

**EXAM 1: Form B**  
**153L Fall 2005**

Name: ANSWERKEY  
 TA: \_\_\_\_\_ Section: \_\_\_\_\_

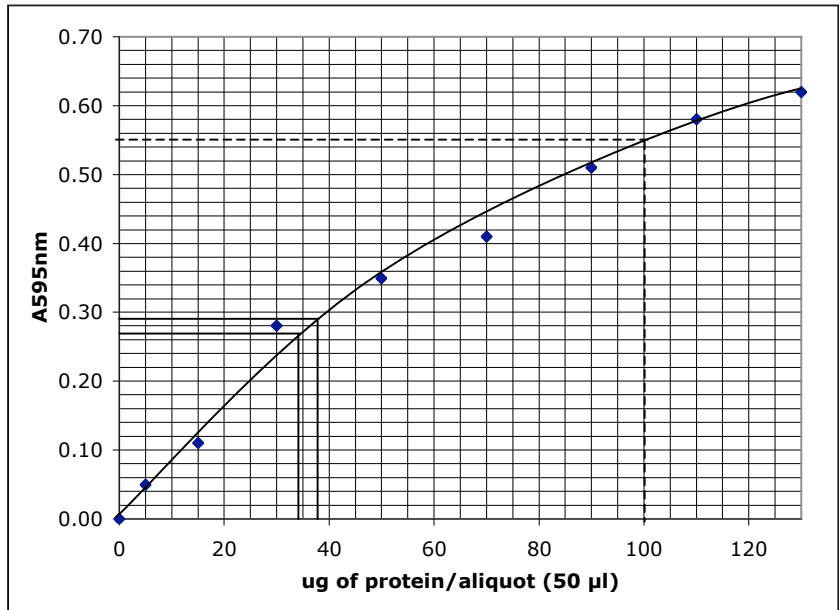
For TA use:

Q.1. \_\_\_\_\_ + Q.2. \_\_\_\_\_ + Q.3. \_\_\_\_\_ = Total Score: \_\_\_\_\_

**Question 1 (30 pts.)**

You have done a dye-binding assay and have obtained the data of your standard curve as shown in the graph. Duplicate assays of the third dilution step in a serial dilution of your unknown gave an absorbance of 0.27 and 0.29. Note that the first and second dilution steps were both 5 fold dilutions, while the third step was a 4 fold dilution.

a) What is the fold difference in concentration of the third dilution fold from the undiluted? Show all work.



**5 x 5 x 4 = 100 (6 pts.)**

b) What is the concentration (in μg of protein/μl) of the undiluted unknown (mg/ml)? Show all work both on the graph above and your calculations below. Report your concentration as an average of the two readings, and give the appropriate significant figures and delta.

**Draw Best Fit Line (2 pts):** Line must be drawn as the average trend and goes to the origin.  
**Interpolate Values (4 pts):** Absorbencies must not be averaged before interpolating. Concentrations must be determined from the best-fit line and not data points.  
 Concentrations of duplicate measurements: 35 μg/50 μl and 38 μg/50 μl.  
 Undiluted concentrations: 35 μg/50 μl x 100 = 70.0 μg/μl and 38 μg/50 μl x 100 = 76.0 μg/μl. (6 pts.)  
 Average Undiluted Concentration: 73.0 μg/μl. (2 pts.) Δ = |73.0 - 70.0| = ±3.00 μg/μl (2 pts.)  
 Reported Value: 73 ± 3 μg/μl (2 pts.)

c) Assuming you wanted an absorbance of your unknown that was around 0.55, what dilution fold would you make of your undiluted unknown? Show all work.

**Using the plot, interpolate the concentration for an absorbance at 0.55. (3 pts.)**  
 Concentration is 100 μg/ 50 μl or 2.00 μg/μl. (2 pts.)  
 The dilution factor is C<sub>1</sub>/C<sub>2</sub> = 73.0 μg/μl / 2.00 μg/μl = 36.5 (3 pts.)

