar proportions.

Table 1: orientative binding capacity.

µmol Aminoethyl /ml gel	mg BSA immobilized / ml gel
3 - 6	~ 5
15 - 25	~ 14
40 - 60	~ 30

If the ligand is not stable at room temperature, run the following steps in a cold room.

4. Coupling:

Add 1ml Aminoethyl Agarose Beads to the previous solution.

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Stir gently, withdraw aliquots of suspension and test the activity and/or absorbance at 280 nm.

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.

Wash the suspension with distilled water to eliminate excessive reagents, then with 1.0 M NaCl, and finally with distilled water.

5. 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

- Guisán, J.M., Rodríguez, V., Soler, G., Santana, C., Fernández-Lafuente, R., Bastida, A. and Rosell, C.-M. (1993) Syntheses of pharmaceutical oligosaccharides catalysed by immobilized-stabilized derivatives of different ß-galactosidases. Journal of Molecular Catalysis, 84, 373-379.

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