SEPHAROSE AFFINITY GELS

(Modified from Cuatrecases, Biochemistry 11:2291 (1972), March et al., Anal. Biochem. 60:149 (1974), Kolb et al., J. Immunol. 122:2103 (1979) and Pharmacia 'Affinity Chromatography' booklet.)

Dialyze proteins extensively vs. 0.1 M $NaHCO_3$, 0.1 M NaCl to remove all small molecules containing $-NH_2$ or $-SH$ groups. Dilute with 0.1 M $NaHCO_3$, 0.1 M NaCl to obtain the same concentration as desired in the affinity gel.

CNBr (Eastman): Lachrymator; handle in fume hood.

To a 25 g bottle (white crystals - no yellow) add 50 ml acetonitrile, to make a 62.5% w/vol solution of CNBr. This may be stored indefinitely at -20°C over desiccant. Allow to warm before opening.

5M potassium phosphate buffer: 1142 g $K_2HPO_4.3H_2O$ and 56 g KOH, make to final volume of 1 liter (pH = 11.8).

Sepharose CL-4B (Pharmacia): Weigh out the desired quantity of settled slurry, assuming density = 1.0. Wash on a filter funnel with D H_2O and reconstitute with 5M phosphate to obtain a 1:1 slurry.

Activation: (in a fume hood). Use 2-4 g CNBr/100 ml Sepharose (for 1 to 20 mg of protein/ml gel). Add the CNBr-acetonitrile dropwise while stirring the slurry magnetically. After 5 min, transfer the slurry to a Buchner funnel containing Whatman No. 1 filter paper and wash with at least 10 vol of 0.1 mM HCl at 0-4°C. Hydrate with 0.1 mM HCl to normal settled bed volume and immediately add weighed aliquots to equal volumes of proteins in 0.1 M $NaHCO_3$ + 0.1 M NaCl on ice. Stir very gently or rotate O/N at 4°C. Add glycine to 0.05 M to saturate remaining reactive groups on the gel, and allow the slurry to settle. Read A280-310 of supernatant to determine the % of coupling.

Wash out glycine and uncoupled protein and store at 4°C in buffer containing 0.05% NaN_3 plus 20 µg/ml gentamicin or 0.01% thimerosol (for antibodies or other proteins lacking free SH groups).