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Product Information

Cyanogen Bromide-Activated Matrices

Catalog Numbers **C9210, C9142, and C9267**
Storage Temperature 2–8 °C

CAS RN 68987-32-6

Product Description
Cyanogen bromide-activated agarose matrices are offered for preparation of resins for affinity chromatography.

Processed agarose has a primary structure consisting of alternating residues of D-galactose and 3-anhydro-galactose. These sugars provide an uncharged hydrophilic matrix. Crosslinked agarose is usually preferred over the non-crosslinked version for most affinity applications that require harsh activation or usage conditions. Unfortunately, the added stability gained by crosslinking results in 30–50% loss of potential reaction sites (consumed in the chemistry of crosslinking).[1] The addition of crosslinks to stabilize beaded agarose does not reduce porosity significantly. Larger beads allow higher flow rates.

Cyanogen bromide (CNBr) in base reacts with hydroxyl groups on agarose to form cyanate esters or imidocarbonates (see Figure 1). These groups react readily with primary amines under very mild conditions; the net result is a covalent coupling of a ligand to the agarose matrix. The preferred resultant structure is an imidocarbonate, which has no net charge.

The isourea bond formed between the activated support and amine ligand is somewhat unstable, so a small but constant leakage of coupled ligand may occur. Isourea derivatives may also act as weak anion exchangers, causing nonspecific binding, especially when small ligands are immobilized.[7]

Advantages of cyanogen bromide-activation:

- Many matrices contain -OH groups.
- The pH conditions needed for coupling are mild enough for many sensitive biomolecules.
- The procedure is relatively simple and reproducible.
- The coupling works for large and small ligands - although for very small ligands, a spacer may be used to reduce steric hindrance.

These products are cyanogen bromide-activated as CNBr is highly toxic and sensitive to oxidation - most researchers prefer to use activated resins. They only need to be swollen, rinsed, and added to coupling buffer.

Cyanogen bromide-activated Agarose (Catalog Number C9210) - 4% crosslinked agarose, 40–165 μm diameter beads. Coupling capacity is ≥10 mg of BSA per mL of packed gel.

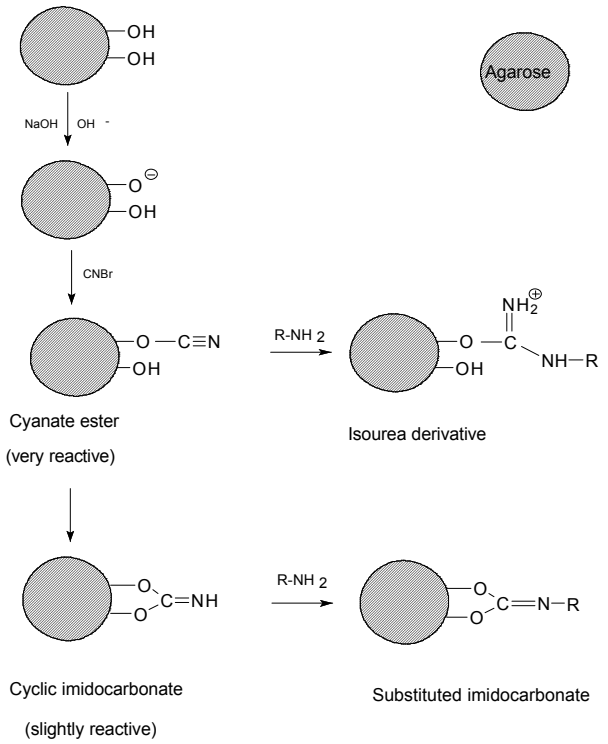
Cyanogen bromide-activated-Sepharose® 4B (Catalog Number C9142) - 4% agarose, 40–165 μm diameter beads. Coupling capacity is 30–40 mg of α-chymotrypsin per mL of packed gel.

Cyanogen bromide-activated−Sepharose 6MB (Catalog Number C9267) - 6% agarose, 200-300 μm diameter macrobeads. Coupling capacity is ≥20 mg of α-chymotrypsin per mL of packed gel.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
Store these powdered products at 2–8 °C. The activated resins are extremely moisture-sensitive.

Figure 1.
Reaction Scheme



Procedure

Although numerous references can be found in the literature for use with specific proteins (antibodies, enzymes, etc.) or nucleic acids, this protocol is written for general purposes.[2,3]

1. Dissolve protein to be coupled in 0.1 M $NaHCO_3$ buffer containing 0.5 M NaCl, pH 8.3–8.5, (5–10 mg protein per mL of gel).
   Note: Other buffers can be used, but avoid amine-containing buffers such as Trizma or other nucleophiles (buffers with amino groups) which will react with the binding sites.
2. Wash and swell cyanogen-bromide activated resin in cold 1 mM HCl for at least 30 minutes. A total of 200 mL per gram of dry gel is added in several aliquots. Remove the supernatant, which contains lactose by gentle filtration between successive additions.
   Note: Lactose is necessary to stabilize the beads during lyophilization, but it will interfere with binding if present during coupling. The use of HCl preserves the activity of the reactive groups, which hydrolyze at high pH.
3. Wash the resin with distilled water, 5–10 column volumes, then wash the resin with the $NaHCO_3$/NaCl coupling buffer (5 mL per gram dry gel) and **immediately** transfer to a solution of the ligand in coupling buffer.
   Note: The reactive groups hydrolyze in basic solution.
4. Mix protein with gel for 2 hours at room temperature or overnight at 2–8 °C. Use a paddle stirrer or end-over-end mixer, but **not** a magnetic stir bar, which may grind the beads.
5. Wash away unreacted ligand using $NaHCO_3$/NaCl coupling buffer.
6. Block unreacted groups with either 0.2 M glycine or 1 M ethanolamine, pH 8.0, for 2 hours at room temperature or 16 hours at 2–8 °C.
7. Wash extensively to remove the blocking solution, first with basic coupling buffer, pH ~8.5, then with 0.1 M acetate buffer, pH 4, containing 0.5 M NaCl.
8. Complete this wash cycle of high and low pH buffer solutions four or five times.
9. If the resin is to be used immediately, equilibrate it in buffer. If not, store the resin in 1.0 M NaCl at 2–8°C with a suitable bacteriostat.

References

1. Hermanson, G.T. et al., *Immobilized Affinity Ligand Techniques*, Academic Press (New York, NY: 1992) p. 6-9.
2. *Affinity Chromatography - Principles and Methods* (GE Healthcare), p. 15.
3. Hermanson, G.T. et al., *Immobilized Affinity Ligand Techniques*, Academic Press (New York, NY: 1992) p. 53-56.
4. *Methods in Enzymology*, **34B**, 77-102 (1974).
5. *Methods in Enzymology*, **104**, 3-11 (1984).
6. *Eur. J. Biochem*., **54**, 411 (1975).
7. Hermanson, G.T. et al., *Immobilized Affinity Ligand Techniques*, Academic Press (New York, NY: 1992) p. 258.

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