Protocol

TD-P Revision 3.0

Creation Date: 8/2/2018
Revision Date: 9/11/2018

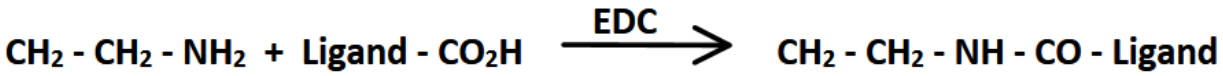
Enzyme & Antibody Immobilization

Utilizing Aminoethyl HTC Agarose Beads

Introduction

GoldBio Aminoethyl resins covalently bond to the carboxyl group present on different biological macromolecules including antibodies and enzymes. During the reaction, the amine groups in the resin react with exposed carboxyl groups in the macromolecule (ligand), and upon addition of a carbodiimide (EDC), the macromolecule is rendered immobile. The resulting resin is stable and reusable in affinity purification in batch, spin column or gravity flow formats and is a powerful tool for use in many different affinity applications. In addition, the use of aminoethyl resins may result in improved characteristics of the immobilized macromolecule. For example, immobilization of enzymes to aminoethyl resins can result in enhanced enzymatic activity and stability. This protocol describes the steps for the immobilization of the macromolecule, information on binding capacity, and helpful troubleshooting tips for a successful immobilization procedure.

Coupling Reaction Scheme:



The agarose bead contains primary amines, which in the presence of the carbodiimide (EDC), covalently bind the carboxyl group present in the ligand (macromolecule).

Materials

- GoldBio Aminoethyl HTC Agarose Beads
- Distilled water
- Deionized water
- Medium porosity sintered glass funnel (for batch immobilization) OR gravity column (for column immobilization) (GoldBio Product # P-301 or P-302)
- EDC Hydrochloride [1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride]
- 1M NaCl
- 0.03% Sodium Azide (if storing)
- Spectrophotometer (to measure activity/absorbance)

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Method

Elimination of the preservative

1. Determine the quantity of aminoethyl resin needed for immobilization.

> **Note: The resin is supplied as a 50% slurry in preservative, meaning that 1 ml of gel corresponds to 2.0 ml of the supplied suspension.**

2. Wash the Aminoethyl Agarose with distilled water using a medium porosity sintered glass funnel or gravity column, for batch or column immobilization, respectively.
    a. For batch immobilization, manually shake the bottle of resin to obtain a homogeneous suspension of beads and preservative. Invert the bottle of resin several times and then filter the resin and place in a container.
    b. For Gravity Column Immobilization: Invert the bottle of resin several times and, placing a funnel in the top of the column, pour the desired volume into an empty gravity column along the side of the column.

Sample Preparation

1. Prepare the ligand (macromolecule) solution and test the activity and/or absorbance at 280 nm.

> **Note: To find an appropriate concentration of ligand, BSA may be used as indicator since it binds in similar proportions. For binding capacity, refer to Table 1.**

2. Prepare a solution containing 8.85 ml of distilled water and 0.19 g EDC Hydrochloride and add the ligand.

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

Coupling

1. Add 1 ml Aminoethyl Agarose beads to the solution prepared above.

2. Stir gently and withdraw aliquots of suspension and test for activity or absorbance at 280 nm.

3. Continue gentle stirring for several hours or until the activity measurements remain constant, which indicates complete immobilization. This may take between 1 to 3 hours.

> **Note: Do not stir for more than 3 hours as the EDC will begin to decompose. However, if the immobilization has to be performed in a cold room, because of the low stability of the ligand, stirring time may increase.**

4. Wash the suspension with distilled water to eliminate excessive reagents.

5. Wash the suspension with 1M NaCl solution.

6. Wash with distilled water.

7. The ligand is now bound to the aminoethyl matrix and can be used immediately for affinity chromatography or stored in 0.03% sodium azide at 4-10°C until further use.

Note: The immobilized biomolecules can be utilized for downstream applications; for example, proteins can be run through gels and blotting techniques for verification while enzymes may be assayed.

Calculations

Table 1. Orientative Binding Capacity

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

Associated Products

Table 2. Applicable to all GoldBio Aminoethyl Ligands (sorted by percent, then density)

GoldBio Catalog #	Product Name
A-500	Aminoethyl 4% (Very Low Density) Agarose Beads, Cross-linked
A-499	Aminoethyl (High Density) 4% Agarose Beads, Cross-linked
A-505	Aminoethyl HTC 4% Very Low Density Agarose Beads
A-504	Aminoethyl HTC 4% High Density Agarose Beads
A-501	Aminoethyl (Low Density) 6% Agarose Beads, Cross-linked
A-503	Aminoethyl (High Density) 6% Agarose Beads, Cross-linked
A-508	Aminoethyl HTC 6% Very Low Density Agarose Beads
A-506	Aminoethyl HTC 6% Low Density Agarose Beads
A-507	Aminoethyl HTC 6% High Density Agarose Beads
A-502	Aminoethyl Agarose Beads-Test Kits

Troubleshooting

This table describes possible causes of problems that could appear during the immobilization procedure and potential resolutions.

Observation	Possible Causes	Recommendation
During the 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 favor the substrate access to the active center.
During the 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 conformational changes causing a non-activated form.	– Test Resins with a lower activation degree that avoids multiple point binding. – Test optimal binding conditions of the enzyme to the resin. For example, reducing contact time in the immobilization process. If work conditions are room temperature, consider lowering the temperature to 4°C.
	The Experimental conditions of the process cause loss of enzyme activity.	– Conditions may be unfavorable, analyze enzyme and adjust conditions.

References

Guisán, J. M., Rodriguez, V., Soler, G., Santana, C., Fernández-Lafuente, R., Bastida, A., ... & Martin-Lomas, M. (1993). Syntheses of pharmaceutical oligosaccharides catalyzed by immobilized-stabilized derivatives of different β-galactosidases. *Journal of molecular catalysis*, 84(3), 373-379.

Zhang, Y., Ge, J., & Liu, Z. (2015). Enhanced Activity of Immobilized or Chemically Modified Enzymes. *ACS Catalysis*, 5(8), 4503-4513. Doi:10.1021/acscatal.5b00996.