生化學

A.

1. Please explain the following terms and describe operation briefly (7.5%)
   a) Western blotting
   b) Salting out
   c) Affinity chromatography

2. How to apply mass spectrometry for the study of proteins and their modifications ? (5%)

B.

1. Purification of enzyme is usually conducted in a 4℃ cold room. On the other hand, enzyme kinetic experiments are performed at either room temperature or 37℃. Why? (2.5 %).

2. The energy used by many cells to maintain homeostasis is produced by the oxidation of **compound A** in the citric acid cycle. During this oxidation process, **compound B** is generated and is used to drive the processes of oxidative phosphorylation, which are responsible for converting **compound B** to the high energy **compound C**. What are the names for compounds **A**, **B**, and **C**? (3 %)

3. When working on a protease, you hypothesize that a cysteine residue is located in the active site and is required for its protease activity. Please design 2 different experiments to test your hypothesis. (4 %)

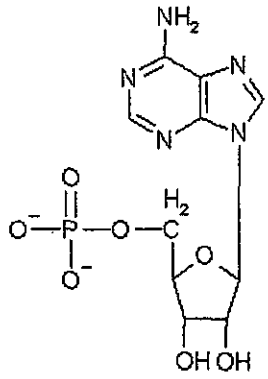
4. Which of the following conditions are assumed during the measurement of initial rate for an enzyme-catalyzed reaction? (1 %)
   I: [substrate] >> [enzyme]; II: [product] → 0;
   III: Reverse reaction is negligible; IV: [substrate] ~ constant
   a) I, II, III.
   b) I, II, IV.
   c) II, III, IV.
   d) I, II, III, IV.

5. In glycolysis , ATP is produced by **process A**. In citric acid cycle, ATP is produced by **process B**. What are the names for **A** and **B**? (1%)

   a) A: substrate-level phosphorylation; B: oxidative phosphorylation
   b) A: oxidative phosphorylation; B: substrate-level phosphorylation
   c) A: oxidative phosphorylation; B: β-oxidation
   d) A: substrate-level phosphorylation; B: substrate-level phosphorylation
   e) A: substrate-level phosphorylation; B: β-oxidation

見背面

6. The compound shown below is called _____. (1 %)



C.

1. What are the functions of lipoprotein lipase (LPL) in lipoprotein metabolism? What would you expect the profile of plasma lipoprotein in human deficient in LPS? (4 %)
2. What is LDL-receptor? Please explain the roles of LDL-receptor in cholesterol metabolism. (4.5 %)
3. What are ketone bodies? How ketone bodies are formed? (4%)

D.

I. 1 point per question (6 %)

1. If cells are treated with proper concentration of carboxin that completely blocks complex II oxidoreductase without induction of cell death and simultaneously added with compound X functioning as proton channels based on diffusion in the inner membrane of mitochondria, please calculate how many ATP will be generated after one glucose goes through glycolysis, TCA cycle, and respiratory chain. (A) 0 (B) 4 (C) 8 (D) 34 (E) 38.

2. How many following compounds are high-energy phosphates? i. phosphoenolpyruvate, ii. 3-phosphoglycerate, iii. creatine phosphate, iv. carbamoyl phosphate, v. glycerol 3-phosphate (A) 1 (B) 2 (C) 3 (D) 4 (E) 5.

3. Phosenolpyruvate carboxykinase can be found in (A) only in cytosol (B) only in mitochondria (C) in endoplasmic reticulum/Golgi (D) endoplasmic reticulum/Golgi /mitochondria (E) cytosol/mitochondria.

4. How many carbon sugar phosphate can not be found in the nonoxidative reactions of the pentose phosphate pathways? (A) 2C (B) 3C (C) 4C (D) 6C (E) 7C.

5. NH_3 intoxication is life-threatening and is toxic to nervous system. NH_3 intoxication is mainly due to the fact that (A) NH_3 and H_2O form NH_4OH to produce OH^-, leading to damaging the nervous system (B) NH_3 will be used to generate glutamate and high concentrations of glutamate will damage neuron cells (C) High

接次頁

concentrations of glutamate will enhance aspartate aminotransferase activity to consume pyruavte to produce alanine, leading to depletion of pyruvate (D) NH_3 reacts with α-ketoglutarate to form glutamate, resulting the depletion of α-ketoglutarate (E) neuron cells consume too much energy to metabolize NH_3, leading to neuron cell damage.

6. If a person has an increase of serum bilirubin in the absence of urine urobilinogen and in the presence of urine bilirubin, he/she may have (A) jaundice due to hemolytic anemia, (B) jaundice due to viral hepatitis, (C) jaundice due to cancer of pancreas, (D) obstructive jaundice, (E) jaundice due to cirrhosis and liver cancer.

II. (6.5%)

a. Please describe how cAMP and Ca^{++} play roles in regulating phosphorylase activity (3%).

b. Please use benzoate as an example to describe how glycine can be used for preservatives or drug excretion into urine. Your answers shall provide intermediate from benzoate and cofactors involved in formation of final product hippurate. (1.5 %)

c. Please briefly describe the four stages of urea biosynthesis from an α-amino acid degradation to generate urea. (2%)

E.

