



Principles and Techniques of
Biochemistry and
Molecular Biology

Sixth edition

Edited by
Keith Wilson
& **John Walker**

A new and expanded edition of the bestselling textbook
Principles and Techniques of Practical Biochemistry

Contents

Preface to the sixth edition xi

List of contributors xiii

List of abbreviations xvi

1 Basic principles 1

K. WILSON (Section 1.7 in association with J. Fyffe)

- 1.1 Biochemical studies 1
- 1.2 Units of measurements 3
- 1.3 Weak electrolytes 10
- 1.4 Buffer solutions – their nature and preparation 15
- 1.5 pH and oxygen electrodes 18
- 1.6 Quantitative biochemical measurements 28
- 1.7 Principles of clinical biochemical analysis 48
- 1.8 Safety in the laboratory 68
- 1.9 Suggestions for further reading 69

2 Cell culture techniques 71

A. R. BAYDOUN

- 2.1 Introduction 71
- 2.2 The cell culture laboratory and equipment 72
- 2.3 Safety considerations in cell culture 76
- 2.4 Aseptic techniques and good cell culture practice 77
- 2.5 Types of animal cell and their characteristics in culture 81
- 2.6 Bacterial cell culture 93
- 2.7 Plant cell culture 97
- 2.8 Potential use of cell cultures 101
- 2.9 Suggestions for further reading 101

Contents

3 Centrifugation 103

K. OHLENDIECK

- 3.1 Introduction 103
- 3.2 Basic principles of sedimentation 104
- 3.3 Types, care and safety aspects of centrifuges 109
- 3.4 Preparative centrifugation 117
- 3.5 Analytical centrifugation 124
- 3.6 Suggestions for further reading 130

4 Microscopy 131

S. W. PADDOCK

- 4.1 Introduction 131
- 4.2 The light microscope 133
- 4.3 Optical sectioning 146
- 4.4 Imaging living cells and tissues. 151
- 4.5 The stereomicroscope 153
- 4.6 The electron microscope 154
- 4.7 Imaging and biochemistry 159
- 4.8 Specialised imaging techniques 160
- 4.9 Image archiving, presentation and further information 162
- 4.10 Suggestions for further reading 163

5 Molecular biology, bioinformatics and basic techniques 166

R. RAPLEY

- 5.1 Introduction 166
- 5.2 Structure of nucleic acids 167
- 5.3 Genes and genome complexity 174
- 5.4 Location and packaging of nucleic acids 178
- 5.5 Functions of nucleic acids 179
- 5.6 The manipulation of nucleic acids: basic tools and techniques 190
- 5.7 Isolation and separation of nucleic acids 191
- 5.8 Molecular biology and bioinformatics 198
- 5.9 Molecular analysis of nucleic acid sequences 200
- 5.10 The polymerase chain reaction 207
- 5.11 Nucleotide sequencing of DNA 216
- 5.12 Suggestions for further reading 224

6 Recombinant DNA and genetic analysis	225
R. RAPLEY	
6.1 Introduction	225
6.2 Constructing gene libraries	225
6.3 Cloning vectors	235
6.4 Hybridisation and gene probes	253
6.5 Screening gene libraries	255
6.6 Applications of gene cloning	259
6.7 Expression of foreign genes	264
6.8 Analysing genes and gene expression	270
6.9 Analysing whole genomes	283
6.10 Pharmacogenomics	288
6.11 Molecular biotechnology and its applications	289
6.12 Suggestions for further reading	291
7 Immunochemical techniques	292
R. THORPE AND S. THORPE	
7.1 Introduction	292
7.2 Production of antibodies	298
7.3 Purification and fragmentation of immunoglobulins	308
7.4 Immunoprecipitation	315
7.5 Labelling antibodies	320
7.6 Immunoblotting	328
7.7 Immunoassays	331
7.8 Immunohisto/cytochemistry	341
7.9 Affinity and avidity	347
7.10 Immunochemical use of surface plasmon resonance	347
7.11 Suggestions for further reading	348
8 Protein structure, purification, characterisation and function analysis	349
J. M. WALKER	
8.1 Ionic properties of amino acids and proteins	349
8.2 Protein structure	353
8.3 Protein purification	356
8.4 Protein structure determination	378
8.5 Proteomics and protein function	392
8.6 Suggestions for further reading	404

