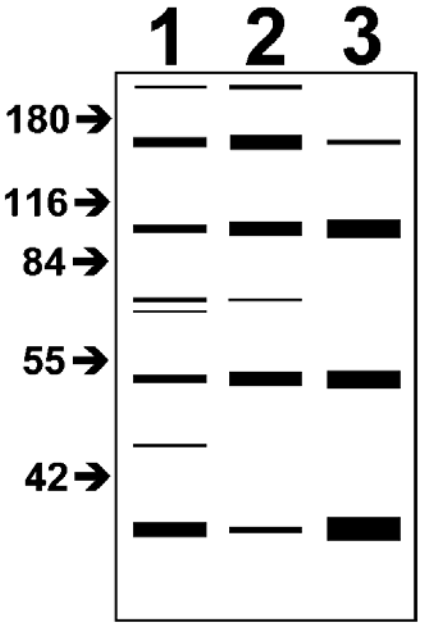
**HOMEWORK #6**

You have partially purified an enzyme using non-denaturing conditions. The preparation still has several bands when analyzed by SDS gel electrophoresis (Figure, lane 1). You want to identify the band(s) that are possibly the protein of interest and to completely purify the protein.

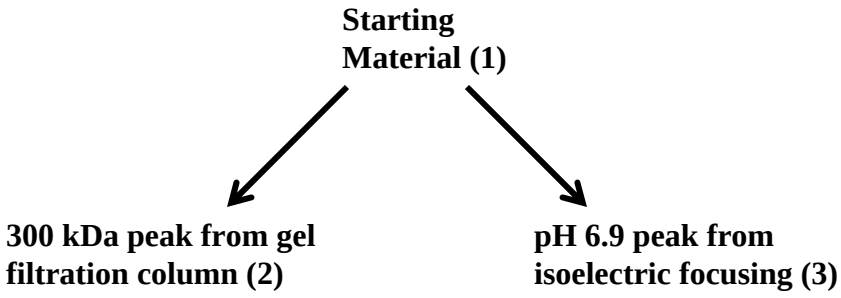
You further analyze one portion of this starting material by gel filtration chromatography and the enzyme activity eludes as a single peak of activity with a molecular mass of approximately 300 kDa. You pool these fractions and determine the specific activity and analyze the protein sample by SDS gel electrophoresis (Table and Figure, lane 2).

You analyze another portion of this starting material by preparative isoelectric focusing and the majority of the enzyme activity is found in a single fraction with a pH 6.9. You determine the specific activity of this fraction and analyze the protein sample by SDS gel electrophoresis (Table and Figure, lane 3).

| Sample | Specific Activity | Lane |
|---|---|---|
| Starting Material | 812 units/mg | 1 |
| Gel Filtration | 1595 units/mg | 2 |
| Isoelectric Focusing | 1933 units/mg | 3 |




Legends. The figure on the right represents a Coomassie-blue stained gel with 3 lanes. Each lane has 10 μg of protein. Lane 1 = original starting material; Lane 2 = after gel filtration; Lane 3 = after isoelectric focusing. The figure below is a schematic showing how the samples were obtained.



1. Identify the protein band(s) on the gel which are most likely to be responsible for the enzyme activity? (You can give approximate sizes or indicate directly on gel with arrows, asterisks, circles, etc.) Explain your answer.

2. What predictions, if any, can you make about subunit composition of the enzyme? Explain your answer.

3. How close to being completely purified will the enzyme be if the gel filtration and isoelectric focusing are carried out as sequential steps? In other words, how many contaminating bands and how much would you still expect in a preparation in which the two procedures were carried out sequentially (i.e., either subject the 300 kDa gel filtration peak to isoelectric focusing or subject the pH 6.9 isoelectric focusing fraction to gel filtration)? You can indicate on the figure the contaminating bands or draw a figure of the expected gel.

4. Assuming that the fold-purification and yields are similar if the steps are done sequentially as compared to being done separately, what would be the expected specific activity following combined gel filtration and isoelectric focusing?