AFFINITY COUPLING

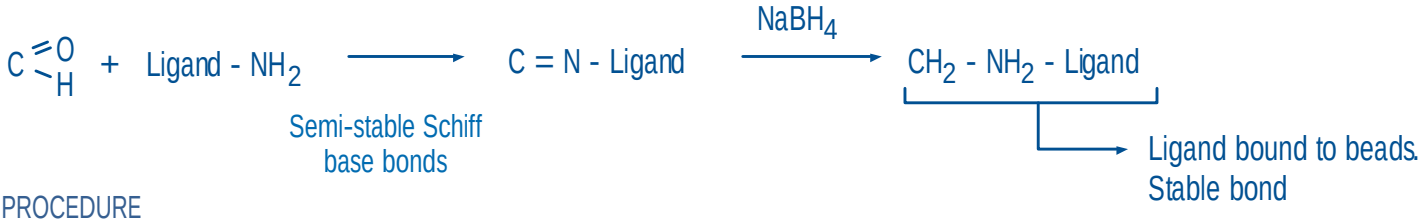
GLYOXAL AGAROSE BEADS & GLYOXAL RAPID RUN™ BEADS PROCEDURE FOR USE

ABT Glyoxal resins allow a covalent binding of agarose to amino groups of ligands (antibodies, antigens, enzymes, proteins or other biomolecules). The aldehyde groups (resin) react with exposed primary amines (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: antibody, antigen, enzyme, affinity proteins (e.g. Protein A or Protein G) 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 Glyoxal Resin needed for your immobilization following the Recommendations bellow.

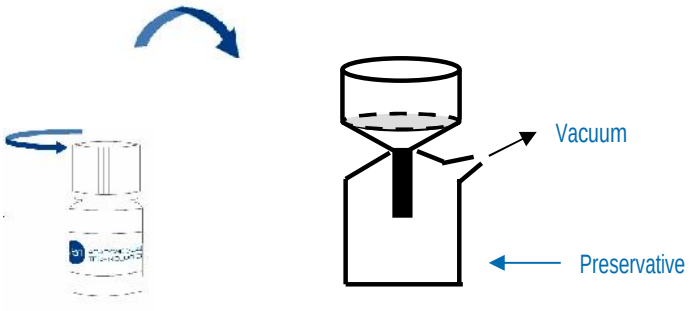
   The Resin is supplied as 50% slurry in preservative.

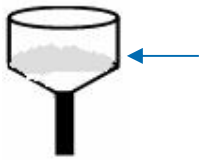
   Note: 1 ml gel corresponds to 2.0 ml of the supplied suspension.

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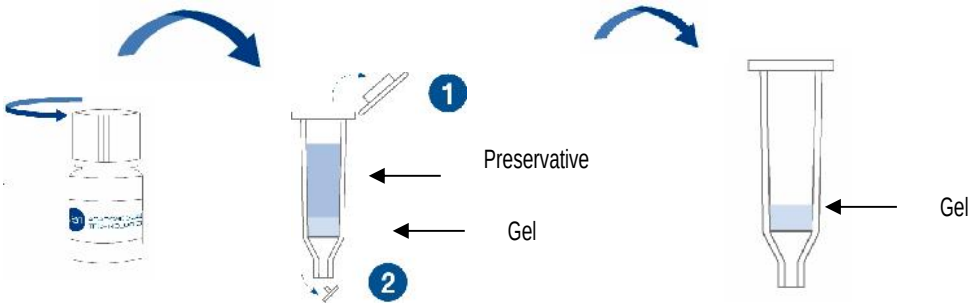
Gravity column immobilization: invert the bottle of the resin several times and then pipette[1] the desired volume into an empty gravity column (CAT. Nº: CXL-50) cutting pipette tip previously.



[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.

- pH:

Selection of the binding buffer depends on the characteristics of the ligand to be immobilized. The coupling efficiency is higher at pH 10.0 (see Table 1).

Note: the majority of the affinity proteins tested are stable at pH 10.0.

The following tables can be used as a guide.

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

	pH 8.0		pH 9.0		pH 10.0	
Cat Nº	mg human IgG immobilized /ml gel	Coupling Efficiency	mg human IgG immobilized /ml gel	Coupling Efficiency	mg human IgG immobilized /ml gel	Coupling Efficiency
6BCL-GM3	1.1	11%	4.9	49%	9.5	95%
4BCL-GH1	1.2	12%	6.1	61%	9.6	96%

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

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

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- Quantity of Ligand:

The quantity of ligand immobilized depends on several factors such as size of ligand, density of Glyoxal groups (resin), density of amino groups (ligand), time and temperature of immobilization and pH.

Table 2: orientative binding capacity.

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

3. Coupling: Add 1 ml Glyoxal Agarose Beads to 9 ml ligand solution in a buffer at pH 10.0. If the ligand is not stable at room temperature, run the following steps in a cold room.

   Stir gently and check pH frequently. Withdraw aliquots of suspension and assay for activity or absorbance at 280 nm.

   Continue gentle stirring for several hours or until the activity measurements remain constant, which indicates complete immobilization (1-6 hours). Avoid magnetic stirring.
   Note: A longer immobilization time favors a strong biomolecule/bead reaction and stability, but may result in unfavorable distortions.

4. Stabilization by incubation with a reducing (reductive amination): When the activity/absorbance is constant, 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. Do not perform this step near an open flame. Run near an extractor fan if possible.

   Wash the suspension with 25 mM phosphate buffer pH 7.0 using a vacuum filter to eliminate the excess borohydride. Subsequently, wash the suspension thoroughly with distilled water.

   In this step the bond is stable and the remaining active sites of the resin have been blocked.

5. The ligand-coupled Glyoxal Agarose Beads is reusable and should be stored at 4-10ºC in a preservative containing a buffer which is suitable for the ligand.

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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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