AGAROSE BEAD TECHNOLOGIES

GLYOXAL AGAROSE

BASE BEADS FOR AFFINITY CHROMATOGRAPHY RESINS

Affinity chromatography

Affinity chromatography is based on selective binding interaction between an immobilized ligand and the target molecule. Affinity chromatography plays a dominant role in the development of simple purification protocols that can be applied at both research and manufacturing scales. Purification processes that rely on affinity chromatography typically yield the desired purity of the target molecule in fewer steps and therefore have higher overall process yields. The high yields are important both at manufacturing scales when process economics are in focus, and at small scales when amounts of samples to be purified are limited.

Affinity chromatography resins are made by immobilization of ligands on the surface of base beads. The type of ligand used will determine the resin selectivity, while the type of beads will determine binding capacities as well as the level of nonspecific adsorption.

Examples of molecular interactions utilized for affinity chromatography include antibody-antigen, enzyme-inhibitor, or lectins-glycoproteins bindings. Among large-scale applications of affinity chromatography, the use of either Protein A or Protein G affinity resins for purification of monoclonal and polyclonal antibodies are good examples of highly successful implementations of affinity chromatography in the increasingly important field of diagnostic and therapeutic applications.

The quality of an affinity resin is determined by the strength of the ligand-target (ligate) interactions, the type of base matrix used, e.g., pore size and pore volume, and last but not least the type of surface chemistry employed for the ligand immobilization.

Preferred properties of base beads for preparation of an affinity chromatography resin are a large accessible surface area for ligand immobilization and subsequent target adsorption, hydrophilic character of the surface to reduce nonspecific interactions between the resin and the sample, and the right surface chemistry enabling ligand immobilization. In fact, immobilization chemistry of affinity ligands on chromatography support very often is the key differentiator between affinity resins using the same ligand, even if all other properties of the base matrix are kept the same.

From the resin quality perspective, the following resin attributes are related to the conjugation chemistry used for immobilizing ligands:

- Level of leached ligand
- Level of nonspecific binding
- Reusability
- Interaction strength (avidity)

Glyoxal agarose

Agarose Bead Technologies offers a family of preactivated agarose base matrices specifically developed for the preparation of affinity chromatography resins.

These base matrices are preactivated with aldehydes groups and are marketed under the trade name Glyoxal Agarose (GA). Glyoxal chemistry enables the coupling of proteinaceous ligands via reductive amination of the primary amino groups on the ligand surface. This chemistry enables stable conjugation of ligands via multipoint covalent attachment and has been proven advantageous over other immobilization chemistries due to the increased stabilization of the coupled ligand.

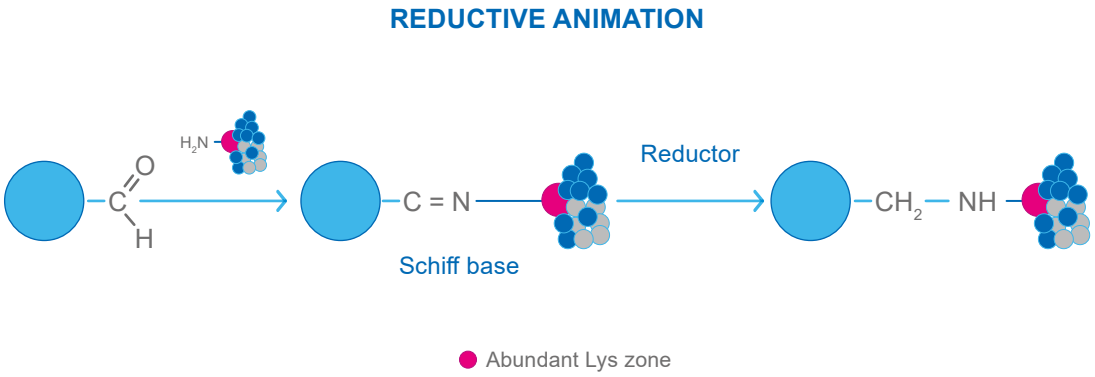


Figure 1. Scheme of protein immobilization process on GA beads.

