DEAE Chromatography of Antibodies

- Plan to start with 5 mls of acites fluid, or an ammonium sulfate cut from 5-10 mls of ascites fluid. If starting with ascites fluid, it must be extensively dialyzed against 10 mM Tris, pH 8.5. Use SpectraPor 2 (12-14 kDal cut off) tubing. Use twice as much dialysis tubing as needed for the dialyzed volume. This will allow enough space for expansion during dialysis. Use tubing clips at the ends. Dialyze overnight at 4°C in at least 100 volumes of buffer. Change buffer three times.

1. Pour a 15 ml column of DEAE matrix (DEAE Sepharose or DE52). Use 10 mM Tris, pH 8.5. Do this in the cold room. Set up as in Figure below.
   - Alternatively, regenerate a previously used DEAE column. See Harlow and Lane for procedure.
   - Tris-HCl at pH 8.5 at 25°C will have a pH of ~9 at 4°C.
   - Use a flow rate = ~1 ml/minute for the steps below.

2. Wash column with 10 volumes of 10 mM Tris, pH 8.5.
3. Bind antibody to column. Save load eluate.
4. Wash with 10 volumes of 25 mM NaCl in 10 mM Tris, pH 8.5.
5. Elute the antibody with a linear gradient in 10 mM Tris, pH 8.5. Usually 25 mM to 250 mM NaCl works well. Total volume of gradient is 150 mls (75 mls of 25 mM + 75 mls of 250 mM).
6. Collect 20-30 X 5-7.5 ml fractions.
   - TCA precipitate 10 µl of each fraction and analyze by SDS-PAGE. Look for heavy chain (55 kDal) and light chain (25 kDal) bands.
7. Pool antibody containing fractions and store at 4°C. Determine protein concentration by $A_{280}$ measurement.
   - For IgG, an $A_{280}$ of 1.35 = 1 mg/ml.
   - For long term storage add sodium azide. Do not add azide if CNBr coupling will be done.