

## **IgG Purification on a Protein G or Protein A Sephacrose Column**

1. Swell Protein G (or A) Sepharose CL-4B (Pharmacia) in H<sub>2</sub>O overnight (or at least 30 min.) @ RT. One gm powder gives 4-5 ml final volume of gel.
2. Pour the column.
3. Wash column with 10 column volumes of 100 mM Tris HCl buffer, pH8.0 (0.135M NaCl, 100 mM Tris HCl).
4. Spin serum at 4°C at 10,000-13,000 rpm for 15 minutes prior to loading on the column. Save 10µl for test at end.
5. Run the serum through the column 2 times. Collect and save the serum after it is run through the column.
6. Wash the column with 10 column volumes of 100mM Tris HCl buffer, pH8.0, and then 10 column volumes of 10mM Tris HCl, pH 8.0.
7. Elute the antibody off the protein G (or A) beads with 100mM glycine, pH3.0. Collect the eluted protein into eppendorfs containing a 15% final fraction volume of 2.0M Tris-HCl, pH8.0. Collect ~2 column volumes. Run an additional 5 column volumes of 100mM glycine, pH3.0, through the column after finished collecting to clean column.
8. Wash the column with 10 column volumes of 100mM Tris HCl buffer, pH8.0 with azide (0.02%). Store at 4°C.
9. Measure the fractions individually on spectrophotometer at OD 280nm using the quartz cuvette (clean well before, but not between fractions).
10. Combine peak fractions.
11. Column can be reused.
12. Test initial serum (prior to purification), eluates, and flow through by western blotting (use 1: 5000 dilutions) to see if column absorbed all the specific reactivity and if that reactivity was recovered in the eluates.