

25 points

Multiple Choice

Circle the correct answer (there may be more than one). Each question worth 1 point (2 total).

1. The spectrophotometer measures A
 A. Absorbance

 B. Reflectance

 C. Concentration

2. Glucose A&C
Should have read as Cu^{++} etc. Full credit for A&C or just C
 A. Can reduce C^{++} to C^+

 B. Can oxidize C^+ to C^{++}

 C. Can change the color of DNS

Short Answer. 2 points each, total 20 points

3. Determining protein concentration by A_{280} can be inaccurate but the BCA assay is very accurate . Why?

A_{280} relies on number of aromatic amino acids which can vary from standard to unknown protein. BCA determines number of peptide bonds which equals number of amino acids

4. What is the purpose of the "Blank" in spectrophotometry. What components go into the blank

To set base point for measuring concentration. Contains everything except that which is to be measured

5. K_m is the most important information you can obtain about an enzyme. What is K_m . What are its units. What does it tell you about an enzyme

The Michealis Constant (a ratio of constants). Units are concentration. It tells the efficiency with which an enzyme can bind substrate, can create a transition complex.

6. How does change in pH and temperature effect the function of an enzyme.

PH and heat can both denature (destroy) an enzyme or place it in a sub optimal chemical or physical environment.

7. In an experiment in which the concentration of substrate is held constant and the concentration of enzyme is changed you can obtain values of V_o that are the same at both high and low enzyme concentration. What explanation can you provide.

Too much enzyme uses all the substrate and give no change in rate because reaction is over. Too little enzyme cant produce enough change inn substrate concentration to measure. In both cases change in amount/ unit time is the same and very low

8. What is the effect on K_m when the concentration of enzyme is increased or decreased?

No effect. K_m is a property of the enzyme itself

10. In the assays of ADH and LDH we measured the change in concentration of NADH with time to determine enzyme activity. What was the major difference in the two assays.

ADH: NADH increased. LDH: NADH decreased

11. Why is it necessary to “blank” or “zero” the spectrophotometer when you are determining concentration but it is not necessary when you are determining kinetic rates.

In determining concentration you are determining an absolute amount of an unknown relative to a standard curve produced by a known and you need a common start point for all measurements. You must also ensure that measurements will be in range of accuracy of the spectrophotometer. In rate determination you look at change with time and do not refer to a reference (standard curve)

12. We used an affinity column which contained AMP sepharose to purify lactate dehydrogenase. What other enzymes could you purify with this column.

Any enzyme that has NADH – NAD⁺ as cofactor

13. What are the units of specific activity?

Amount of substrate per unit time per amount of enzyme.

Moles/ Min/Milligram

Define 1point each total 3 points

14. Standard Curve

Plot of absorbance versus amount for known dilution of a standard (protein-carbohydrate)

15. K_{cat}: The rate constant for the production of product.

16. Kosmotrope; A salt which aids protein solubility and stabilizes protein structure and function