

ACS BIOCHEMISTRY EXAM

QUESTIONS AND ANSWERS @ 2023

Henderson-Hasselbach Equation - Answer $\text{pH} = \text{pKa} + \log ([\text{A}^-] / [\text{HA}])$

FMOC Chemical Synthesis - Answer Used in synthesis of a growing amino acid chain to a polystyrene bead. FMOC is used as a protecting group on the N-terminus.

Salting Out (Purification) - Answer Changes soluble protein to solid precipitate. Protein precipitates when the charges on the protein match the charges in the solution.

Size-Exclusion Chromatography - Answer Separates sample based on size with smaller molecules eluting later.

Ion-Exchange Chromatography - Answer Separates sample based on charge. CM attracts +, DEAE attracts -. May have repulsion effect on like charges. Salt or acid used to remove stuck proteins.

Hydrophobic/Reverse Phase Chromatography - Answer Beads are coated with a carbon chain. Hydrophobic proteins stick better. Elute with non-H-bonding solvent (acetonitrile).

Affinity Chromatography - Answer Attach a ligand that binds a protein to a bead. Elute with harsh chemicals or similar ligand.

SDS-PAGE - Answer Uses SDS. Gel is made from cross-linked polyacrylamide. Separates based off of mass with smaller molecules moving faster. Visualized with Coomassie blue.

SDS - Answer Sodium dodecyl sulfate. Unfolds proteins and gives them uniform negative charge.

Isoelectric Focusing - Answer Variation of gel electrophoresis where protein charge matters. Involves electrodes and pH gradient. Protein stops at their pI when neutral.

FDNB (1-fluoro-2,3-dinitrobenzene) - Answer FDNB reacts with the N-terminus of the protein to produce a 2,4-dinitrophenol derivative that labels the first residue. Can repeat hydrolysis to determine sequential amino acids.

DTT (dithiothreitol) - Answer Reduces disulfide bonds.

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Table of Contents Acs Standardized Exam For Biochemistry Practice Test

1. Understanding the eBook Acs Standardized Exam For Biochemistry Practice Test
 - The Rise of Digital Reading Acs Standardized Exam For Biochemistry Practice Test
 - Advantages of eBooks Over Traditional Books
2. Identifying Acs Standardized Exam For Biochemistry Practice Test
 - 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 Acs Standardized Exam For Biochemistry Practice Test
 - User-Friendly Interface
4. Exploring eBook Recommendations from Acs Standardized Exam For Biochemistry Practice Test
 - Personalized Recommendations
 - Acs Standardized Exam For Biochemistry Practice Test User Reviews and Ratings
 - Acs Standardized Exam For Biochemistry Practice Test and Bestseller Lists

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 - Acs Standardized Exam For Biochemistry Practice Test eBook Subscription Services
 - Acs Standardized Exam For Biochemistry Practice Test Budget-Friendly Options
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 - ePub, PDF, MOBI, and More
 - Acs Standardized Exam For Biochemistry Practice Test Compatibility with Devices
 - Acs Standardized Exam For Biochemistry Practice Test Enhanced eBook Features
7. Enhancing Your Reading Experience
 - Adjustable Fonts and Text Sizes of Acs Standardized Exam For Biochemistry Practice Test
 - Highlighting and Note-Taking Acs Standardized Exam For Biochemistry Practice Test
 - Interactive Elements Acs Standardized Exam For Biochemistry Practice Test
8. Staying Engaged with Acs Standardized Exam For Biochemistry Practice Test
 - Joining Online Reading Communities
 - Participating in Virtual Book Clubs
 - Following Authors and Publishers Acs Standardized Exam For Biochemistry Practice Test
9. Balancing eBooks and Physical Books Acs Standardized Exam For Biochemistry Practice Test
 - Benefits of a Digital Library
 - Creating a Diverse Reading Collection Acs Standardized Exam For Biochemistry Practice Test
10. Overcoming Reading Challenges
 - Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
11. Cultivating a Reading Routine Acs Standardized Exam For Biochemistry Practice Test
 - Setting Reading Goals Acs Standardized Exam For Biochemistry Practice Test
 - Carving Out Dedicated Reading Time
12. Sourcing Reliable Information of Acs Standardized Exam For Biochemistry Practice Test
 - Fact-Checking eBook Content of Acs Standardized Exam For Biochemistry Practice Test
 - Distinguishing Credible Sources
13. Promoting Lifelong Learning

- Utilizing eBooks for Skill Development
- Exploring Educational eBooks
- 14. Embracing eBook Trends
 - Integration of Multimedia Elements
 - Interactive and Gamified eBooks

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