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Protein Biotechnology (240353)

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PART I: Circle the letter of the most appropriate answer of each of the followings

1	A mutation results in a single amino acid substitution of a protein. One technique that is **more likely** to be useful in differentiating between the normal and the mutant forms of the enzyme is a. Denatured SDS polyacrylamide gel electrophoresis. b. Native polyacrylamide gel electrophoresis. c. Gel filtration chromatography that separate based on sizes of proteins. d. Optical density of the normal and the mutant proteins at 220 nm. e. All of the above.
2	Proteins are usually purified to a. Identify a biological function of the target protein. b. Identify the structure of the target protein. c. Produce a commercial product. d. Purify an enzyme to use in generating a desired product. e. All of the above.
3	In the experiment where RNase activity was lost after treatment with 8 M urea and 2-mercaptoethanol, the RNase activity was restored after removing both chemicals. This experiment was **used to confirm** that; a. 8 M urea denatures RNase. b. 2-Mercaptoethanol breaks disulphide bridges. c. Denatured proteins are enzymatically inactive. d. Native proteins have enzymatic activity. e. Refolding of RNase was directed by the primary sequence.
4	Select the amino acid that has around α-carbon only one bond that can freely rotate: A. Valine B. Serine C. Glutamine D. Proline E. Asparagine
5	Which amino acid is most likely to be on the core of a water soluble protein? A. Serine B. Histidine C. Cysteine D. Glutamic Acid E. Phenylalanine
6	All the following statement are true regarding α-helix **except**: A. Each turn consists of 3.4 amino acids. B. Proline is not favourable amino acid to form a helix. C. The stabilizing force is the ionic interaction of side chains of charged amino acids. D. Helix formed to minimize satiric interference.. E. Helix is formed to maximize H-bonding of the backbone.
7	Number of **free amine group** present in the peptide (Pro-Phe-Asp-Ser-Arg) is: A. 0 B. 1 C. 2 D. 3 E. 4
8	Which is **not correct** regarding proteomic studies? a. 2D-SDS-PAGE can be used to separate the proteins. b. Study structure and function just for one protein.

	c. Digest proteins with a proteinase before mass spectroscopy (MS) analysis. d. Sequence of as short as 6 amino acids can be a unique sequence. e. Study the whole expressed proteins in a tissue.
9	Which of the followings is/are most likely **a contributing factor** in <u>increasing</u> possibility of producing an inclusion bodies in *E. coli*: a. Expressing a hydrophobic protein. b. High rate of protein expression. c. Growing the bacteria at high temperature. d. Expressing a protein of high molecular weight. e. All of the above.
10	For 100 kDa protein, number of grams in **<u>one mole</u>** is a. 100 b. 1000 c. 10,000 d. 100,000 e. 1,000, 000
11	<u>Increasing salt concentrations</u> does not significantly affect the **<u>strength</u>** of: A. H-bonding. B. Hydrophobic interaction C. Ionic bonds D. Covalent bond. E. None of the above.
12	Which of the followings can be used as ligands in affinity protein purification a. Substrate analogs of a specific enzyme. b. Antibody against a protein of interest. c. Antigen to purify the corresponding antibodies. d. Hormones to purify a corresponding receptor. e. All of the above.
13	The **correct order** of connecting the components of HPLC is: a. buffer tanks, column, pumps, detector, fraction collector. b. pumps, buffer tanks, column, detector, fraction collector. c. buffer tanks, pumps, column, detector, fraction collector. d. pumps, buffer tanks, column, fraction collector, detector,. e. column, buffer tanks, pumps, detector, fraction collector.
14	Which of the following detectors can be used in HPLC for **protein purification**: a. UV spectrophotometer. b. Fluorometer c. Mass spectroscopy d. Diode array e. All of the above

<u>PART 2</u>: Short answers:

What is the net charge of aspartate at pH 10? ……..

Why expressing insulin in bacteria is not considered as protein engineering?

A mixture of five typical globular proteins (each with a single polypeptide chain) with the following molecular weights and pIs.

A. The name of the protein that has the smallest **elution time** on gel filtration chromatography at pH 7.5 is: ……

B. The name of the first protein eluted from a **positively charged** column chromatography using increasing salt concentration at pH 7.5……

Name	Mol. Wt.	pI
A	100,000	6.5
B	125,000	6.0
C	150,000	7.0
D	175,000	6.8
E	140,000	8.0

Answer the followings regarding the inducible lac operon/T7 expression system in *E. coli*:

A. What is the **source** of T7 promoter…….

B. **Name the promoter** used to control expression of the gene of interest……..

C. **Name the gene** that is controlled by the inducible lac operon…….

D. What the repressor protein **binds** in presence of the inducer ……..

Answer the following regarding finding amino acid sequence of a polypeptide:

E. Edman degradation determine one amino acid at a time from the…… terminal of the peptide.

F. How do you produce peptide fragments with overlapping sequences?

In a protein purification scheme, the following data were obtained:

Purification step	Total protein (mg)	Specific activity (u/mg)
Crude protein extract	10000	5
Salt precipitation (fraction 20-40%)	4000	10
Ultrafiltration	2000	15
A peak from affinity chromatography	100	200
Gel filtration chromatography	50	400

. Calculate how many fold purification is obtained in all steps?

. Which step in which the recovery of the target (specific) protein was 100% without losing any specific protein? Why?