GURUDAS COLLEGE

DEPARTMENT OF BIOCHEMISTRY

3rd SEMESTER HONOURS

INTERNAL ASSESSMENT-2020

PAPER-SEC A2

Answer all the questions:

1.The purity of an enzyme at various stages of purification is best measured by:

a. Total protein

b. Total enzyme activity

c. Specific activity of the enzyme

d. Percent recovery of protein

e. Percent recovery of the enzyme

2. Which would be best to separate a protein that binds strongly to its substrate?

a. Gel filtration

b. Affinity chromatography

c. Cation exchange

d. Anion exchange

e. Cation or anion exchange

3. In a mixture of the five proteins listed below, which should elute second in size-exclusion (gel filtration) chromatography?

a. cytochrome c Mr = 13,000

b. immunoglobulin G Mr = 145,000

c. ribonuclease A Mr = 13,700

d. RNA polymerase Mr = 450,000

e. serum albumin Mr = 68,500

4. The first step in two-dimensional gel electrophoresis generates a series of protein bands by isoelectric focusing. In a second step, a strip of this gel is turned 90 degrees, placed on another gel containing SDS, and electric current is again applied. In this second step:

a. proteins with similar isoelectric points become further separated according to their molecular weights.

b. the individual bands become stained so that the isoelectric focus pattern can be visualized

c. the individual bands become visualized by interacting with protein-specific antibodies in the second gel

d. the individual bands undergo a second, more intense isoelectric focusing

e. the proteins in the bands separate more completely because the second electric current

is in the opposite polarity to the first current

Assumption for the following questions:

Your "assignment" is to purify the enzyme "BC-1ase". It is an enzyme found in the cytosol of yeast cells. Its molecular weight is 25,000 Da; its pI = 5.1; and its absorption coefficient = 0.65 at 280 nm (1mg/mL solution). This protein is responsible for the synthesis of ATP in the cell from AMP and inorganic phosphate.

5. After disrupting the yeast cells, which of the following might you add to stabilize the protein?

a. NaCl

b. Protease Inhibitor.

c. ATP.

d. AMP.

e. All of the above.

6. Fractional precipitation is the next step. BC-1ase precipitates between 2.5-3.5 M added salt. Which of the following salts do you choose to use?

a. silver chloride, AgCl.

b. ammonium perchlorate, NH_4ClO_4.

c. ammonium sulfate, $(NH4)_2SO_4$.

d. barium perchorate, Ba $(ClO_4)_2$.

e. guanidinium chloride, CN_3H_6Cl.

7. The precipitate from step 2 is dissolved in buffer, pH=7.0. The high salt concentration is removed by passing the solution through a gel filtration column. The protein is expected to:

a. elute from the column after the residual salt.

b. elute before the residual salt.

c. stick to the column.

d. remain at the top of the column.

e. Any of the above choices are possible.

8. The desalted protein solution from the gel filtration column is next applied to an ion exchange column. The best results are expected from a column that contains

a. anion exchange resin, pH 7.0.

b. anion exchange resin, pH 3.0.

c. cation exchange resin, pH 7.0.

d. cation exchange resin, pH 8.0.

e. cation exchange resin, pH 10.0.

9. The fractions obtained from the ion exchange column are nearly pure BC-1ase. To estimate the homogeneity of the preparation which of the following tests are suitable:

a. polyacrylamide gel electrophoresis (PAGE), native conditions.

b. SDS-PAGE.

c. isoelectric focusing.

d. determination of specific activity.

e. All of the above choices are valuable tests for purity.

10. What would be the best way to determine the location of this protein in the column fractions?

a. UV absorption.

b. Changes in the refractive index.

c. Measure the rate of ATP synthesis.

d. SDS gel electrophoresis of the protein.

e. Mass spectroscopy of the protein.