AFFINITY COUPLING
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:

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.

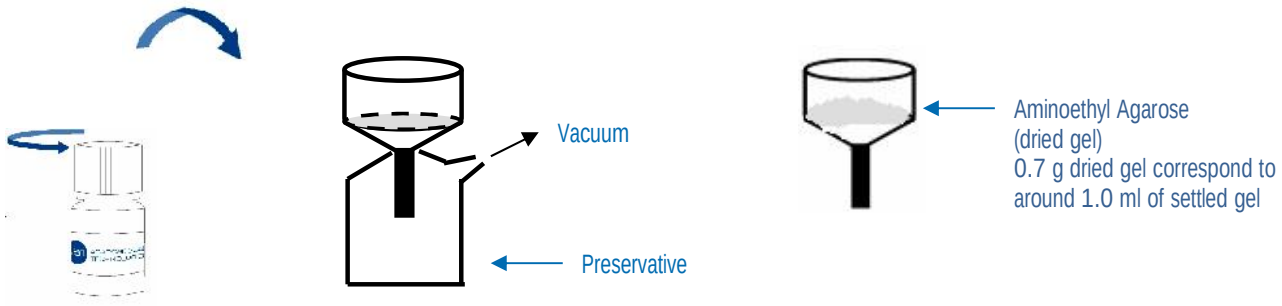
   The Resin is supplied as 50% slurry in preservative.

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

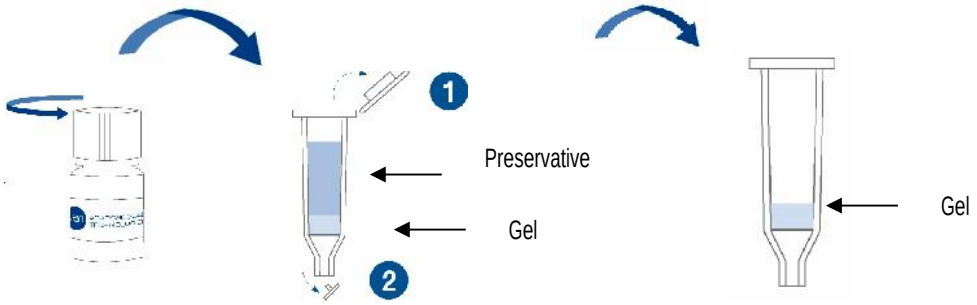
   Wash the Aminoethyl Agarose Beads with distilled water using a medium porosity sintered glass funnel (for batch immobilization) or a gravity column (for column immobilization).

   ### 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.



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 (EDC) 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.

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.

TROUBLESHOOTING GUIDE

Problems and Solutions

This table describes the possible causes of problems that could appear during the immobilization procedure steps and the recommendations for resolution.

OBSERVATION	POSSIBLE CAUSES	RECOMMENDATION
DURING IMMOBILIZATION STAGE THE ENZYMATIC ACTIVITY VARIES	The substrate diffusion towards the active center can be crippled.	- Increase the substrate concentration, increase the mixing/agitation to favour the substrate access to the active center.
	Steric hindrance of the substrate.	- Ask for information about "tailor made resins with spacer arms" (they will favour the access of the substrate to the active center of the enzyme).
DURING IMMOBILIZATION STAGE THE ENZYMATIC ACTIVITY DECREASES DRASTICALLY	The enzyme immobilization takes place through amino acid linkage that forms the essential part of the active center or essential amino acids for the enzymatic activity.	- Test using another activated resin.
	Immobilization can generate comformational changes causing a non-activated form.	- Test resins with a lower activation degree avoiding multipuntual binding. - Test optimal binding conditions of the enzyme to the resin. E.g: Reduce contact time in the immobilization process. If work conditions are at room temperature, make the immobilization at 4ºC.
	The experimental conditions of the process cause loss of enzyme activity.	- It is necessary to know the conditions in which the enzyme is stable.

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