STAPH A PURIFICATION OF ANTIBODIES

1. Resuspend 1.0 g protein A/Sepharose CL-4B in 6 ml **PBS**. Leave at 4°C
   for about 2 h to swell. (Binding capacity is approximately 140mg IgG per gram of resin. Serum contains on average, 8-16 mg IgG per ml).

2. Wash 3 ml of slurry on sintered glass filter with 10 ml **PBS**.

3. Dilute 5 ml serum 10 x in **PBS** and take OD_{278} (OD_{278} of 1 = 0.8mgml^{-1}).

4. Add resin to serum and incubate shaking overnight at 4°C.

5. Transfer resin/serum to a column and collect flow through.

6. Wash column with 20 ml **PBS**.

7. Elute antibody with 1 ml aliquots of 0.1 M glycine-HCl pH 2.5.

8. Collect 10 fractions into tubes containing 200 µl 2 M Tris pH7.6.

9. Measure OD_{278} of flow through, wash and fractions.

10. Pool protein containing fractions and concentrate if necessary. Add sodium azide to 0.02%. Store at –70°C for long term, –20°C for short term.

11. Wash column with 10 ml PBS/0.02% azide and store at 4°C. If want to use resin for a different antibody, recycle by washing on a sintered glass filter with 200 ml 1M acetic acid and then 500 ml **PBS**.

10 x PBS (1L)

100 mM sodium phosphate pH 7.0	100 ml 1 M
1.5 M NaCl	300 ml 5 M