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Product Information

CYANOGEN BROMIDE ACTIVATED MATRICES

Sigma Prod. Nos. C9210, C9142, C9267

Exact replacement for Product Code 16777

CAS NUMBER: N/A

Sigma offers three very similar cyanogen bromide-activated agarose matrices for use in making agarose beads for affinity chromatography. The general procedure used to couple ligands is the same for each, and is found on p. 3. General information and references are given on pages 1-2.

Product Number	C9210 ¹	C9142	C9267
Matrix Description	4% agarose cross linked	Sepharose ² 4% agarose	Sepharose ² 6% agarose macrobeads
Bead diameter	40-165 μm	40-165 μm	200-300 μm
Binding capacity per mL gel	≥ 35 mg BSA ³	~ 30 -40 mg chymotrypsinogen	≥ 20 mg chymotrypsinogen ⁴
Appearance	white powder	white powder	white powder

STORAGE / STABILITY AS SUPPLIED:

These products are stable for at least eighteen months at 2-8°C if kept very dry; gradually over five years, about 50% loss in binding capacity can be expected.³ The activated resins are extremely moisture-sensitive.

ABOUT THE AGAROSE RESINS:

Processed agarose has a primary structure consisting of alternating residues of D-galactose and 3! anhydrogalactose. These sugars provide an uncharged hydrophilic matrix.

Cross-linked agarose is usually preferred over the non-cross-linked variety for most affinity applications that require harsh activation or usage conditions. Unfortunately, the added stability gained by cross-linking results in 30-50% loss of potential sites (consumed in the chemistry of cross-linking).⁷ The addition of cross-links to stabilize beaded agarose does not reduce porosity significantly. Larger beads allow higher flow rate; Sepharose 6 MB beads are significantly larger (200-300 μm diameter) than most beaded agaroses (40-165 μm).

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ABOUT THE ACTIVATION / COUPLING:

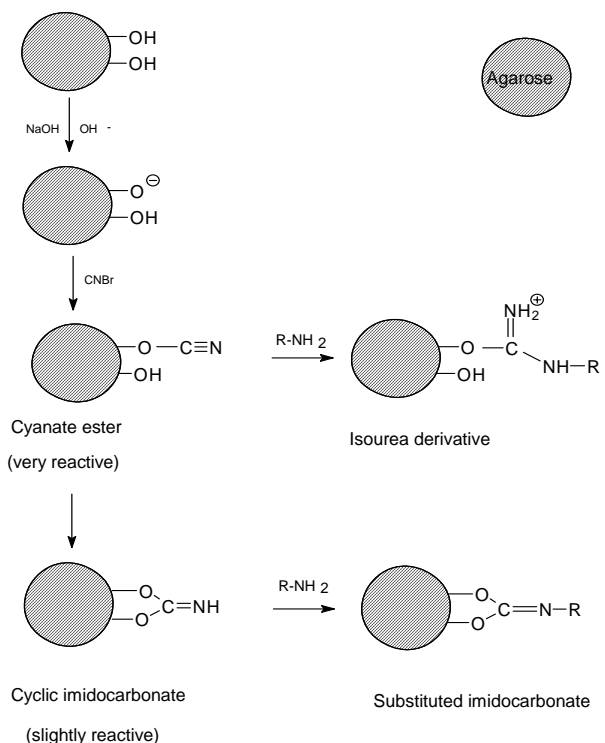
Cyanogen bromide in base reacts with -OH groups on agarose to form cyanate esters or imidocarbonates. These groups react readily with primary amines under very mild conditions; the net result is a covalent coupling of a ligand to the agarose matrix. The preferred resultant structure is an imidocarbonate,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

Why CNBr activation? The advantages:

- a) Many matrices contain -OH groups.
- b) The pH conditions needed for coupling are mild enough for many sensitive biomolecules.
- c) The procedure is relatively simple and reproducible.
- d) The coupling works for large and small ligands- although for very small ligands, a spacer may be used to reduce steric hindrance.
- e) the Sigma products are *already CNBr-activated*, so only need to be swollen, rinsed and added to coupling buffer.

The disadvantages:

- a) CNBr is highly toxic and sensitive to oxidation - most researchers prefer to use these *pre-activated* resins.
- b) The isourea bond formed between 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



REFERENCES:

1. C9210 replaces C1150; both produced by Sigma, but C9210 has higher binding capacity and better swelling stability.
2. Sepharose is a trademark of Pharmacia.
3. Sigma quality control or production department.
4. Supplier information.
5. *Affinity Chromatography - Principles and Methods* (Pharmacia Inc.), p. 15.
6. Hermanson, G.T., Mallia, A.K. and Smith, P.K., *Immobilized Affinity Ligand Techniques* (Academic Press, 1992), p. 53-56.
7. *Ibid.*, p. 6-9.

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ADDITIONAL REFERENCES:

General information on procedure and usage: *Methods in Enzymology*, 34B, 77-102 (1974); 104, 3-11 (1984).

Preparation of DNA-agarose and use in enzyme purification: *Eur. J. Biochem.*, 54, 411 (1975).

Hermanson, G.T., Mallia, A.K. and Smith, P.K., *Immobilized Affinity Ligand Techniques* (Academic Press, 1992), p. 258.

Preparing antibody resins: *Affinity Chromatography: A Practical Approach*, eds. Dean, Johnson and Middle (Oxford Press, 1985), p. 31-34, 119. Hermanson, *ibid.*, p. 223.

GENERAL PREPARATION / USE:^{3,5,6}

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.

1. Dissolve protein to be coupled in 0.1 M NaHCO₃ buffer containing 0.5 M NaCl, pH 8.3-8.5 (about 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 (contains lactose) by gentle suction in a Büchner or suction funnel between successive additions. Note: Lactose is necessary to stabilize the beads during freeze-drying, 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 NaHCO₃/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 4°C. Use a paddle stirrer or end-over-end mixer, but **not** a magnetic stir bar (which may grind beads).
5. Wash away unreacted ligand using NaHCO₃/NaCl coupling buffer described above.
6. Block unreacted groups with either 1 M ethanolamine or 0.2 M glycine, pH 8.0 for 2 hours at room temperature or 16 hours at 4°C.
7. Wash extensively to remove the blocking solution, first with basic coupling buffer at pH ≈ 8.5, then with acetate buffer (0.1 M, pH 4) containing NaCl (0.5 M).
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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