

Figure 11. To test whether the % Triton X-100 affect the smearing of protein. Five micrograms of BSA were suspended in buffer A containing 0.3M NaCl and 0, 0.1, 1 and 10% Triton X-100. The samples were mixed with 5 microliters of 10 x SDS loading dye and loaded onto a 5% SDS gel from lane 2-5, respectively. Lane 1 contained standard protein marker. After electrophoresis, the gel was stained with Coomassie blue. The result shows that high %Triton X-100 (10%) could cause the protein smearing.

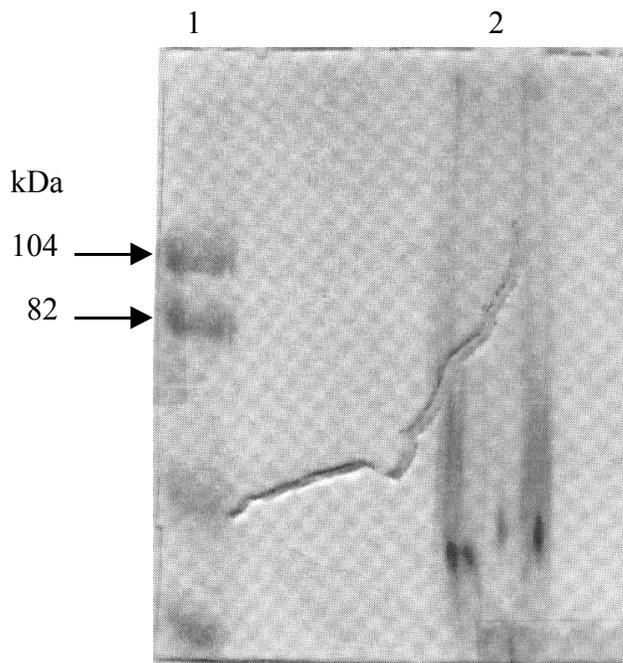


Figure 12. Test to determine if buffer A containing 0.3M NaCl and 0.1% Triton X-100 caused smearing. The first lane contained standard marker protein. Lane 2 contained 5 micrograms of BSA in 6 ml buffer A with 0.3M NaCl and 0.1% Triton X-100. The sample was concentrated by a YM100 spin column then washed with milli Q H₂O twice. After electrophoresis, the gel was stained with Coomassie blue. The result shows that the buffer A containing Triton X-100 caused the smear.

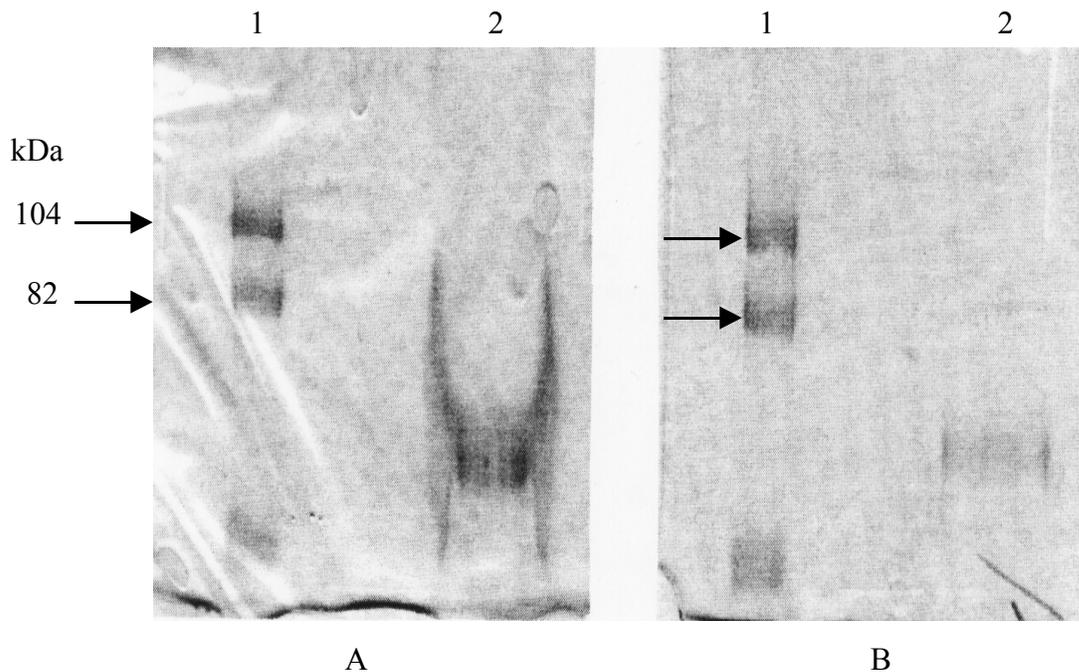


Figure 13. The effect of treating Triton X-100 containing samples with Bio-Beads. Two gels of 5% SDS were prepared. First lanes of both contained standard marker protein. In figure 13A, lane 2 contained 5 micrograms of BSA in 6 ml buffer A with 0.3M NaCl and 0.1% Triton X-100. The sample was shaken at RT for 2 h and concentrated by a YM10 column. In figure 13B, lane 2 contained 5 micrograms of BSA and 0.1 g Bio-Beads. The sample was treated the same as the sample from figure 13A. Before loading, the samples were concentrated by speed vacuum to decrease the volume 20 microliters then mixed with 5 microliters of 10 xSDS loading dye containing 8M urea, 2-ME and a few crystals of sucrose. After electrophoresis, the gels were Coomassie blue stained. The result shows that Bio-Beads could remove the smear caused by Triton X-100.

