

CNBr Activation of Agarose.

(heparin agarose) (Also see; Cuatrecasas et.al. Analytical Biochemistry 60. 149-152 (1974))

- 1) Use approximately 250 ml of settled agarose beads (Biorad Biogel A-15m agarose 100-200 mesh Handle agarose gently).
- 2) Wash with several volumes of distilled deionized water. (A 600 ml sintered glass funnel is best for washes [Kimax 900ml-90c]).
- 3) To 250 ml settled beads add 750 ml of 2N Na Carbonate and mix by gentle stirring at 25° (Use 2 L plastic beaker and magnetic stirrer).
- 4) Increase the stirring rate and add 25 ml of CNBr/acetonitrile solution (50g CNBr in 25 ml acetonitrile stir with glass rod to dissolve, use CNBr in chemical hood). Stir the mixture vigorously for 2 minutes.
- 5) The suspension is immediately collected by vacuum filtration.
- 6) The resin is quickly washed with 1 L each :
 - a) 1 time with 0.1 M Na Bicarbonate (pH 9.5)
 - b) 1 time with water
 - c) 1 time with 0.2 M Na Bicarbonate (pH 8.5)
- 7) Quickly after the last wash transfer the agarose to a plastic bottle containing 3 gm Heparin dissolved in 250 ml of 0.2 M Na Bicarbonate (pH 8.5).
- 8) Shake at 4° C for 20 hours on rotary wheel.
- 9) Add Glycine to 1M (18.77 gm) and continue shaking for 4 hours at 25°.
- 10) Collect the slurry and wash with 2 L of :
 - a) 0.1 M Na Acetate 0.5M NaCl (pH4).
 - b) 2M Urea 0.5M NaCl.
 - c) 0.1 M Na Bicarbonate 0.5 M NaCl (pH10).
 - d) H₂O.

Solutions for Heparin-Agarose:

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|-----------------------------------------|-------------------------------|------------|
| 1) 2 M Na Carbonate | 212g in 1L | |
| 2) 0.1 M Na Bicarbonate (pH 9.5) | 16.8 g in 2L | pH w/ NaOH |
| 3) 0.2 M Na Bicarbonate (pH 8.5) | 33.6 g in 2L | pH w/ NaOH |
| 4) 0.1 M Na Acetate (pH4) + 0.5M NaCl | 27.2 g + 58.44 g in 2L | pH w/ HOAc |
| 5) 2 M Urea + 0.5 M NaCl | 240 g + 58.44 g in 2L | |
| 6) 0.1 M Na Bicarb (pH 10) + 0.5 M NaCl | 16.8 g + 58.44 g in 2L | pH w/ NaOH |
| 7) 100mM Tris-HCl (pH7.9) | (2 M) 12.5 ml | |
| 2 mM EDTA | (.5M) 1 ml | |
| <u>0.04% NaN₃</u> | <u>(2%) 5 ml</u> | |
| | to 250 ml w/ H ₂ O | |
| 8) 1M Glycine (pH8) | 75.07 g in 1L | |

