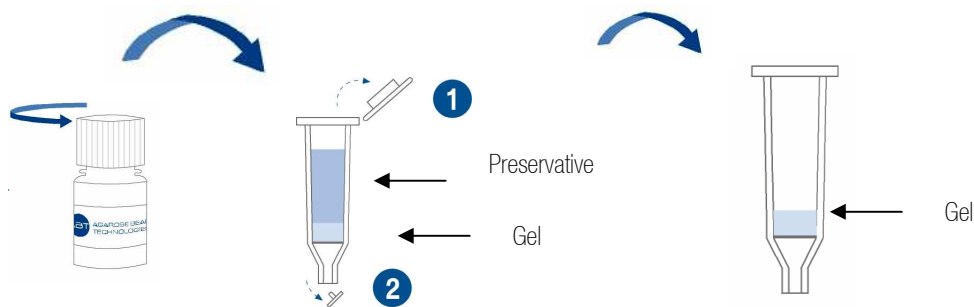


GLYOXAL AGAROSE BEADS & GLYOXAL RAPID RUN™ BEADS PROCEDURE FOR USE

Gravity column immobilization: invert the bottle of the resin several times and then pipette⁽¹⁾ the desired volume into an empty gravity column (CAT. N°: 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.

Cat N°	pH 8.0		pH 9.0		pH 10.0	
	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-GH1	1.5	15%	8.5	85%	9.4	94%
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 PBS.

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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 4-10°C in a preservative containing a buffer which is suitable for the ligand.

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