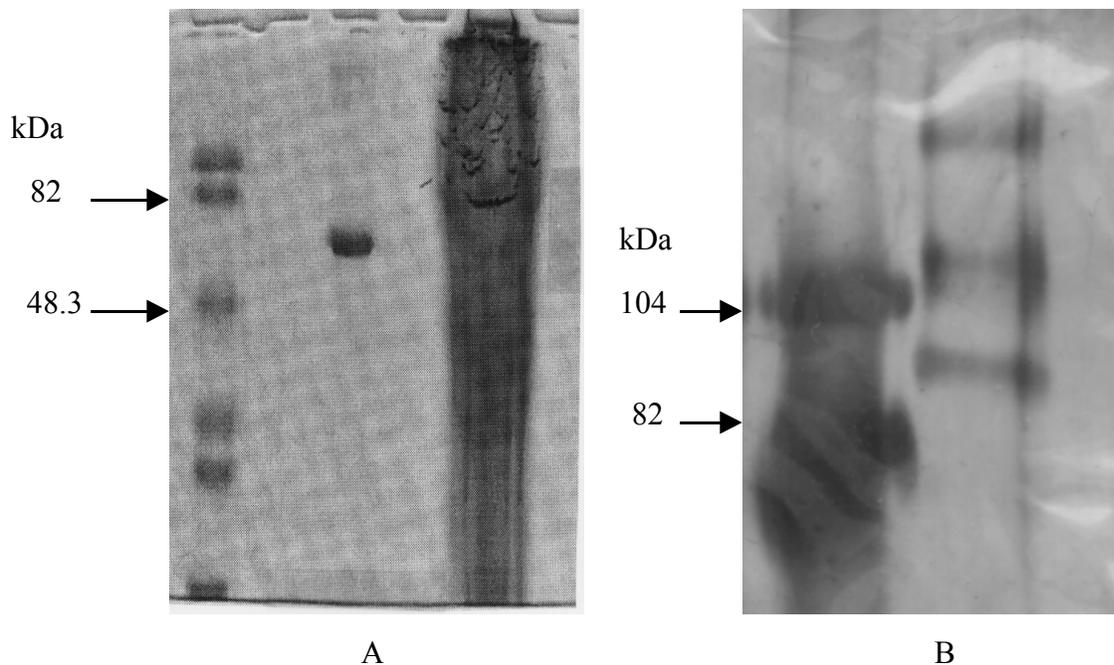


Figure 14. The effect of Bio-Beads treatment on SDS PAGE of concentrated 300SW fractions. Three purification of 5NU were pooled together and concentrated to decrease the volume. The concentrated sample was loaded on 5% SDS gel (lane 3, A); lane 2 (A) contained 5 micrograms of BSA. In B, the pooled purified protein was treated with Bio-Beads, concentrated and loaded onto 5% SDS gel. The bands of proteins could be seen. Lane 1 contained standard protein marker.

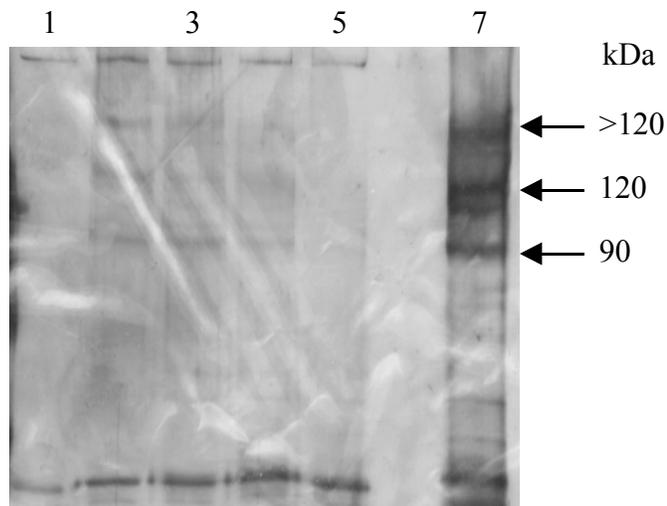


Figure 15. Silver stain of active fractions from 300SW column on 5% SDS gel. Lane 1-5 contained 20 microliters of active fraction # 14-18, respectively. Lane 7 contained pooled active fractions from 300SW column, treated with Bio-Beads and concentrated by a YM 100 spin column. Three bands of 90, 120 and >120 kDa were seen in lane 2, 3 and 7.