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Score _____

Exam 3, BICH 440, Monday, November 21, 2005

You MUST sign the following academic integrity statement:

On my honor, as an Aggie, I have neither given nor received unauthorized aid on this academic work. Signed: _____

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) (15 pts) A relaxed, circular, double-stranded DNA molecule in B-form contains 6300 bp.
- (A) What is the linking number of this relaxed DNA molecule?
 - (B) If DNA gyrase (+ATP) introduced 12 negative supercoils, what would be the values for L , W , and T ?
 - (C) What is the superhelical density for the DNA molecule in (B)?
 - (D) If the starting, relaxed DNA molecule was in Z-form, what would be the linking number?

- 2) (10 pts) An enzyme follows Michealis-Menten kinetics. Indicate (with an "X") which of the kinetic parameters in the table would be altered by the following conditions.

condition	Km	Vmax	neither	both
(a) doubling [S]				
(b) a competitive inhibitor				
(c) 6M urea				
(d) a pure, noncompetitive inhibitor				
(e) a mixed, noncompetitive inhibitor				

- 3) (15 pts) The following RNA fragment and DNA oligonucleotide are annealed, then mixed with reverse transcriptase to synthesize cDNA. Write out the DNA fragment(s) synthesized from the primer when nucleotide mixes are added as described in each condition. You do not need to write out the primer sequence – just write "primer" followed by the correct sequence of synthesized DNA.

RNA: 5'-CACCGAUAAGCGUCACGGUUCGACGACUGGUUUAC-3'

DNA oligonucleotide: 5'-CCAGTCGTCGAACCGTG-3'

(A) dATP, dGTP, dCTP, dTTP, ddTTP (in limiting concentration)

(B) dCTP, dATP, dGTP, ddGTP (in limiting concentration)

(C) dATP, dGTP, dCTP, ddTTP

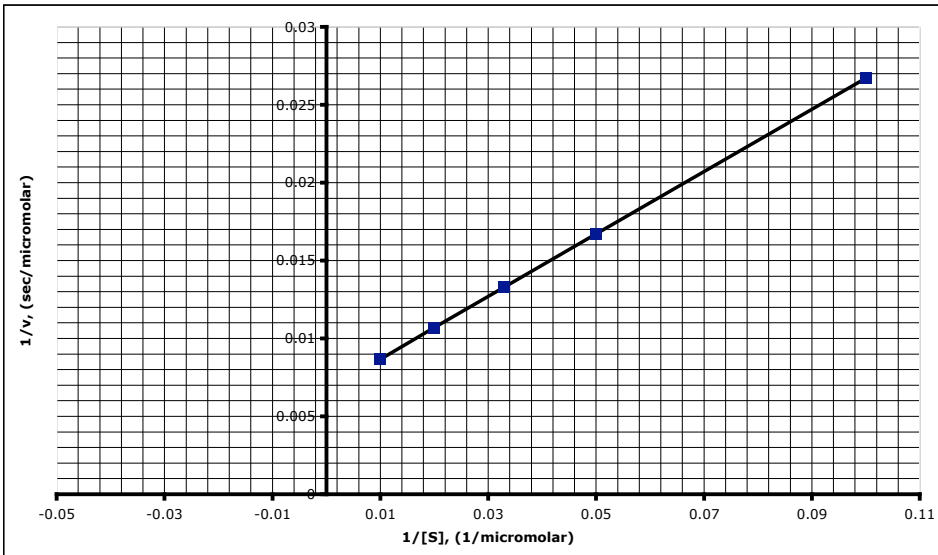
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4) (15 pts) The mechanism of lysozyme.

- (A) Draw the mechanism of catalysis for this enzyme for the model that invokes a covalent intermediate.
- (B) Explain in words the role of the glutamic acid in the mechanism.
- (C) What would be the result of using a mutant enzyme with a glutamic acid to glutamine substitution in the active site and a synthetic fluorinated substrate as discussed in lecture and your book? Explain the roles of glutamine (versus glutamic acid) and the fluorinated substrate that lead to this result.

5) (15 pts) (A) 20 micrograms of an enzyme with molecular weight of 50,000 are used in 1 mL (for each reaction) to generate the double-reciprocal plot shown below. Calculate the ratio of k_{cat}/K_m for this enzyme in units of $\text{sec}^{-1}\text{M}^{-1}$.

(B) On the plot draw a line corresponding to addition of a mixed, noncompetitive inhibitor that results in decrease of V_{max} by one-half, and doubling of K_m .



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6) (5 pts) Given the following double-stranded oligonucleotide:

5' -GAACTATCTTAAGCCATTCC-3'

3' -CTTGATAGAATTCGGTAAGG-5'

A hypothetical restriction endonuclease recognizes the sequence CTTAAG and cuts with b-type specificity leaving 3' overhangs of 2nt. Draw the two fragments after cleavage with this enzyme, indicating the positions of the phosphates at the cleavage site.

7) (7 pts) Draw a diagram that shows important characteristics for a bacterial expression vector. Make sure that you include signals on the plasmid that are required for plasmid maintenance and selection, and for protein expression from the inserted DNA.

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8) Shorter answer

(A) (5 pts) Write the pathway of equilibrium reactions for an enzyme-catalyzed bisubstrate reaction ($A + B \leftrightarrow P + Q$) that follows an ordered, sequential pathway.

(B)(3 pts) Name three examples of aspartic proteases.

(C)(3 pts) Name three examples of serine proteases.

(D)(2 pts) What two amino acid residues act as general acids/bases in the mechanism of ribonuclease A?

(E)(3 pts) Why is penicillin called a suicide substrate for the enzyme glycoprotein transpeptidase?

(F)(2 pts) Theoretically, how many times would a DNA fragment be amplified after 20 cycles of PCR?