Name _____

I.D. _____

Score _____

Exam 3, BICH 440, Section 500, Monday, November 20, 2000

Write your name on each page. Write concise answers to demonstrate effectively your mastery of the subject. Show your work in order to receive maximum credit where applicable.

gas constant R 8.315 J/mol-K Faraday constant F 96.5 kJ/mol-volt

1) (10 pts) Calculate the activation energy for an enzyme-catalyzed reaction if the rate enhancement for the catalyzed reaction is 10^7, and the activation energy for the uncatalyzed reaction is +60.0 kJ/mole (assume 37°C).

2) (10 pts) On the same set of axes draw velocity vs. [S] ([phosphate] in this case) plots demonstrating K-system allosteric regulation of glycogen phosphorylase by a) ATP and b) AMP. Show how $K_{0.5}$ is determined from each plot. Be sure to label the axes.

Name _____

3) (5 pts) Why is it advantageous to use a genomic library carried in a bacteriophage lambda vector rather than a plasmid DNA vector in order to screen for a particular human gene?

4) (15 pts) Initial velocities were determined for an enzyme-catalyzed reaction at a range of substrate concentrations where 10 nanograms (1ng = 10^{-9} g) of the enzyme (MW = 60,000) were added to each sample. Answer the following questions after plotting this data in the Lineweaver-Burk (double reciprocal) form on the graph paper (next page).

   a) What is k_{cat} / K_m (in M^{-1} sec^{-1}) for this enzyme?
   b) On the same graph in which you plotted this data, sketch a line that would represent this same amount of enzyme to which was added a competitive inhibitor such that the apparent K_m = 5.0 mM.

[S], mM	**v_0, nanomoles/sec**
0.25	13.3
0.5	24
1.0	40
2.0	60
5.0	86
10.0	100

Name _____

5) (10 pts) Starting at the hormone receptor, enumerate (that means 1,2,3, etc), and briefly describe, the cascade of proteins/enzymes that ultimately result in phosphorylation (and activation) of glycogen phosphorylase.

6) (10 pts) Given the following sequence of a single-stranded segment of DNA:

5' - GGACTCGAAGCTCTTAAAGGCGGGACTGTG -3'

Using an oligonucleotide DNA primer of sequence: 5'-CACAGTCCCGCCTTT-3', for a dideoxy sequencing reaction with DNA polymerase, write the complete set of DNA fragments that are synthesized for each reaction to which the designated nucleotides are added. You do not have to write the entire primer sequence for each DNA fragment - just write "5'-primer" followed by the rest of DNA in that fragment.

a) ddGTP, dATP, dTTP, dCTP (1 mM of each)
b) dGTP, dATP, dTTP, dCTP (1 mM of each); plus ddTTP 0.03 mM)
c) dGTP, dATP, dCTP (1 mM of each); plus ddGTP (0.03 mM)

Name _____

7) (40 pts) Short answer and fill-in:

a) Write the names of the six classes of enzymes included in the international classification system.

b) Approximately how many nucleosomes exist within a chromatin fragment that includes 10^7 base-pairs of DNA?

c) Define isozyme.

d) Write out the linking number equation and define in one or several words each term of the equation.

e) What are the axes for a Hill plot that describes binding of oxygen to hemoglobin? What is determined from the maximum slope of this Hill plot? What is the meaning of the latter value (max. slope)?

f) What is a suicide inhibitor (suicide substrate) of an enzyme? Name an example.

g) What is the difference in subunit structure between adult hemoglobin and fetal hemoglobin?

h) If the superhelical density of a particular segment of B-form DNA is -0.05, approximately how many superhelical turns exist in 1000 bp?

Name _____

i) Describe how the activities of bacterial topoisomerase I (nicking-closing enzymes) and topoisomerase II (DNA gyrase) counteract each other to maintain a constant value of DNA supercoiling.

j) What three steps must be repeated in every cycle of a polymerase chain reaction (PCR)?

k) Write the Michaelis-Menten equation.

l) Which is a more effective inhibitor? - A, with a K_I of 1 mM, or B, with a K_I of 10 mM? (hint: remember the K_I describes the dissociation constant for binding of the inhibitor to the enzyme).