Protocol

TD-P Revision 3.0

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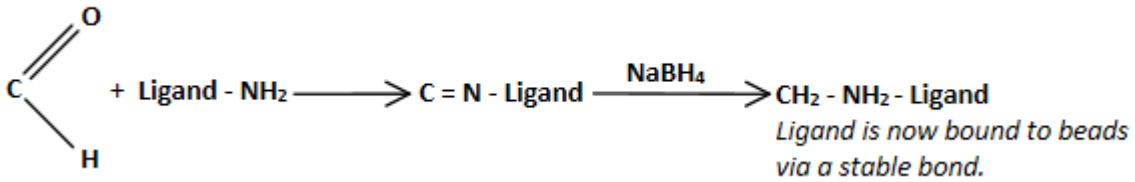
Enzyme & Antibody Immobilization Protocol

Utilizing Glyoxal Agarose Beads

Introduction

GoldBio Glyoxal Agarose beads/resins covalently bond to the amino group present on different biological macromolecules including antibodies, antigens, enzymes, protein A, protein G or other macromolecules. During the reaction, the aldehyde groups of the beads react with exposed primary amines on the macromolecule. This immobilization process creates semi-stable Schiff bases that are reduced with the addition of sodium borohydride. 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. This protocol describes the steps for the immobilization of a macromolecule (ligand) on Glyoxal agarose beads, information on binding capacity, and helpful troubleshooting tips for a successful immobilization procedure.

Coupling Reaction Scheme:



Materials

- Glyoxal Agarose Beads
- Glass filter
- dH_2O
- Spectrophotometer set to 280 nm
- 0.1M sodium bicarbonate at pH 10.0
- 25mM phosphate buffet at pH 7.0
- Vacuum filter
- pH meter
- Sodium Borohydride

Elimination of the preservative

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

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Note: The resin is supplied as a 50% slurry in preservative. Thus, 1 ml of gel corresponds to 2 ml of the supplied suspension.

2. Wash the glyoxal agarose beads with distilled water using a medium porosity sintered glass funnel or gravity column, for batch and 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, filter the resin, and place in a container.
    b. For gravity column immobilization, invert the bottle of resin several times and pipette (cut pipette tip before transferring the volume) the desired volume into an empty gravity column (GoldBio Catalog # P-301).

Note: This resin is supplied in an aqueous slurry containing preservative (50:50), so it's necessary to pipette double volume of liquid to get the desired amount of gel.

Sample preparation

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

2. Select the appropriate binding buffer, which depends on ligand characteristics. The coupling efficiency is higher at pH 10.0. The majority of affinity proteins tested are stable at this pH. Use Table 1 as a guide.

Table 1. Immobilization efficiencies of human IgG on 1 ml of Glyoxal Resin.

	pH 8.0		pH 9.0		pH 10.0	
Glyoxal resin category number:	mg human IgG immobilized per ml of gel	Coupling Efficiency	mg human IgG immobilized per ml of gel	Coupling Efficiency	mg human IgG immobilized per ml of gel	Coupling Efficiency
6% High Density Agarose G-303	1.1	11%	4.9	49%	9.5	95%
4% High Density Agarose G-301	1.2	12%	6.1	61%	9.6	96%

Note: The recommended coupling buffer is 0.1M sodium bicarbonate pH 10.0. Coupling efficiency with antibody is around 95%.

Note: It is important to avoid amine buffers such as Tris.

3. The quantity of immobilized ligand depends on several factors such as ligand size, glyoxal group (resin) density, amino group (ligand) density, time and temperature of immobilization, and pH. Refer to Table 2 for orientative binding capacity of these resins.

Table 2. Orientative binding capacity.

µmol Glyoxal per ml gel	mg BSA immobilized per ml gel	mg Protein A per ml gel	mg Protein G per ml gel
15 - 25	~ 10	~ 3	~ 3
40 - 60	~ 20	~ 3	~ 3
80 - 100	~ 30	N/A	

Coupling

1. Add 1 ml of glyoxal agarose beads to 9 ml ligand solution in a buffer at pH 10.0.

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

2. Stir gently and check pH frequently.

3. Collect aliquots and assay for activity or absorbance at 280 nm.

4. Continue stirring gently for several hours (1-6 hours) or until activity measurements remain stable indicating complete immobilization. Avoid magnetic stirring.

Note: A longer immobilization time results in a strong macromolecule/bead reaction and stability. However, unfavorable distortions may result.

Stabilization by incubation with reducing agent (reductive amination)

1. When activity/absorbance remains stable, 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.

Note: Do not perform this step near an open flame. Complete near an extractor fan if possible.

2. Wash the suspension with 25mM phosphate buffer at pH 7.0 using a vacuum filter to eliminate the excess borohydride.

3. Wash the suspension thoroughly with distilled water. Here, the macromolecule/bead bond is stable and the remaining active sites of the resin have been blocked.

4. The ligand-coupled Glyoxal Agarose Beads can be used immediately or stored at 4-10°C in preservative containing a buffer suitable for the ligand until further use. These beads are reusable.

Associated Products

- GoldBio Crosslinked Glyoxal Agarose Beads

References

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Troubleshooting

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 avoiding multipoint 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 4°C)
	The Experimental Conditions of the process cause loss of enzymatic activity	Check the conditions in which the enzyme is stable.