GA is a highly activated support and, therefore, immobilization of ligands on GA beads occurs at alkaline pH. However, if the ligands have several exposed terminal amino groups on the same plane, they can be immobilized even at neutral pH (Figure 1).

The high activation level is one of the main advantages of GA support as compared to other types of activated beads. In reductive amination, ligand immobilization is driven by the higher density of reactive groups (primary amino groups, lysins) on the protein surface. Consequently, ligands are "self-directed" onto the agarose surface by the area where multipoint covalent immobilization is more favorable.

For this reason, GA has been successfully used for the stabilization of enzymes and proteins via multipoint covalent attachment with a low impact on the properties of immobilized ligand. For instance, between 1 to 4-logs improvement in the stability of the immobilized enzymes with at least 70% of their activity have been reported for enzymes immobilized on glyoxal agarose[1].

Advantages

Important attributes that make glyoxal agarose a preferred support for the preparation of a good affinity resin include:

- Very high yield of ligand immobilization
- Minimal ligand leakage
- Long shelf life characterized by the constant density of active groups
- No need for any special resin pretreatment prior to ligand immobilization
- Ethanol as a preservative agent

Other attributes that are directly associated with the glyoxal chemistry include:

- Very strong multipoint covalent ligand conjugation
- Ligand immobilization via primary amines (-NH2)
- Preferential ligand orientations (binding happens on abundant Lys zones)
- Very reproducible immobilization process
- Higher binding specificity as no cationic groups are present on the conjugated surface
- Very stable covalent binding with the formation of secondary amines enabling resin reusability
- Controllable ligand density

[1]Hussain, Fouzia et al., Molecules 23.12 (2018); Guisan, Jose M. et al., Methods in Molecular Biology. Vol. 2100, (2020), p. 83–92.

Density of glyoxal groups (preactivation levels)

Agarose Bead Technologies offers various types of glyoxal agarose base matrices differing in agarose concentrations, crosslinking degree, size of the bead (standard and fine), and with either high or low density of glyoxal groups.

Beads with a low density of glyoxal groups (15 - 25 µmol/mLresin) are recommended if higher ligand mobility after its immobilization is required from the ligand accessibility perspective. This is especially important in the case of enzyme immobilization processes where the three-dimensional structure of the enzyme could be affected by too many covalent bindings with the support surface.

Beads with a high density of glyoxal groups (40 - 60 µmol/mLresin) are recommended for applications where a stronger covalent binding of ligands to the agarose beads is required. The stronger binding improves ligand stability and thus resin reusability.

Examples of final ligand densities achievable with the ABT agarose glyoxal resins with the low and high density of the glyoxal groups are shown in Figure 2. The figure shows that the amount of immobilized protein-ligand on the surface of the beads is correlated with the number of glyoxal groups on the surface. Furthermore, the figure also shows that conjugation is a quick process. More than 90% of the ligand is conjugated within the first hour even at 4°C.

A.

