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PURIFICATION OF RAT MONOCLONAL ANTIBODIES BY $(NH_4)_2SO_4$ PRECIPITATION, DE-52 CHROMATOGRAPHY, AND SEPHADEX G-200 OR BIOGEL A-5 m FILTRATION

Quantitation: At all stages of purification, fraction volume, A_{280} and A_{310} absorbance, and rat Ig concentration by single radial immunodiffusion should be quantitated. Obtain this data for starting material, 2.2M $(NH_4)_2SO_4$ precipitate (before and after dialysis for volume and rat Ig, after only for A_{280} and A_{310}), selected DE-52 fractions, selected G-200 fractions, and final concentration pool. Each monoclonal antibody is different, and thus may behave differently at any step. The yield and purification factor at each step should therefore be calculated.

$(NH_4)_2SO_4$ Precipittion: To spent tissue culture medium containing 5% fetal calf serum and monoclonal antibody, add while stirring in the cold over a period of about 15 min 33.7 g of finely divided $(NH_4)_2SO_4$/100 ml to bring to 2.2M. Avoid foaming. After 30 min centrifuge at 10,000 RPM in Sorvall GSA rotor for 10 min. Save both supernatant and pellet. The latter should contain antibody. Suspend the pellet (it does not have to dissolve) in 1 to 2 ml/100 ml of starting material of 0.1 M tris HCl buffer pH 7.8 at 20°C (made by adding HCl to tris base - no NaOH). Measure volume, reserve 50 µl for Mancini assay, and dialyze vs. 3 changes of 0.1 M tris-HCl pH 7.8. The pH and concentration of this buffer have been designed to prevent precipitation of euglobulins (IgM and IgG2c in the rat), but absence of precipitation has not yet been much tested in practice.

DEAE Chromatography: Taking $A_{280}-A_{310}/1.5$ of the dialyzed $(NH_4)_2SO_4$ precipitate as mg/ml, calculate total mg of protein and use 1 ml DE-52 (Whatman, Reeve-Angell) packed volume per 8 mg protein. Weigh out approx. 0.6 g of pre-swollen DE-52/1 ml of desired bed volume. Make about a 2:1 slurry with 0.1 M tris-HCl buffer pH 7.8 and adjust pH (DE-52 will change it) to 7.8. Prepare a chromotography column with glass wool at bottom and add about a 1:1 slurry (some supernatant having been decanted) and allow coarse particles to settle to bottom for 10 min. before opening the stopcock and commencing packing. Pack to predetermined volume (level) with DE-52. Equilibrate with 2-3 volumes of 0.1 M tris-HCl buffer and check pH and conductivity of effluent vs. buffer. If they are within 0.1 unit and 5%, respectively, the column is ready for use; otherwise, continue equilibration. During equilibration and application of the sample under gravity, use a safety loop of the line from reservoir to column which dips below the column outlet to prevent it from running dry; if this happened, the column would have to be repacked. Apply the sample to the column. Typically, 100 drop fractions are collected. Wash the sample off sides of column, etc. and into bed with buffer (typically 5-10 ml). More extensive washing is omitted to avoid dilution of the rat IgG, which usually elutes in these fractions. Commence elution with a linear gradient made from 3-4 column volumes each of 0.1 M tris-HCl pH 7.8 and the same buffer containing 0.05 M NaCl. The Pharmacia P-3 pump may be used to form the gradient; two columns may be eluted simultaneously. Pump at a rate comparable to that obtained by gravity flow. Assay fractions for IgG content by Mancini. Pool and

concentrate appropriate fractions to 10-20 ml for gel filtration. The main goal is to separate the Ig from later eluting fractions; lower specific activity early fractions may be retained since the contaminating material in these fractions is well separated from IgG in gel filtration.

Gel Filtration: These columns require special care in packing but this will not be described as they are generally ready for use. Deaerate fresh column buffer, 0.01 M tris-HCl, pH 7.8 to 8.0, 0.14 M NaCl, 0.05% NaN_3, before each run. The sample in 10 to 20 ml is made 2.5% in sucrose (use a sterile stock 50% solution). Most of the reservoir buffer is removed from over the gel bed, and the sample is layered under the remaining buffer with a bent pasteur pipette. Filtration is carried out at standard pump flow rate (2-3 days/bed volume). Use Sephadex G-200 fine for IgG and Biogel A-5m 200-400 mesh for IgM. (Or more recent S series or Sephacryl gels).