This method has been revised by Wes + Derek see new procedure

Sepharose 4B Conjugation

~ 5 ml of packed Sepharose 4B made up to 10 ml with dist. H_2O (15 ml small beaker) (25 mg/ml final conc.) - 0.5 gm cyanogen bromide (weigh in the hood) dissolved in 10 ml* dist H_2O (50 ml beaker) (Can be dissolved in 8 ml and the extra 2 ml used to rinse sepharose beaker).

Combine: the two ingredients in the 50 ml beaker stirring continuously over a magnetic stirrer and using a pH meter to monitor pH. Adjust pH with 2.5N NaOH to 11.0-11.5 and keep constant for 8 min. by adding NaOH dropwise. pH will stabilize

The sepharose is then transferred to a scintered glass funnel and washed with ice cold water (rinse out beaker). then with 20 vols. of 0.1M $Na_2CO_3/NaHCO_3$, pH 9.0 buffer at +4°C (~ 200 ml).

Transfer washed gel to a tube or vial, add material (We have been successful in binding up to approx 10 mg protein/1 ml bed.)to be conjugated in 0.1M $Na_2CO_3/NaHCO_3$ pH 9.0 to a total volume not greater than twice the packed-bed volume. Rotate tube at +4°C for 16-24 hrs. Wash conjugated sepharose in a scintered glass funnel with a small amount of buffer and collect, if O.D. determination have to be done. Wash well with 0.1M $Na_2CO_3/NaHCO_3$, pH 9.0 and water (20 vols). Finally wash with 1M glycine buffer pH 9.0 to block any free activated sites then wash well with PBS pH 7.0.

Before use the column should be treated with 10 vol. 0.2 M acetic acid in saline (9 gm/lt) then neutralized by washing with PBS (~ 20 vols) to remove any loosely bound protein.

A non-cross reacting protein e.g. BSA should then be passed through the column to block any non-specific sites (~ 2 ml 3% BSA in 0.05M Tris-HCL buffer pH 7.6 was used or normal serum is just as good).

The column is then washed with PBS until free of OD_{280} absorbing material.

The column is now ready for use.

When the column is not in use it should be stored at +4°C in PBS with 0.1% sodium azide.