

Contents

Preface	vii
---------	-----

Section I Basic Techniques of Experimental Biochemistry 1

Introduction	3
Requirements for a Student of Experimental Biochemistry	3
Laboratory Safety	5
Units of Biochemistry	5
Analysis and Interpretation of Experimental Data	6
Presentation of Experimental Data	10
To the Instructor	11
EXPERIMENT 1 Photometry	15
EXPERIMENT 2 Chromatography	25
EXPERIMENT 3 Radioisotope Techniques	45
EXPERIMENT 4 Electrophoresis	61

Section II Proteins and Enzymology 79

Introduction	81
Amino Acids: Identification and Quantitative Determination	81
Amino Acids: Ionic Properties	82
Protein Structure	83
Determination of Protein Structure	86
Determination of Molecular Weights and Quaternary Structures of Protein	88
Protein Denaturation	89

Protein Purification	90
Quantitative Determination of Proteins	93
Enzymology	95
Kinetics of Inhibition of Enzymes	98
Other Factors Affecting Enzyme Activity	100
Enzyme Assay	101
Assay Procedure during Enzyme Purification	102
EXPERIMENT 5 Acid-Base Properties of Amino Acids	105
EXPERIMENT 6 Sequence Determination of a Dipetide	111
EXPERIMENT 7 Study of the Properties of β -Galactosidase	123
EXPERIMENT 8 Purification of Glutamate-Oxaloacetate Transaminase from Pig Heart	135
EXPERIMENT 9 Kinetic and Regulatory Properties of Aspartate Transcarbamylase	149
EXPERIMENT 10 Affinity Purification of Glutathione-S-Transferase	157

Section III Biomolecules and Biological Systems 163

Introduction	165
Carbohydrates	165
Lipids	183
Membranes	189

EXPERIMENT 11	Microanalysis of Carbohydrate Mixtures by Isotopic, Enzymatic, and Colorimetric Methods	195
EXPERIMENT 12	Glucose-1-Phosphate: Enzymatic Formation from Starch and Chemical Characterization	205
EXPERIMENT 13	Isolation and Characterization of Erythrocyte Membranes	217
EXPERIMENT 14	Electron Transport	227
EXPERIMENT 15	Study of the Phosphoryl Group Transfer Cascade Governing Glucose Metabolism: Allosteric and Covalent Regulation of Enzyme Activity	243
EXPERIMENT 16	Experiments in Clinical Biochemistry and Metabolism	253

Section IV Immunochemistry 261

Introduction	263
Types of Antibodies	263
Polyclonal and Monoclonal Antibodies	266
Purification of Antibodies	270
Use of Antibodies in Biochemical Studies	271
Advantages and Disadvantages of Polyclonal and Monoclonal Antibodies	276
EXPERIMENT 17 Partial Purification of a Polyclonal Antibody, Determination of Titer, and Quantitation of an Antigen Using the ELISA	279
EXPERIMENT 18 Western Blot to Identify an Antigen	291

Section V Nucleic Acids 301

Introduction	303
Structural Features of Nucleic Acids	303
Cellular Localization and Roles of Nucleic Acids	306
Chemical Properties of Nucleic Acids	309

Physical Properties of Nucleic Acids	310
Plasmids and Other Cloning Vectors	314
Restriction Endonucleases and Other Modifying Enzymes	318
Ligation of DNA Fragments	321
Transformation of Host Cells	326
Selection and Screening for Transformed Cells	329
EXPERIMENT 19 Isolation of Bacterial DNA	333
EXPERIMENT 20 Transformation of a Genetic Character with Bacterial DNA	339
EXPERIMENT 21 Constructing and Character- izing a Recombinant DNA Plasmid	345
EXPERIMENT 22 In Vitro Transcription from a Plasmid Carrying a T7 RNA Polymerase-Specific Promoter	359
EXPERIMENT 23 In Vitro Translation: mRNA, tRNA, and Ribosomes	369
EXPERIMENT 24 Amplification of a DNA Fragment Using Polymerase Chain Reaction	385

Section VI Information Science 397

Introduction	399
Specialized Biological Databases	401
Computer Software Programs for Analyzing Sequences	402
The Protein Data Bank (PDB) and RasMol	404
EXPERIMENT 25 Obtaining and Analyzing Genetic and Protein Sequence Information via the World Wide Web, Lasergene, and RasMol	405
Appendix Supplies and Reagents	409
Index	437