Coupling Peptides To Resin For Affinity Purification

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We use Affigel-10, converting its functional group first to amino and then to iodoacetyl. You can also buy amino resin (it's more expensive) and start at step 6. All washes are performed on a glass-fritted filter funnel sucking until a wet cake is formed. Do not dry the resin completely or you will introduce lots of air bubbles. All reactions up to peptide addition are performed in the funnel by covering the spout with parafilm. This minimizes loss. Assume the affigel is a 50% slurry.

1. Wash with 5 volumes of 100% cold EtOH.
2. Wash with 5 volumes of 50% cold EtOH.
3. Wash with 5 volumes of cold water.
4. Add 5% ethylene diamine in water. Incubate 15' at RT.
5. Wash with 10 volumes water. At this point you have amino-affigel.
6. Wash with 3 volumes of 0.1 M NaPi pH 7.8.
7. Resuspend resin in 0.1 M NaPi pH 7.8. Add iodoacetic acid-NHS ester, and incubate for 10 min at RT. The resin has about 10 5mol groups/ml, so add about 20 5mol reagent per ml (7 mg/ml resin). Dissolve the IAA-NHS ester in dry DMSO and add it while stirring the resin. This step and subsequent steps up to the blocking of residual iodoacetate groups should be done in dim light since the iodo group is light sensitive.
8. Wash with 10 volumes of 0.1 M NaPi pH 7.8= buffer.
9. Resuspend the resin as a 50% slurry in buffer. Add the peptide. If the peptide is soluble directly in buffer, add it as a solid. Many peptides go in better added as a 100 mg/ml stock in DMSO. Generally, if your peptide was readily soluble when you checked for thiol groups, it will be soluble in the 50% slurry because the concentration is the same. Some hydrophobic peptides will crash out. It is possible to couple such peptides in 20% buffer, 80% DMSO. In an extreme case you can use 100% DMSO containing triethylamine. The amount of peptide to add is a question. We usually add 1-2 mg peptide/ml resin, hoping to make a resin that will bind 10-100 mgs/ml of specific antibody.
10. Mix gently on a rotating wheel ON at 4 !C. Add

betamercaptoethanol to 0.2% to block residual iodoacetate groups. Incubate 1 hr at RT.

11. Wash resin sequentially with 5 volumes 0.1 M NaHCO3, 5 volumes of 1 M Na2CO3, 5 volumes of water, 5 volumes of 0.2 M glycine, pH 2.0; 150 mM NaCl, 5 volumes of TBS and 5 volumes of 6 M Guan-HCl in TBS. Reequillibrate into TBS + 0.1% NaN3.

Before adding valuable peptide, we recommend checking the resin chemistry using a quick eyeball test. The amino resin will react with an NHS ester, whereas the original affigel and the iodoacetate will not. Take an aliquot (50ul) of resin at each step. Resuspend in 100 ul of buffer. Add 1 ul of 0.1 M NHS fluoroscein or NHS-rhodamine in DMSO. Incubate 5' RT. Wash the resin twice in buffer by centrifugation. The original resin, and the resin after step 8 should be only lightly labeled, wherease the resin after step five should be heavily labeled.