

Crosslinking Peptide to Affigel

1. Dissolve 10 mg of desalted (or 20 mg non-desalted) peptide in 1 ml DMSO (anhydrous).
2.
 - a). Put 1.7 ml of Affigel bead matrix into a disposable 10 ml column.
 - b) Drain the supernant.
 - c) Wash with 5 ml isopropanol and then 2 ml DMSO. Bead volume should shrink to 1 ml.
 - d) Using extra DMSO, transfer beads to a 15 ml "green cap" DNA tube (polypropylene) cut off halfway. Remove excess DMSO after beads settle.
3. Add peptide/DMSO solution to the beads and rock 2-4 hrs at RT.
4. Let the gel settle and remove excess peptide and DMSO and save. Add 1 ml of 100 mM Ethanolamine (Fisher), pH 8.0, and rock slowly @ RT for 2 hours to block all remaining active crosslinks.
5. Either put back in a disposable column and wash as specified below or transfer to two 2 ml microcentrifuge tubes and wash.
 - a). For column, wash with 20 ml PBS or TBS, pH 7-8. Then wash with 2 ml PBS or TBS with 1 mM PMSF, 0.03 TIU aprotinin and 0.02% azide.
 - b). For microcentrifuge tube, wash 5 times with 1 ml PBS or TBS, pH 7-8. Use PBS/TBS with PMSF, aprotinin and azide from the last wash.
 - c). Store at 4°C.

Affinity Purifying Antibody Using An Affigel/ Peptide Column

1. This procedure can be performed using Protein-A purified IgG or crude serum. The former probably gives cleaner Ab but the latter works just fine and is what we usually do.
2. Use Affigel-linked peptide column in a 2-10 ml disposable column (depending on matrix volume; we usually use 0.5 ml-1.5 ml matrix with 1 mg peptide coupled per ml).
3. Pre-spin serum or purified IgG in 50 ml Nalgene Hi-speed polycarbonate tubes (Fisher # 05-528) at 12,000 RPM in a Beckman JA-20 or Sorvell SS-34 rotor in a RC-5 or J2-21M centrifuge to remove insoluble protein aggregates.
4. Save 10 ul to compare by western blotting with the purified Ab and post adsorption flow through.
5. Apply serum or purified Ig G to the column and let flow through slowly. Save flow through and reapply a second time and let flow through slowly again. Save flow through until you know all the Ab was absorbed by the column (test at the end).
6. Wash with 10 ml per ml of column, of 100 mM Tris-HCl, pH 8.0, 10 ml per ml of column, of 500 mM NaCl, 10 mM Tris-HCl, pH 8.0 and then, 10 ml per ml of column, of 10 mM Tris-HCl, pH 8.0.

7. Before elution, put 150 μ l 1M Tris, pH 7.4, in each well. Elute with 5 ml 100 mM Triethylamine (TEA), pH 11.4 (TEA and ddH₂O, no pHing is necessary), and collect 0.5 ml per tube. If TEA doesn't work well, use 0.1M glycine, pH 3.0.
8. Measure OD of the elution @ 280 nm and test the Ab specificity and yield by Western Blot. (Test crude Ab, final flow through, and purified Ab on strip blots).
9. Regenerate the column for reuse by washing with 10 ml 0.1M glycine, pH 3.0, and then 10 ml 0.1M PBS or TBS containing 1 mM PMSF, 0.03 TIU aprotonin, and 0.027 sodium azide. Store at 4°C in the latter solution.