9 Mass spectrometric techniques	405
A. AITKEN	
9.1 Introduction	405
9.2 Ionisation	407
9.3 Mass analysers	413
9.4 Detectors	430
9.5 Structural information by tandem mass spectrometry	430
9.6 Analysing protein complexes	443
9.7 Computing and database analysis	444
9.8 Suggestions for further reading	447
10 Electrophoretic techniques	449
J. M. WALKER	
10.1 General principles	449
10.2 Support media	453
10.3 Electrophoresis of proteins	457
10.4 Electrophoresis of nucleic acids	473
10.5 Capillary electrophoresis	478
10.6 Microchip electrophoresis	483
10.7 Suggestions for further reading	484
11 Chromatographic techniques	485
K. WILSON	
11.1 Principles of chromatography	485
11.2 Chromatographic performance parameters	489
11.3 Liquid chromatography (LPLC and HPLC)	500
11.4 Adsorption chromatography	515
11.5 Partition chromatography	518
11.6 Ion-exchange chromatography	524
11.7 Molecular exclusion (gel filtration) chromatography	529
11.8 Affinity chromatography	533
11.9 Gas-liquid chromatography	541
11.10 Thin-layer (planar) chromatography	546
11.11 Selection of a chromatographic system	549
11.12 Suggestions for further reading	550

12 Spectroscopic techniques: I Atomic and molecular electronic spectroscopy 551

D. B. GORDON

- 12.1 Introduction 551
- 12.2 γ -Ray spectroscopy and γ -ray resonance spectroscopy 554
- 12.3 X-ray spectroscopy 556
- 12.4 Ultraviolet and visible light spectroscopy 557
- 12.5 Spectrofluorimetry 571
- 12.6 Circular dichroism spectroscopy 579
- 12.7 Turbidimetry and nephelometry 583
- 12.8 Luminometry 584
- 12.9 Atomic spectroscopy 586
- 12.10 Lasers 591
- 12.11 Suggestions for further reading 592

13 Spectroscopic techniques: II Vibrational spectroscopy and electron and nuclear spin orientation in magnetic fields 593

D. B. GORDON

- 13.1 Introduction 593
- 13.2 Infrared and Raman spectroscopy 594
- 13.3 Electron spin resonance spectroscopy 596
- 13.4 Nuclear magnetic resonance spectroscopy 603
- 13.5 Suggestions for further reading 620

14 Radioisotope techniques 621

R. J. SLATER

- 14.1 The nature of radioactivity 621
- 14.2 Detection and measurement of radioactivity 628
- 14.3 Other practical aspects of counting radioactivity and analysis of data 651
- 14.4 Inherent advantages and restrictions of radiotracer experiments 655
- 14.5 Safety aspects 656
- 14.6 Applications of radioisotopes in the biological sciences 659
- 14.7 Suggestions for further reading 664

15 Enzymes	<i>665</i>
K. WILSON	
15.1 Characteristics and nomenclature	<i>665</i>
15.2 Analytical methods for the study of enzyme reactions	<i>668</i>
15.3 Enzyme steady-state kinetics	<i>679</i>
15.4 Enzyme active sites and catalytic mechanisms	<i>702</i>
15.5 Control of enzyme activity	<i>709</i>
15.6 Suggestions for further reading	<i>718</i>
16 Cell membrane receptors	<i>719</i>
K. WILSON	
16.1 Receptors for cell signalling	<i>719</i>
16.2 Quantitative aspects of receptor–ligand binding	<i>720</i>
16.3 Techniques for the study of receptor–ligand binding	<i>729</i>
16.4 Molecular structure of receptors	<i>746</i>
16.5 Mechanisms of signal transduction	<i>751</i>
16.6 Receptor desensitisation and trafficking	<i>763</i>
16.7 Suggestions for further reading	<i>767</i>
<i>Index</i>	<i>769</i>

The colour figure section is between pp. 142 and 143.