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**Question 2** (26 pts.)

**Limit your answers to a maximum of 3-4 sentences. Avoid being too succinct in your answer. Remember, we cannot assume your understanding.**

a) Why would a 2 mg/ml bottle of free amino acids (such as: yeast protein hydrolysate) not be a sufficient standard for a protein assay? Explain completely.

**Free amino acids, namely the nonpolar amino acids, do not provide a sufficient hydrophobic pocket (3-dimensionally surround the coomassie blue), and thus there would not be any significant interaction between the amino acids and the dye. (6 pts.)**

b) What structural changes occur on the protein when it is added to the protein dye-binding reagent, and how do these changes cause protein unfolding?

**The dye binding reagent is sufficiently acidic to protonated all ionizable groups. There are two general classes of ionizable groups on a protein: the amine groups and the carboxylic acid groups. When these groups are protonated, the amine groups will have a positive charge and the carboxylic acid groups will lose their charge. Changes in the charges will disrupt interactions within the protein that help maintain the native structure of the protein which result in more unfolding and exposure of hydrophobic pockets. (8 pts.)**

c) In our protein assay, why are hydrophilic interactions between the protein and coomassie blue not the driving force for the interaction between these two molecules? Are the two opposite charges even important? Explain.

**Charged molecules in aqueous solution will be completely surrounded by shells of water molecules which would prevent an significant attraction let alone direct interactions between the two molecules (assuming the concentration of molecules is far less than the concentration of water). (6 pts.)**

**The opposite charges still play an important role in the stability of binding after the hydrophobic regions of the blue form of coomassie and the hydrophobic pockets of the protein bring the two molecules in close proximity (thus increasing the probability of interaction between the two charges). (6 pts.)**

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**Question 3: (24 points)**

For each protein assay scenario, an errant step is listed. Explain the nature of the problem for each of scenarios and the effect it will have in the data and the measurement of the concentration of the unknown. Assume we carry out the protein assay as described in our 153L lab. **LIMIT YOUR ANSWER TO NO MORE THAN 3-4 SENTENCES.**

a) You let your standards and samples incubate in coomassie for more than one hour before measuring at the spectrophotometer.

**Degradation via oxidation will occur for all samples. (4 pts.)**

**There will be a systematic drop in absorbance that may be significant enough to affect the ability to get a reliable standard curve, reproducible unknown values, and accurate readings of the unknown concentrations. (4 pts.)**

b) A student uses a solution of D.I. water to blank their protein assay standards and unknown samples. The student attempts to resolve this situation by measuring the absorbance of a sample containing an appropriate volume of the dye-reagent and 50  $\mu\text{l}$  of water (this sample was assayed using a solvent blank). The student then subtracted this absorbance from their standards and unknown samples. (Assume incubation time of the standards, unknowns, and this additional sample were the same.)

*There two problems with this experiment, however one is roughly corrected by the student. The incorrect blank was used with the standards and unknowns causing a systematic increase in values and a loss of the origin point for the standard curve. The student corrects for this by measuring the absorbance of the reagent blank (blanked with water) and subtracts the absorbance from each point to retain the origin. (6 pts.) But a problem still exists in which the measurement of the blank could have been done under different temperature conditions which could affect the noncovalent interactions of the protein and coomassie. As a result, the accuracy of the reagent blank value could still be in error. (2 pts.)*

c) You add the coomassie dye-binding reagent directly to your stock solutions of BSA standards and unknown solutions.

**Addition of coomassie to your stock solutions of BSA standards and unknowns will result in first a difference in coomassie concentrations in your standards, plus increased stabilization of the blue form (more protein in the stock). (4 pts.)**

**Varying concentrations of coomassie will negate any comparison between any of the samples or standards because rather than protein amounts being the only variance, there is also a difference in coomassie concentration. (4 pts.)**

**One additional effect that is not expected on this exam is the alteration of pH.**