

**EXAM 5a: Form A**  
**153L Fall 2005**

Name: \_\_\_\_\_ Answer Key \_\_\_\_\_  
TA: \_\_\_\_\_ Section: \_\_\_\_\_

For TA use:

Q.1. \_\_\_\_\_ + Q.2. \_\_\_\_\_ = Total Score: \_\_\_\_\_

Write your name on each page.

**Question 1: (20 pts.) Limit your answer to 1-2 sentences.**

You set out to run a gel exclusion column on your unknown globular protein sample with a set of 5 standards.

a) How will you monitor the elution of your proteins? Describe the method and how it detects the presence of proteins.

**Using absorbance spectroscopy (2 pts.) to detect the absorbance of tyrosine and tryptophan at 280 nm (3 pts.).**

b) You will need two column runs to determine your molecular weight of your unknown. What is the nature of the two runs and why are they necessary?

**One run consists of standards alone on the column, and the other consists of standards plus unknown (2 pts.). Necessary to adequately identify the peak elution times of the standards and peak elution times of the unknown (3 pts.).**

c) If your elution profile gave three distinct peaks at the earlier time points, but one very broad and tall peak at the later time points, then what adjustment do you need to make to the beads? Explain why you will make this adjustment.

**Increase the concentration of sugar (or polysaccharides) in the beads (2 pts.) in order to decrease the pore sizes so that you can allow for greater collisions to the beads. This will result in resolution of the three unresolved proteins (3 pts.).**

d) Suppose SDS, urea, and beta mercaptoethanol were added to the sample (unknown and standards), the sample was heated at 100°C for three minutes, and then loaded into the column that also has SDS in the column buffer. How will the proteins be separated, and what impact would it have on the molecular weight determination of the unknown?

**The proteins will not longer separated by their native molecular weight but rather their subunit molecular weight. (2 pts.) If the subunit molecular weights are known for the standards, the pore size in the beads allow for proper resolution, and you run the standards alone under the same denaturing conditions, then there may be no impact on determining the subunit molecular weight of the unknown protein. (3 pts.)**

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**Question 2: (20 pts.) Limit your answer to 2-3 sentences.**

Provide why the following are important components for the following principles of the SDS-PAGE procedure:

a) Binding SDS to the protein for reducing non-molecular weight variables.

**SDS bound to proteins results in a net negative charge for all proteins, and because SDS binds to proteins in a constant mass ratio of SDS to amino acids, this results in approximately identical charge to mass ratios for all proteins (3 pts.).**

**SDS also provides uniform shape as it denatures (unfolds) proteins to the same linear/cylindrical shape (2 pts.).**

b) A pH 6.8 solution at the Cl<sup>-</sup> and glycine interface for resolution of protein bands.

**The pH 6.8 is required to keep glycine protonated at the amine group so that the charge is predominantly neutral. Approximately 1:1000 glycines will be negatively charged which makes glycines average charge to mass ratio less than the SDS-protein complexes (3 points). As a result SDS-protein complexes will move faster than the glycine layer which will enable the stacking of protein in the ion deficient zone (2 pts.).**

c) Stacking gel for resolution of protein bands.

**A stacking gel which has large pore sizes is required to ensure that proteins have time to stack into the ion deficient zone before reaching the resolving gel. In order for this to be possible, all proteins must negotiate through the gel with the same velocity regardless of size. (5 pts.)**

d) Using the correct % acrylamide for resolution and determining the molecular weight.

**A correct % acrylamide is required for the gel in order to obtain proper stacking and ensuring that varying size proteins run at varying velocities (3 pts.). The varying velocities will allow one to plot the log Mr vs. the distance of migration for the standards in an approximately linear fashion which the unknown can then be interpolated into the standard curve for the determination of the Mr of LDH. (2 pts.)**