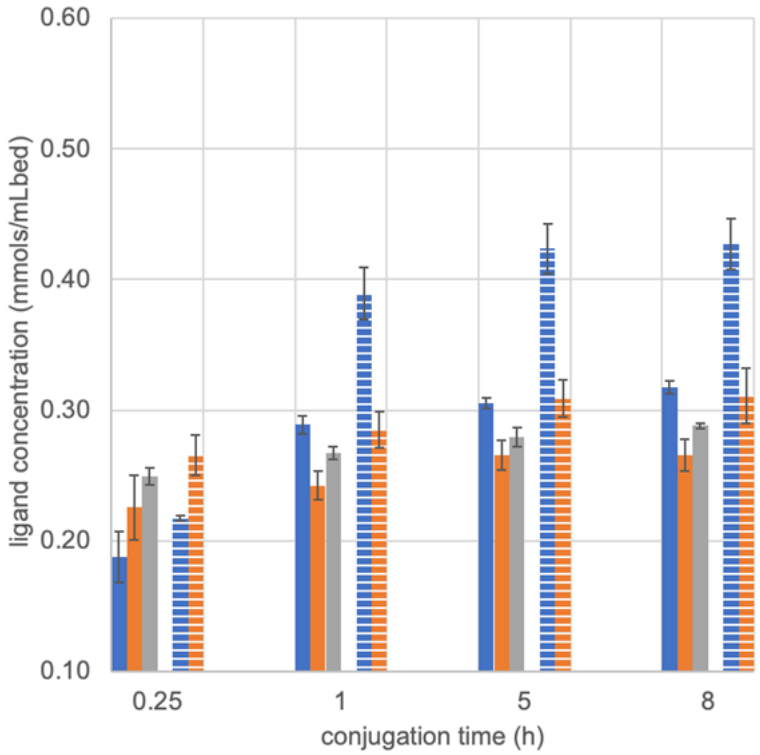
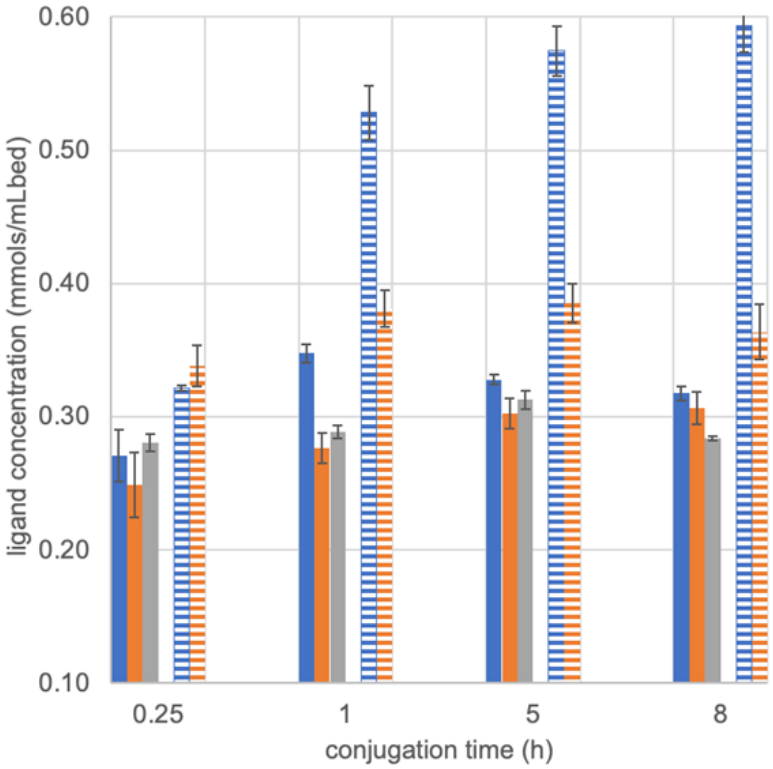
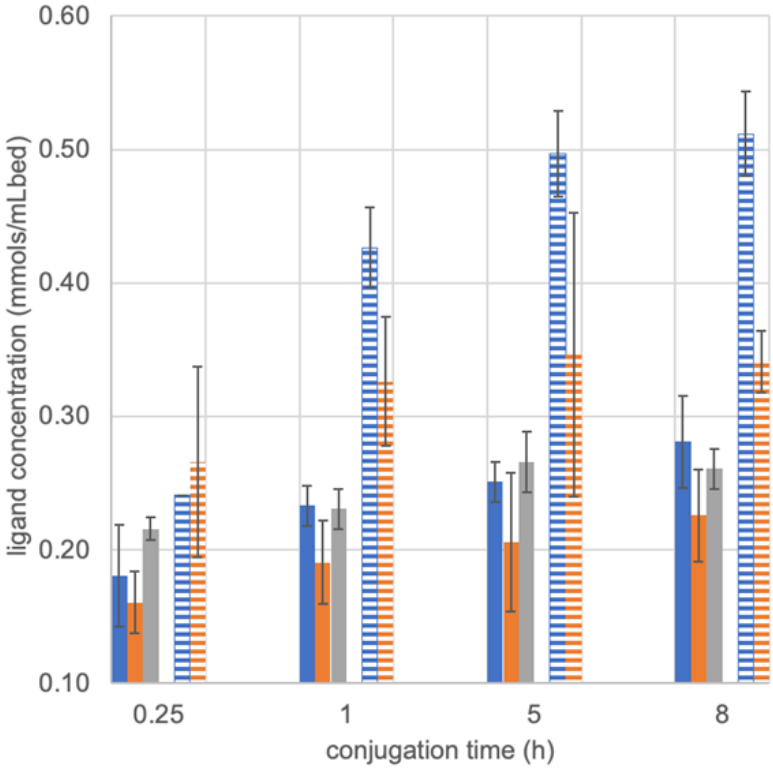


