

1. Design an experiment to express human insulin in *E. coli*. (10%)
2. A method of preparation of monoclonal antibodies has been developed by Milstein and Kohler in 1975. (10%)
  - (a) Please describe their procedures step by step.
  - (b) List and explain at least three potential applications of monoclonal antibodies.
3. The binding affinity of isolated heme for CO is 25,000 times higher than O<sub>2</sub>, but the binding affinity of hemoglobin for CO is only 200 times higher than O<sub>2</sub>. What makes the difference? (10%)
4. When the substrate concentration is equal to K<sub>m</sub>, the catalytic velocity is equal to half of the V<sub>max</sub>. Please use Michaelis-Menten equation to explain this result. (10%)
5. Several proteases have a catalytic triad in their active site. Please draw the structure of a catalytic triad and indicate how does the proton transfer occurred? (10%)
6. (a) Circular dichroism measurements have shown that poly-L-lysine is a random coil at pH 7 but becomes  $\alpha$ -helical as the pH is raised above 10. Account for this pH-dependent conformational transition. (b) Predict the pH dependence of the helix-coil transition of poly-L-glutamate. (10%)
7. It has been estimated that an animal can generate at least one million different kinds of antibody. Please explain why an animal can use limited numbers of antibody genes to generate such diversify antibodies? (10%)
8. Please explain following terms: (30%)
  - (i) Polymerase chain reaction (PCR)
  - (ii) Western blotting analysis
  - (iii) Enzyme-link immunosorbent assay (ELISA)
  - (iv) Sanger dideoxy sequencing method
  - (v) Salmonella test for mutagens
  - (vi) Lineweaver-Burk plot