Please briefly describe following questions regarding the eukaryotic translation:

1. General characteristics of RNA templates for protein synthesis (4%).
2. What is the anticodon of tRNA for the start codon (2.5%)
3. What is the function of the aminoacyl tRNA synthetase (3%).
4. Please calculate the approximate molecular weight of protein X that is encoded by an mRNA with 810 nucleotides including a start codon at nucleotides 82-84 and a stop codon at nucleotides 448-450 (3%).

F.

Please describe significances of proteases in cancer cell signaling or tumor formation. What are biochemical methods to determine their amount, activities in these events (12.5%)

G.

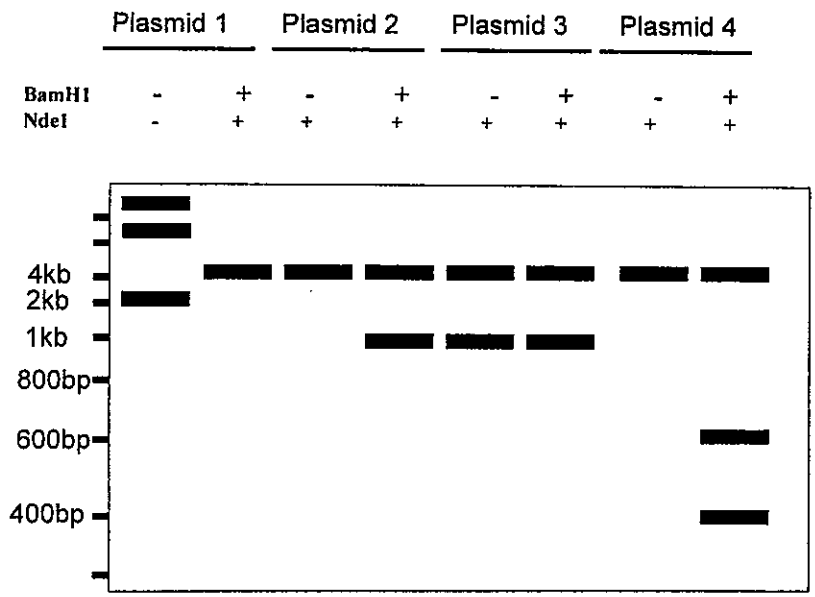
An enzymeX consists of two distinct functional domains, which are the substrate binding and catalytic domains. What domain should be mutated so that one can generate a dominant negative mutant of enzyme X? Why? (12.5%)

H.

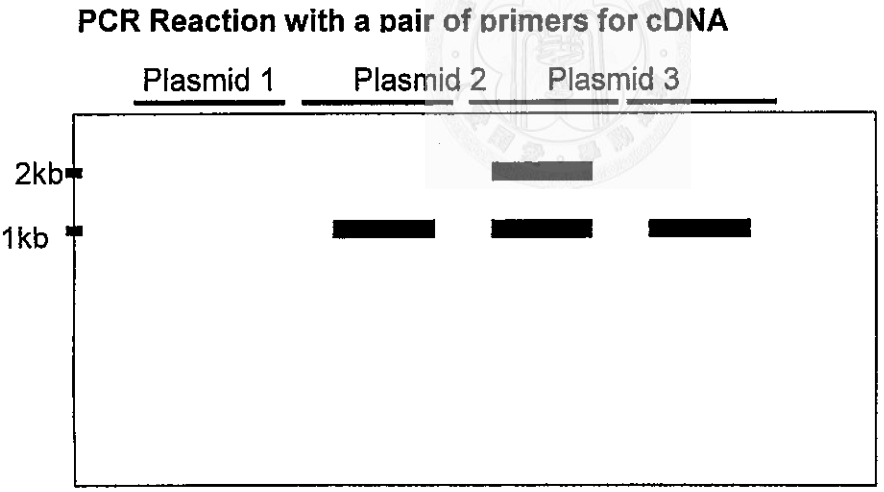
One investigator had done the ligation reaction for the subcloning the cDNA inserts into expression vector and he/she want to check the expression constructs with restriction enzymes digestion by using Nde1 and BamH1 digestions followed by PCR reaction. Please carefully examine the data below and answer questions:

Experiment 1 : restriction enzymes digestion

見背面



Experiment 2: PCR reaction for checking plasmids



Question 1: How many kb/ or bp of this empty expression vector and How many bp of the cDNA insert? (2 %)

Question 2: Which plasmid is a **correct construct** and can be used to express cDNA? (2%)

Question 3: Why there are multiple bands in restriction enzymes digestions for **plasmid 4 (lane 8) and what does PCR result for plasmid 4 tell us**? (2.5 %)

Question 4: Why there are multiple bands are in the **lane 1** of the restriction enzymes digestion data? (2 %)

Question 5: Please draw a gene maps with restriction enzymes cleavage sites and inserts for each vectors (4 %)

試題隨卷繳回