

Aldehyde Agarose Beads

Catalog # AA-101

PRODUCT DETAILS

Matrix	4% highly cross-linked agarose (45 – 150 μ m beads)
Active Groups	Aldehydes (35 – 50 μ mol/ml)
Spacer	1 atom (when amino ligands are coupled with the aldehydes)
Coupling Capacity	30 – 40 mg/ml (BSA)
Form	Aqueous suspension with 20 % ethanol
Size	10 ml (20 ml suspension; cat. # AA-101-10), 5 ml (10 ml suspension; cat. # AA-101-5).
Storage Temperature	2 – 8 $^{\circ}$ C

Aldehyde Agarose Beads allow the covalent binding of agarose to the amino groups of the chosen biomolecule (peptides, enzymes, antibodies and etc.). Unlike to other resins which are available for this application, the bond between the Aldehyde Agarose Beads and the biomolecule is **irreversible**. Aldehyde agarose beads are ready to use and require no activation.

Coupling efficiencies with the aldehyde resin exceed those obtained with cyanogen bromide (CNBr) activated supports. Furthermore, aldehyde forms a bond with the amine-containing ligand that is more stable than with the CNBr method and is uncharged. These properties provide a more leak-resistant immobilization and lower nonspecific binding when used for affinity purification procedures.

The coupling reaction involves spontaneous formation of Schiff base bonds between aldehydes groups (on the support) and amines (on the ligand) and their subsequent stabilization by incubation with a reductant (sodium cyanoborohydride or sodium borohydride). The entire coupling reaction occurs in 1 to 4 hours in simple non-amine buffers such as PBS or bicarbonate. Coupling efficiency with proteins is generally greater than 85%, resulting in 1 to 12 mg of immobilized protein per milliliter of agarose resin.

Highlights:

- **Ideal for antibodies and other proteins** – immobilize molecules via primary amines ($-\text{NH}_2$)
- **Flexible coupling conditions** – efficient coupling over a wide range of pH (7-10) and buffer conditions (PBS or other non-amine buffer)
- **Stable, permanent immobilization** – Coupling reaction results in stable, leak-resistant secondary amine bond between resin and ligand
- **Better than immobilization to CNBr-activated agarose** – bond is more stable and uncharged, resulting in less nonspecific binding in affinity purification procedures
- **Versatile and reusable** – prepared affinity resin is adaptable to column and batch affinity techniques and the resin is reusable for typical applications based on protein binding interactions

Usage:

This material is offered for laboratory use only.

Procedure

1. Wash the Aldehyde Agarose Beads with distilled water using a glass filter.
2. Prepare the ligand solution and test the activity and/or absorbance at 280 nm.
3. Add 1 ml Aldehyde Agarose Beads to 9 ml ligand solution in a buffer at pH 10 (sodium carbonate-bicarbonate or borate buffers). If the ligand is not stable at room temperature, run the following steps in a cold room.
4. Stir gently and check pH frequently. Withdraw aliquots of suspension and assay for activity or absorbance at 280 nm.
5. Continue gentle stirring for several hours or until the activity measurements remain constant, which indicates complete immobilization. Avoid magnetic stirring. (**Note:** A longer immobilization time favors a strong biomolecule/bead reaction and stability, but may result in unfavorable distortions.)
6. 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.
7. 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, and filter to dryness.
8. The ligand-coupled Aldehyde Agarose Beads should be stored at 2-8 $^{\circ}$ C in a preservative containing a buffer which is suitable for the ligand.