Recommended carbodiimide coupling procedure for CH Sepharose® 4B and AH Sepharose® 4B

The following method may be used as a guide to the best coupling procedure.

1. Weigh out the required amount of freeze dried powder and suspend it in 0.5 M NaCl. (1 g gives about 4 ml swollen gel).
2. To remove additives wash with 0.5 M NaCl for 15 minutes, (200 ml/g freeze dried powder) on a sintered glass filter.
3. Wash the gel with distilled water (adjusted to pH 4.5) to remove NaCl.
4. Dissolve the ligand to be coupled in water (adjusted to pH 4.5). Purified dioxane or ethyleneglycol up to a final concentraion of 50 % may be used if the ligand does not dissolve in water.
5. Add the ligand solution to the gel and adjust the pH to between 4.5 and 6.0. A final gel concentration liquid: gel, 2:1 makes an acceptable slurry for stirring.
6. Stir the mixture gently at room temperature. Use slow-moving propeller stirring. Do not use a magnetic stirrer.

52-1167-00

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7. Add solid carbodiimide powder, not lumps, to a final concentration of 0.1 M. Alternatively add an aqueous solution of carbodiimide, dropwise.

   Recommended carbodiimides: N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) or, N-cyclohexyl-N'-2(4'-methyl-morpholinium) ethyl carbodiimide-p-toluene sulphonate (CMC).

8. Maintain at pH 4.5–6.0 for 1 h (by addition of dilute NaOH). After 1 h changes observed in pH are small.

9. Allow the reaction to proceed for 24 h at room temperature or at 4°C (dependent on coupled ligand).

10. Wash the gel thoroughly. Buffered solutions of alkaline and acid pH with a high concentration (1 M) of added salt should be used alternately.

    N.B. If organic solvent was used to dissolve the ligand it is necessary to wash the gel with an organic solvent to remove unreacted ligand. Dioxane, ethyleneglycol, ethanol, methanol and acetone may be used.

11. Was with distilled water.

12. If the gel is to be used immediately, equilibrate with the buffer of choice. Otherwise it is recommended to store in buffer of neutral or slightly acidic pH with a high concentration of salt (1 M) at 4–8°C in the presence of a suitable bacteriostatic agent.