AFFINITY PURIFICATION OF ANTIBODIES ON CNBr-SEPHAROSE/ANTIGEN COLUMNS

1. Transfer resin to sintered glass filter and wash with 10 bed volumes of 10 mM Tris pH 7.5.

2. Wash with 10 bed volumes of 100 mM glycine pH 2.5. (This step is not necessary if the column has been used before - you can proceed directly from step 1 to step 4).

3. Wash with 10 mM Tris pH 7.5 until the pH reaches 7.5.

4. Spin crude serum to pellet debris and dilute supernate 10 x in 10 mM Tris pH7.5. Check OD_{278} and then add resin to diluted serum (OD_{278} of 1 = 0.8 $mgml^{-1}$). Shake at 4°C o/n.

5. Load serum/resin into a column and collect flow through.

6. Wash column with 20 bed volumes 500 mM NaCl, 10 mM Tris pH7.5.

7. Elute antibodies with 1ml aliquots of 100 mM glycine pH 2.5. Collect the fractions in tubes containing 50µl 2M Tris pH 8.0. Check OD_{278} of fractions and stop collecting once OD_{278} = 0.

8. Wash the column with 10 mM Tris pH7.5 until the pH is 7.5 and then store in 10mM Tris pH7.5 plus 0.02% sodium azide.

9. Combine the antibody containing fractions. Concentrate if necessary in centricons. Check OD_{278} of

final sample and if protein concentration is below 1mgml^{-1}, add nuclease-free BSA to bring it up to 1mgml^{-1} (this concentration prevents protein degradation). Add 10 x PBS and 0.2% sodium azide to bring final concentration to 1 x PBS, 0.02% sodium azide.