Figure 2. Protein ligand (BSA) immobilization kinetics performed at two temperatures on glyoxal agarose resins with different densities of glyoxal groups. A) 21°C and 4% agarose base beads; B) 21°C and 6% agarose base beads; C) 4°C and 6% agarose base beads. Legend: bars from left to right: Cross-Linked (CL) low ligand density; Rapid Run (RR) low ligand density; RR-Fine low ligand density; CL high ligand density; RR high ligand density. See the table at the end of the document for detailed resin specifications. Note: x-axis is not linear.

B.



C.



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Stable immobilization of a ligand on GA is a simple procedure that can be accomplished following a basic, 4-steps, ligand immobilization protocol:

1. Replace EtOH with the immobilization buffer (pH 10) by either filtration or decanting.
2. Prepare a 10% resin slurry in the immobilization buffer.
3. Add the ligand and follow the ligand immobilization process under gentle mixing conditions for at least 1 hour, or until the desire level of ligand, immobilization is achieved.
4. Add the reducing agent (sodium borohydride) to transform Schiff's bases into stable secondary amine bonds and to block the remaining active sites of the resin.
5. Wash the resin with an excess of phosphate buffer pH 7.0 to eliminate the excess of borohydride. Subsequently, wash the resin thoroughly with distilled water.
6. Store the resin with the corresponding storage solution, e.g., 20% EtOH.

Particle and pore sizes

ABT offers glyoxal agarose beads that are well suited for either batch or column-based purifications. The 4% agarose base beads are better suited for applications where low to moderate pressure drops are expected, e.g., batch adsorption or column chromatography at low flow rates and/or short bed heights. The beads based on 6% agarose are harder than the 4% beads and, therefore, can be used in columns packed to higher bed heights and/or be operated at higher flowrates. The 6% agarose beads can also be used for batch adsorption applications.

Since the capacity of an affinity resin will depend on the number of accessible ligands, for each combination of the ligand-ligate pair there will be an optimum pore size that will result in the highest binding capacity. For instance, a pore large enough to accommodate a ligand but too small to allow for the target protein to diffuse into the pore and bind to the ligand will not contribute with its surface to the binding capacity of the resin. As a rule of thumb, base beads with pores that are 10 times larger than the ligand-ligate complex should be chosen to obtain the right capacity of the affinity resin under typical flowrates used in column chromatography.

In order to provide this level of flexibility in designing an affinity resin, ABT offers glyoxal beads characterized by different pore sizes. These beads differ not only in their cutoff values but also in the volume of pores accessible for large proteins, e.g., Thyroglobulin. In Figure 3, a comparison of size exclusion selectivity curves for the 6 types of agarose glyoxal base beads is shown.

