

**Multiple Choice Quiz proteins (enzymes) purification**

1. During successful purification scheme, this may be expected that the

- A. specific activity increases      B. specific activity decreases  
C. number of proteins in the sample decreases      **D. both (a) and (c)**

2. In ion-exchange chromatography

- A. proteins are separated on the basis of their net charge**  
B. proteins are separated on the basis of their size  
C. proteins are separated on the basis of their shape  
D. either (b) or (c)

3. Gel-filtration chromatography separates on the basis of

- A. size and shape using porous beads packed in a column**  
B. size using porous beads packed in a column  
C. shape using porous beads packed in a column  
D. none of the above

4. Affinity chromatography deals with the

- A. specific binding of a protein constituents for another molecule**  
B. protein - protein interaction  
C. protein - carbohydrate interaction  
D. none of the above

5. A purified protein sample contains 10 µg of protein and has an enzyme activity of 1 m mole of ATP synthesized/sec (1 unit). What is the specific activity of the final purified sample?

- A. 1,000 units/mg      B. 10,000 units/mg.      **C. 100,000 units/mg**  
D. 1,000,000 units/mg

6. Proteins separation can be carried out on the basis of

- A. net charge      B. solubility in salt solutions      C. size or mass      **D. all of these**

7-Which of the following statements about protein separation by gel filtration are correct? Please select all that apply.

- a) When a mixture of proteins is separated by gel filtration, the smallest molecular weight protein is eluted first .
- b) When a mixture of proteins is separated by gel filtration, the largest molecular weight protein is eluted first .
- c) When a mixture of proteins is separated by gel filtration, the smallest molecular weight protein is eluted last .
- d) When a mixture of proteins is separated by gel filtration, the smallest molecular weight proteins flow around the beads .

**8. For the study of a protein in detail, an effort is usually made to first:**

- a) Conjugate the protein to a known molecule.
- b) Determine its amino acid composition.
- c) Determine its amino acid sequence.
- d) Determine its molecular weight.
- e) Purify the protein.

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

- a) cytochrome  $M_r = 13,000$
- b) immunoglobulin G  $M_r = 145,000$
- c) ribonuclease A  $M_r = 13,700$
- d) RNA polymerase  $M_r = 450,000$
- e) serum albumin  $M_r = 68,500$

**10. By adding SDS (sodium dodecyl sulfate) during the electrophoresis of proteins, it is possible to:**

- a) Determine a protein's isoelectric point.
- b) Determine an enzyme's specific activity.
- c) Determine the amino acid composition of the protein.
- d) Preserve a protein's native structure and biological activity.
- e) Separate proteins exclusively on the basis of molecular weight.

**11. To determine the isoelectric point of a protein, first establish that a gel:**

- a) contains a denaturing detergent that can distribute uniform negative charges over the protein's surface.
- b) exhibits a stable pH gradient when ampholytes become distributed in an electric field.
- c) is washed with an antibody specific to the protein of interest.

- d) neutralizes all ionic groups on a protein by titrating them with strong bases.  
e) relates the unknown protein to a series of protein markers with known molecular weights,  $M_r$ .

**12. 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.

**13. The term *specific activity* differs from the term *activity* in that specific activity:**

a) is measured only under optimal conditions.

b) is the activity (enzyme units) in a milligram of protein.

c) is the activity (enzyme units) of a specific protein.

d) refers only to a purified protein.

e) refers to proteins other than enzymes.

**14. In isoelectric focusing, separation of proteins are based on**

a) relative content of positively charged groups

b) relative content of negatively charged groups

c) both a and b      d) pH      e) None of these

**15. Which of the following statement is incorrect**

a) In affinity chromatography, lectins are used to purify a glycoprotein

b) The separation in gel filtration chromatography is based on size, shape and net charge of the protein

c) In Ion exchange chromatography, the bound proteins are eluted using NaCl solution

d) In affinity chromatography, the binding of a protein to a ligand is by specific non-covalent interactions

## Protein Purification

### Isolation of Proteins from Cells

- 1) First step is? - Cell Fractionation.
- 2) Second step is? - Salting Out.

### Cell Fractionation

#### 1) What is done first?

- Proteins are released from cells using Homogenization (Blender Processing or Tissue Sonicator).

