AFFINITY PURIFICATION OF ANTIBODY

I. PREP COLUMNS

1. Bring Sulfolink gel to RT
2ml = 1ml of gel volume because it is in a 50% slurry

1a. Pack into small Bio-Rad disposable column

2. Equilibrate column with 15ml of coupling buffer
Coupling buffer = 50mM Tris, 5mM EDTA pH 8.5
3. Dissolve 1mg of peptide in 1ml of coupling buffer
4. Add dissolved peptide to column and save 20ul (do not put this into column)
4a. Cover top and bottom of column
5. Mix at RT for 15 minutes
6. Add 2ml of coupling buffer and incubate upright for 30min at RT
7. After 30 minutes collect all the sample = "load"
8. Wash the column with 6ml of coupling buffer
8a. Can do all of the above in batch instead of one column
9. Determine coupling efficiency eg below

	Ab280	dilution	volume	Ab. Units
Start	0.122	1:10	1ml	1.22
Load	0.122	1X	2.9ml	0.354
Wash	0.028	1X	6ml	0.168

You have lost 0.698 Ab Units in the above example

10. Wash column again with 10 ml of coupling buffer
11. Add 3ml of coupling buffer with 0.05M cysteine
12. Incubate at RT for 30min
13. Wash with 20ml of 1M NaCl
14. Wash with 20ml coupling buffer + 0.05% NaN3 degased
15. Column can be stored overnight at 4 degrees with a constant flow of degased coupling buffer + 0.05% NaN3

II. Preparing Samples for Purification

1. Spin at 5000 rpm for 30min
2. Decant Supt. and store pellet at –20
3. Add 18% Na_2SO_4 and continuously stir at 37 degrees for 30 min
4. Spin 8000rmp 15 min at RT
5. Decant Supt. and store at –20
6. Wash pellet with 18% Na_2SO_4 in water
7. Spin 8000rpm 15min at RT
8. Decant supt and store at –20
9. Redissolve pellet in 1/3 volume of water
10. Dialyse at 4 degrees with 2 changes of PBS or TBS
11. Spin dialysed serum at 5000rpm 4 degrees
12. Store any pellet at –20
13. Load the supt on column (may want to only load 1/2)
14. Mix column at RT for 1 hour
15. Keep column upright at RT for 30min
16. Collect the load
17. Wash column with 20ml of coupling buffer and collect the wash
18. Elute in cold room with 10ml of 0.1M glycine pH 2.7 ::
    collect 10 fractions 1ml each
19. Neutralize fractions with 50ul of 1M Tris pH 9.5
20. Measure absorbance at 280
21. Wash the column overnight at 4 degree with coupling buffer + 0.05% NaN_3
22. Elute again if you want