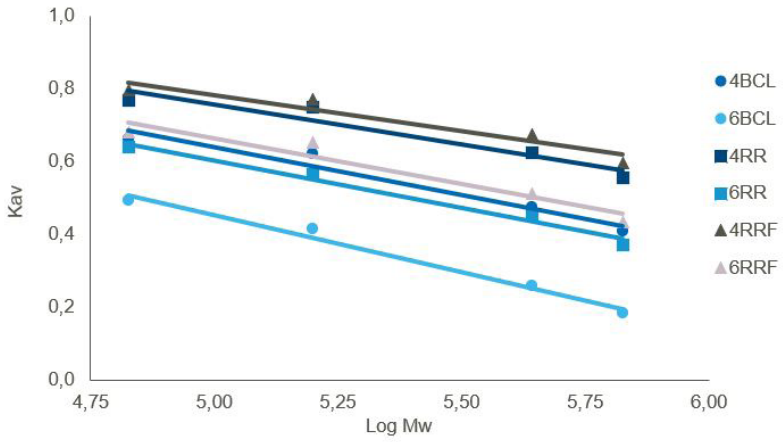

Figure 3. Size exclusion selectivity curves for the indicated base matrices varying in agarose content, level of cross-linking, and particle size. The graph shows the relationship between the average distribution constant (Kav) versus the logarithm of the molecular weight (Log Mw) of known proteins. Legend: abbreviations - BCL: crosslinked agarose beads; RR: rapid run agarose beads; RRF: rapid run fine agarose beads. The numbers in front of the abbreviations represent the agarose content.

Summary

The preactivated Glyoxal Agarose base beads offered by Agarose Bead Technologies possess all the required properties of preferred supports for the preparation of affinity resins. The superiority of these beads is associated with the character of glyoxal conjugation chemistry and with the vast selection of particle and pore sizes enabling either high capacities of the target and/or process scenarios.

Properties of agarose beads	Properties of glyoxal groups
Good chemical stability	Very good chemical stability
Good pressure-flow properties	Controllable density and spacer arm
Minimal non-specific binding	Very low non-specific binding
High accessible surface area	Low leaching of coupled ligand

Table 1. Properties of Glyoxal Agarose (GA) matrices offered by ABT.

The preactivated supports are available in various combinations of agarose content, particle size, rigidity, and levels of glyoxal activations making the glyoxal agarose family of activated resins a perfect choice of base beads for the preparation of affinity resins suitable for various types of purification tasks regardless of the scale of operation. All products within the Glyoxal Agarose family are summarized in the table below (Table 2).

You can also find more information about each type of Glyoxal resin from our webpage.

Product	Low Density Glyoxal						High Density Glyoxal			
	4BCL	6BCL	4 Rapid Run™	6 Rapid Run™	4 Rapid Run™ Fine	6 Rapid Run™ Fine	4BCL	6BCL	4 Rapid Run™	6 Rapid Run™
CAT. No.	4BCL-GL0-X	6BCL-GL0-X	4RR-GL0-X	6RR-GL0-X	4RRF-GL0-X	6RRF-GL0-X	4BCL-GH1-X	6BCL-GM3-X	4RR-GH1-X	6RR-GM3-X
Bead size	~50-150 µm				~20-50 µm		~50-150 µm			
Agarose content	4%	6%	4%	6%	4%	6%	4%	6%	4%	6%
Exclusion limit	>2x10^7	>4x10^6	~3 x 10^7	~4x10^6	~3 x 10^7	~4x10^6	>2x10^7	>4x10^6	~3 x 10^7	~4x10^6
Accessible volume										
Glyoxal Activation degree (µmol/mL$_{resin}$)	15-25						40-60			
Coupling capacity (mg BSA/ml$_{resin}$)	~21	~21	~18	~20	~19	~21	~28	~39	~21	~27
Bead geometry	Spherical									
Active groups	Diols oxidized to aldehydes									
Antimicrobial agent	20% ethanol									
Storage temperature	2 - 8°C									

Table 2. Technical Specifications for Glyoxal Agarose (GA) product family offered by Agarose Bead Technologies.

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If you are interested in agarose resins for the purification, separation or immobilization of biomolecules, contact us today at

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