

BIOSE10 - Lecture 3 Practice Problems - Answers

1-

- (a) Ion-exchange chromatography separates proteins on the basis of their charges. (b) Size-exclusion or gel filtration chromatography separates on the basis of size. (c) Affinity chromatography separates proteins with specific, high affinity for some ligand (attached to an inert support) from other proteins with no such affinity.

2-

- (1) Use Edman degradation to determine the sequence of each peptide. (2) Create a second set of peptides by treatment of the protein with a specific protease (e.g., trypsin), and determine the sequence of each of these. (3) Place the peptides in order by their overlaps.

3-

From the percentage recovery of activity (units), we calculate percentage yield and specific activity (units/mg).

Procedure	Protein (mg)	Activity (units)	% Yield	Specific activity (units/mg)	Purification factor (overall)
1	20,000	4,000,000	(100)	200	(1.0)
2	5,000	3,000,000	75	600	x 3.0
3	4,000	1,000,000	25	250	x 1.25
4	200	800,000	20	4,000	x 20
5	50	750,000	19	15,000	x 75
6	45	675,000	17	15,000	x 75

(b) Step 4, ion-exchange chromatography; this gives the greatest increase in specific activity (an index of purity and degree of increase in purification).

(c) Step 3, pH precipitation; two-thirds of the total activity from the previous step was lost here.

(d) Yes. The specific activity did not increase further after step 5. SDS polyacrylamide gel electrophoresis is an excellent, standard way of checking homogeneity and purity.

4- HSEGTFSNDYSKYLEDRKAQDFVRWLMNNKRSGAEE

19 3 Practice Problems Answers

Lorraine J. Doucette



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Table of Contents 19 3 Practice Problems Answers

1. Understanding the eBook 19 3 Practice Problems Answers
 - The Rise of Digital Reading 19 3 Practice Problems Answers
 - Advantages of eBooks Over Traditional Books
2. Identifying 19 3 Practice Problems Answers
 - Exploring Different Genres
 - Considering Fiction vs. Non-Fiction
 - Determining Your Reading Goals
3. Choosing the Right eBook Platform
 - Popular eBook Platforms
 - Features to Look for in an 19 3 Practice Problems Answers
 - User-Friendly Interface
4. Exploring eBook Recommendations from 19 3 Practice Problems Answers
 - Personalized Recommendations
 - 19 3 Practice Problems Answers User Reviews and Ratings
 - 19 3 Practice Problems Answers and Bestseller Lists
5. Accessing 19 3 Practice Problems Answers Free and Paid eBooks
 - 19 3 Practice Problems Answers Public Domain eBooks
 - 19 3 Practice Problems Answers eBook Subscription Services
 - 19 3 Practice Problems Answers Budget-Friendly Options

6. Navigating 19 3 Practice Problems Answers eBook Formats
 - ePub, PDF, MOBI, and More
 - 19 3 Practice Problems Answers Compatibility with Devices
 - 19 3 Practice Problems Answers Enhanced eBook Features
7. Enhancing Your Reading Experience
 - Adjustable Fonts and Text Sizes of 19 3 Practice Problems Answers
 - Highlighting and Note-Taking 19 3 Practice Problems Answers
 - Interactive Elements 19 3 Practice Problems Answers
8. Staying Engaged with 19 3 Practice Problems Answers
 - Joining Online Reading Communities
 - Participating in Virtual Book Clubs
 - Following Authors and Publishers 19 3 Practice Problems Answers
9. Balancing eBooks and Physical Books 19 3 Practice Problems Answers
 - Benefits of a Digital Library
 - Creating a Diverse Reading Collection 19 3 Practice Problems Answers
10. Overcoming Reading Challenges
 - Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
11. Cultivating a Reading Routine 19 3 Practice Problems Answers
 - Setting Reading Goals 19 3 Practice Problems Answers
 - Carving Out Dedicated Reading Time
12. Sourcing Reliable Information of 19 3 Practice Problems Answers
 - Fact-Checking eBook Content of 19 3 Practice Problems Answers
 - Distinguishing Credible Sources
13. Promoting Lifelong Learning
 - Utilizing eBooks for Skill Development
 - Exploring Educational eBooks
14. Embracing eBook Trends
 - Integration of Multimedia Elements

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