

DEAE SEPHAROSE FAST FLOW COLUMN

1. If the volume of the crude extract is larger than 5 mls use the 200 ml DEAE column, otherwise use the 30 ml DEAE column. In either case the sample is passed over the column at 4° C using the following FPLC protocol:
  - I. the sample is loaded onto the column at a flow rate of 1 ml/min
  - II. the column is flushed with 20 mM Tris pH 6.9 (at r.t.), 1 mM EDTA and 0 mM NaCl (TEN<sub>0</sub>) until the chart recorder reaches base line--the flow thru is collected in a separate container
  - III. a salt gradient from TEN<sub>0</sub> to TEN<sub>0.35</sub> (350 mM NaCl) is passed over the column in a total of 10 column volumes and at 10 ml/min
    - a. fractions are collected during the gradient period (25 mls for the 200 ml DEAE column)
  - V. a salt jump to TEN<sub>1.0</sub> is then done for 2 column volumes at 10 ml/min
    - a. fractions collection continues through half the high salt wash
  - VI. the column is equilibrated with TEN<sub>0</sub> for 2 column washes at 10 ml/min
  
2. Aliquots (12 µl) are taken of fractions 1, 5, 10, 12, 15, 17, 20, 22, 25, 27, 30, 35, 40 and 45
  - A. The aliquots are resolved on a 10% SDS-PAGE gel to determine the location of the hsp70 protein peak
  - B. if the strain utilized contains dnaK, take caution not to pool fractions containing dnaK (elutes from the column around 220 mM NaCl)