Product Information

Cyanogen bromide-activated Matrices

C9142 (Cyanogen bromide-activated-Sepharose® 4B), C9210 (Cyanogen bromide-activated Agarose)

Product Description

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. However, the added stability gained by crosslinking results in 30-50% loss of potential reaction sites, which are 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, as shown in 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.[2]

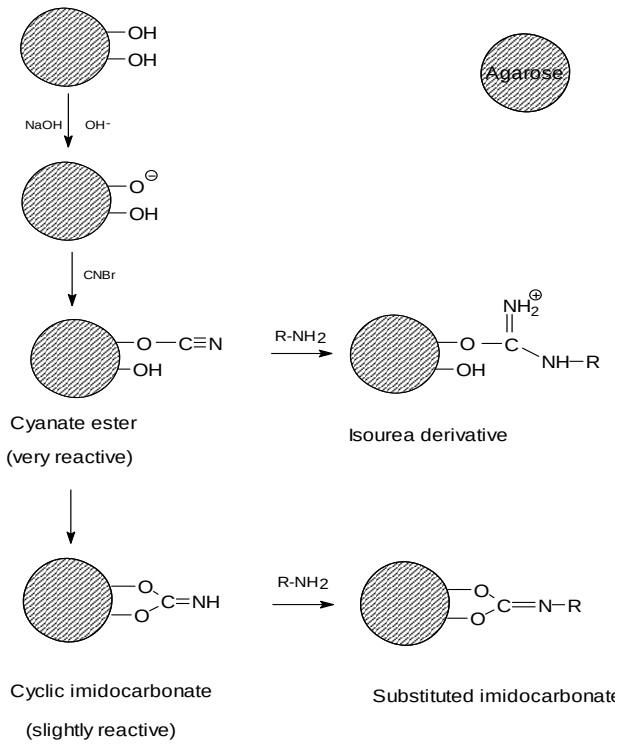
Cyanogen bromide-activated agarose matrices are offered for preparation of resins for affinity chromatography. CNBr-activation has various advantages:

- 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. However, for very small ligands, a spacer may be used to reduce steric hindrance.

These products are cyanogen bromide-activated, as CNBr itself is highly toxic and sensitive to oxidation. Most researchers prefer to use pre-activated resins rather than doing their own protocol to activate agarose with CNBr. These CNBr-activated products only need to be swollen, rinsed, and added to coupling buffer.

Figure 1.

General reaction scheme for CNBr-activation of agarose



Storage/Stability

Store these powdered products at 2-8 °C.
These CNBR-activated resins are extremely moisture-sensitive.

Cyanogen bromide-activated Sepharose® 4B

Cat. No. C9142

- 4% agarose, 40-165 µm diameter beads
- Coupling capacity is 30-40 mg of α-chymotrypsin per mL of packed gel.

Several theses[3] and dissertations[4-6] cite use of this specific CNBr-activated Sepharose® 4B product.

Cyanogen bromide-activated Agarose

Cat. No. C9210

- 4% crosslinked agarose, 40-165 µm diameter beads
- Coupling capacity is ≥10 mg of BSA per mL of packed gel.

Several publications,[7] theses,[8] and dissertations[9-12] cite use of this specific CNBr-activated agarose product.

Procedure

Although numerous references[13-15] can be found in the literature for use of activated agaroses with specific proteins (antibodies, enzymes, etc.) or with nucleic acids, this protocol is written for general purposes.[16,17]

1. Dissolve the protein to be coupled in 0.1 M $NaHCO_3$ buffer containing 0.5 M NaCl, pH 8.3–8.5, at 5–10 mg protein per mL of gel.

   Note: Other buffers can be used. However, avoid amine-containing buffers, such as Tris/Trizma®, or buffers with other nucleophiles 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. However, lactose 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 (a) with basic coupling buffer, pH ~ 8.5, then (b) 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.

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