College of the Canyons: Introduction to Biotechnology
Protein Standard Curve: Post Lab

1. What is meant by the term "assay?"

Assay means a carefully controlled comparison, generally a series of standards is compared to unknowns in order to determine concentration, amount, features of unknown.

2. Describe two different types of assays other than the one you worked on in this lab.

ELISA, DNA Assay, Hormone Assay, Microbial Amount Assay (virtually anything that needs to be quantified)

3. Describe how the protein color development solution yields a color change.

The Biuret's agent reacts with the amine group in the peptide bond. Specifically a Cu++ ion is changed from brown to blue as the ion changes charge.

4. If you had an 80μl sample of 1.5 mg/ml concentration, what would the final concentration be if the final sample is diluted to 150μl? (hint: C1V1=C2V2).

1mg= 1000μg, 1.5mg/ml= 1500μg/ml so (1500μg/ml)(0.08ml)= (0.150ml)(C2)

$\quad$ 80μl= 0.08ml $\qquad\qquad\qquad\qquad$ C2 = 800μg/ml or 0.8mg/ml

$\qquad\qquad\qquad$ *makes sense roughly 1:1 dilution so concentration goes down by 1/2

5. Describe an application where a protein curve using a "*known unknown*" would be assesses. Describe a situation where a protein concentration would be looked at with an "*unknown unknown*" be used. Cite lab manual as needed.

A known curve would be one where the researcher has a reasonable expectation as to what the value will be. Blood samples, many environemental samples often fall with in expected ranges and would give us a value somewhere in the expected range. In the case of unknown curves, the value is likely to be way outside the range. Fatal poisenings, toxic spills, etc are a few examples where the amount of subtance could range wildly..

6. <u>What is the R value?</u> How does repeating an experiment affect the R value. R stands for regression coefficient. It is bascailly a measure of how precise the line drawn through your data points (your standard curve) represent the true data. When the line is very clear and the poits line up, the line is easy to draw and has a very high R value. This means you are very confident in the data. If the points do not line up so neatly, the R value drops are the confidence in the line become less.

7. <u>Cite the two types of curves in question 5 describe the axis of both curves.</u> <u>Which curve is more accurate and why?</u> <u>If one curve is more accurate than the other, why is the inaccurate curve used?</u> A curve with a known known is best. The range is less and small movement to the left or right only slightly change the value. By comparision, the known with in an unknown is less accurate. The very small movements could result in very large changes. The axis are different as the possible ranges for the samples are different. Unknown knowns are only used in a very crude way to get a basic read on the data. After this curve gives a ballpark estimate..the other "known known" may be used to get a more accurate picture of the sample…

8. Use a series of numbers (1,2,3) to list and briefly describe the **steps** involved in getting the protein concentration of an unknown. Hint: Review lab and annotate the steps.

1) Create standards	5) Make best fit line
2) Develop with protein solution	6) Get ABS of unknown
3) Use spectrophotometry to get ABS	7) Use graph to interpolate
4) Graph data	