#### 2) What is done second?

- Differential Centrifugation.

### Salting out

#### 1) what compound helps soluble proteins in the the cytosol separate?

- Ammonium Sulfate.

#### 2)What does the salt take away? Leads to protein being what?

- Makes protein less soluble because hydrophobic interactions among proteins increases.

### Column Chromatography

#### 1) What is the Basis of Chromatography?

- Different compounds interact/distribute themselves in different phases.

#### 2) What are the 2 phases?

- Stationary and Mobile.

#### 3) What is the Stationary phase?

- Samples interacts with this phase.

#### 4) What is the Mobile phase?

- Flows over the stationary phase and carries along with it the sample to be separated.

### Size-Exclusion Chromatography

1) Proteins are separated how in Gel Filtration Chromatography (Size-Exclusion Chromatography)? - Size.

2) What is the Stationary phase composed of?

- Cross-linked gel particles.

3) Which particles come out first?

- Smaller molecules enter the pores and are delayed in elution time. Larger molecules do not enter and elute from column before smaller ones.

### Affinity Chromatography

1) How does it separate proteins/molecules?

- Separates Proteins by their Binding properties.

2) What does the Stationary phase have? What can the Polymer do?

- A Polymer. Can be covalently linked to a compound called a Ligand that specifically binds to protein. After the unwanted proteins are eluted, more ligand or salt is added

3) after the unwanted proteins are eluted, what is added? What does this do?

- Salt. It weakens the binding of protein to the ligand and extra free ligand competes with the column ligand in protein binding.

### Ion-Exchange Chromatography

1) what is in the Column?

- Hydrophobic Polymer (Polystyrene).

2) What is a Cation Exchange Column?

- Negative Charges on the surface.

3) What is an Anion Exchange Column?

- Positive Charges on the surface.

### Electrophoresis

1) which particles move? Move towards what?

- Charged Particles migrate in Electric Field toward opposite Charge.

**2) Mobility is affected by?**

- Charge, Size and Shape

**3) What is used as a matrix for nucleic acids?**

- Agarose.

**4) What is used as a matrix for proteins?**

- Polyacrylamide

**5) Why is Polyacrylamide used for proteins?**

- Polyacrylamide has more resistance towards larger molecules than smaller.

**6) Proteins are treated with what? What does this do?**

- Protein is treated with detergent (SDS) sodium dodecyl sulfate. This gives all proteins a uniform charge

.

**7) Which proteins go through the gel faster? .**

- Smaller proteins move through faster (charge and shape usually similar).

### **Isoelectric Focusing**

**1) What is Isoelectric Focusing?**

- This technique separates proteins according to their isoelectric points.

**2) How is the gel prepared has pH gradient that is parallel to? What is used to make the pH stable?**

- Gel is prepared with pH gradient that parallels electric-field. A stable pH gradient is established in the gel by the addition of appropriate ampholytes.

**3) What is on the protein as it migrates?**

- Charge on the protein changes as it migrates.

**4) What does the protein do when it gets to its pI?**

- When it gets to pI, it has no charge and stops.

**5) What happens when the pH is equal to the pI?**

- The net charge of a protein is zero.

## Will fill out this critical table

| Steps   | Vol. (ml) | Activity (U/ml) | Total (U) | Protein (mg/ml) | Specific Activity (U/mg) | Yield (%) | Purification fold |
|---|-----------|-----------------|-----------|-----------------|--------------------------|-----------|-------------------|
| <b>Extract</b>  | 7000      |                 | 19,950    | 35.5            |                          |           |                   |
| <b>Chloroform supernatant</b>                           | 5800      | 3.6             |           | 19.2            |                          |           |                   |
| <b>30-55 (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub></b> |           | 11.25           | 16,875    | 21.4            |                          |           |                   |
| <b>DEAE – Cellulose</b>                                 | 2380      |                 | 9090      | 1.0             |                          |           |                   |
| <b>Affinity column</b>                                  | 450       | 15.05           |           |                 | 16.7                     |           |                   |
| <b>Gel filtration</b>                                   | 52        | 98              | 5096      | 2.15            | 45.6                     | 25.5      | 570               |