1. Enzymes are classified and named according to the kind of reaction they catalyze and their specificity for the substrates they accept.

a. Amylase and invertase are both hydrolases that cleave the glycosidic bond of sugars to create new reducing ends. Because they hydrolyze different substrates, the two classes of enzyme can be distinguished solely on the basis of their substrate specificity. Outline an experiment that would allow you to determine whether an unknown enzyme has the specificity of invertase or α-amylase (i.e., to distinguish between them). Be sure to indicate how you would detect the disappearance of substrate or the appearance of product. (4 pts.)

b. Catalase and peroxidase (e.g., horseradish peroxidase) both recognize hydrogen peroxide as substrate but they catalyze different reactions. Outline an experiment that would allow you to determine whether an unknown enzyme has catalase activity or peroxidase activity (i.e., to distinguish between the two activities). (4 pts.)

2. Outline an experiment that you might use to determine the pH dependence of the kinetics of an enzyme-catalyzed reaction that arise from binding or from a catalytic step. Be sure to indicate any necessary control experiments you would do. (5 pts.)
3. Most phosphatases catalyze the hydrolysis of $p$-nitrophenol phosphate. We used this artificial substrate in lab to assay alkaline phosphatase. It can also be used for assays of neutral phosphatases, but not acid phosphatases.

Dissociation of the phenolic proton of $p$-nitrophenol occurs with a $pK_a$ of 7.1. The extinction coefficient at 403 nm of $p$-nitrophenolate ion (measured at pH 10) is $18,000 \text{ M}^{-1} \text{•cm}^{-1}$. At pH 7.5, with saturating levels of this substrate, a 3mL assay of a neutral phosphatase gave a $\Delta$Absorbance of 0.25 A/min (the pathlength was 1 cm).

a. What is the $V_{\text{max}}$ of the phosphatase using this substrate? (10 pts.)

b. If the assay contained 0.01 mg enzyme, what is the specific activity of chymotrypsin for this substrate (careful)? (5 pts.)
4. You have just isolated an enzyme that has never before been studied.
   a. How would you establish whether or not you had purified the enzyme to
      homogeneity? (4 pts.)

   b. Assuming it is pure, how would you determine its molecular weight? Be
      specific, don't just name a technique. (4 pts.)

   c. Assuming it is pure, how would you determine whether it was a single
      polypeptide chain or was composed of subunits? (Be specific, don't just name
      a technique). (4 pts.)

5. a. Describe an experiment that you would use to determine $K_I$ for a
    noncompetitive inhibitor of an enzyme-catalyzed reaction. Be as specific as
    you can. (4 pts.)
b. What calculations would you do to analyze the data obtained in part a? You need to establish that the inhibition is noncompetitive and determine $K_I$. (4 pts.)

6. What is the physical basis for separation of proteins using the following techniques (10 pts.)

a. Affinity chromatography

b. Ion exchange chromatography

c. SDS-Polyacrylamide gel electrophoresis

d. Gel filtration chromatography

e. Native polyacrylamide gel electrophoresis

7. You have set up an enzyme assay for pyruvate dehydrogenase. Your stock solutions contain

- 5 mg/mL enzyme
- 10.0 mM NAD$^+$ dissolved in water
- 2.00 mM pyruvate (substrate), dissolved in water
- buffer made from 2.27 g KH$_2$PO$_4$ plus 5.80 g K$_2$HPO$_4$ dissolved in 100 mL water. (molecular weights: KH$_2$PO$_4$, 136.1; K$_2$HPO$_4$, 174.2)

a. If the pK$_a$ of H$_2$PO$_4^-$ is 7.2, what is the pH of the buffer? (5 pts.)
b. What is the concentration of the buffer? (4 pts.)

c. If you used 0.05 mL of the substrate solution in an assay with a total volume of 1.0 mL, what is $[S]_o$? (4 pts.)

d. If you expected the $K_M$ for this substrate to be about 100 $\mu$M ($0.1 \times 10^{-3}$ M), show what volumes of each solution you would add to various assay runs in order to make such a measurement. (10 pts.)

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<th>buffer (mL)</th>
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8. a. List at least five factors that one should consider when designing a procedure to extract an enzyme from a plant tissue. (5 pts.)
b. If you tried several different solutions to extract the enzyme, how would you measure and compare the efficiency of the extraction procedure? (4 pts.)

9. You want to study the occurrence of a toxic protein found in a South American bean. Since you do not have a convenient assay for the protein (it has no enzymatic activity and your only functional assay is that is causes rats to starve to death), you choose to use ELISA. You worked several months to purify the protein, and you now have enough to inject into rabbits to obtain an antibody toward the protein. You can purchase goat anti-rabbit antibodies with peroxidase (not phosphatase) attached.

a. Describe how you would design an Enzyme-Linked Immunosorbant Assay for this protein to be used to assay the protein in extracts of related beans (use any reagents you need), and describe how you would run an actual assay. (10 pts.)

b. (bonus question) Suggest a quick and easy method that might be used to purify the first antibody from the blood of the rabbit. Be brief; you don’t need all the details.