

Q1.5 PRJ
HD H232

C. Revy. Courtesy of

ience Photo Library,
k Image/Science

urtesy of Science

Evans. Courtesy of

Principles and Techniques of Biochemistry and Molecular Biology

Seventh edition

Edited by

KEITH WILSON AND JOHN WALKER



CONTENTS

| | |
|----------------------------------------------------------------------|----------------|
| <i>Preface to the seventh edition</i> | <i>page</i> xi |
| <i>List of contributors</i> | xiii |
| <i>List of abbreviations</i> | xv |
| | |
| 1 Basic principles | 1 |
| K. WILSON | |
| 1.1 Biochemical and molecular biology studies | 1 |
| 1.2 Units of measurement | 3 |
| 1.3 Weak electrolytes | 6 |
| 1.4 Quantitative biochemical measurements | 16 |
| 1.5 Safety in the laboratory | 35 |
| 1.6 Suggestions for further reading | 37 |
| | |
| 2 Cell culture techniques | 38 |
| A.R. BAYDOUN | |
| 2.1 Introduction | 38 |
| 2.2 The cell culture laboratory and equipment | 39 |
| 2.3 Safety considerations in cell culture | 43 |
| 2.4 Aseptic techniques and good cell culture practice | 44 |
| 2.5 Types of animal cell, characteristics and maintenance in culture | 49 |
| 2.6 Stem cell culture | 61 |
| 2.7 Bacterial cell culture | 68 |
| 2.8 Potential use of cell cultures | 71 |
| 2.9 Suggestions for further reading | 72 |
| | |
| 3 Centrifugation | 73 |
| K. OHLENDIECK | |
| 3.1 Introduction | 73 |
| 3.2 Basic principles of sedimentation | 74 |
| 3.3 Types, care and safety aspects of centrifuges | 79 |
| 3.4 Preparative centrifugation | 86 |
| 3.5 Analytical centrifugation | 95 |
| 3.6 Suggestions for further reading | 99 |

| | | |
|--------------------------------------------------------------------|-----|----------------|
| 4 Microscopy | 100 | 7.3 Immun |
| S. W. PADDOCK | | 7.4 Immun |
| 4.1 Introduction | 100 | 7.5 Lateral |
| 4.2 The light microscope | 103 | 7.6 Epitop |
| 4.3 Optical sectioning | 116 | 7.7 Immu |
| 4.4 Imaging living cells and tissues | 123 | 7.8 Fluore |
| 4.5 Measuring cellular dynamics | 126 | 7.9 Cell a |
| 4.6 The electron microscope (EM) | 129 | 7.10 Immu |
| 4.7 Image archiving | 133 | 7.11 Immu |
| 4.8 Suggestions for further reading | 136 | 7.12 Antib |
| 5 Molecular biology, bioinformatics and basic techniques | 138 | 7.13 Ther |
| R. RAPLEY | | 7.14 The f |
| 5.1 Introduction | 138 | 7.15 Sugg |
| 5.2 Structure of nucleic acids | 139 | |
| 5.3 Genes and genome complexity | 145 | 8 Prote |
| 5.4 Location and packaging of nucleic acids | 149 | and |
| 5.5 Functions of nucleic acids | 152 | J. WA |
| 5.6 The manipulation of nucleic acids – basic tools and techniques | 162 | 8.1 Ionic |
| 5.7 Isolation and separation of nucleic acids | 164 | 8.2 Prote |
| 5.8 Molecular biology and bioinformatics | 170 | 8.3 Prote |
| 5.9 Molecular analysis of nucleic acid sequences | 171 | 8.4 Prote |
| 5.10 The polymerase chain reaction (PCR) | 178 | 8.5 Prote |
| 5.11 Nucleotide sequencing of DNA | 187 | 8.6 Sugg |
| 5.12 Suggestions for further reading | 194 | |
| 6 Recombinant DNA and genetic analysis | 195 | 9 Mas |
| R. RAPLEY | | A. A |
| 6.1 Introduction | 195 | 9.1 Intro |
| 6.2 Constructing gene libraries | 196 | 9.2 Ionic |
| 6.3 Cloning vectors | 206 | 9.3 Masi |
| 6.4 Hybridisation and gene probes | 223 | 9.4 Dete |
| 6.5 Screening gene libraries | 225 | 9.5 Stru |
| 6.6 Applications of gene cloning | 229 | 9.6 Anal |
| 6.7 Expression of foreign genes | 234 | 9.7 Con |
| 6.8 Analysing genes and gene expression | 240 | 9.8 Sug |
| 6.9 Analysing whole genomes | 254 | |
| 6.10 Pharmacogenomics | 259 | 10 Ele |
| 6.11 Molecular biotechnology and applications | 260 | J. V |
| 6.12 Suggestions for further reading | 262 | 10.1 Ge |
| 7 Immunochemical techniques | 263 | 10.2 Su |
| R. BURNS | | 10.3 Ele |
| 7.1 Introduction | 263 | 10.4 Ele |
| 7.2 Making antibodies | 273 | 10.5 Ca |
| | | 10.6 Mi |
| | | 10.7 Su |

| | | |
|-----|----------------------------------------------------------------------------------|-----|
| 100 | 7.3 Immunoassay formats | 283 |
| 100 | 7.4 Immuno microscopy | 291 |
| 103 | 7.5 Lateral flow devices | 291 |
| 116 | 7.6 Epitope mapping | 292 |
| 123 | 7.7 Immunoblotting | 293 |
| 126 | 7.8 Fluorescent activated cell sorting (FACS) | 293 |
| 129 | 7.9 Cell and tissue staining techniques | 294 |
| 133 | 7.10 Immunocapture polymerase chain reaction (PCR) | 295 |
| 136 | 7.11 Immunoaffinity chromatography (IAC) | 295 |
| 138 | 7.12 Antibody-based biosensors | 296 |
| 138 | 7.13 Therapeutic antibodies | 297 |
| 138 | 7.14 The future uses of antibody technology | 299 |
| 138 | 7.15 Suggestions for further reading | 299 |
| 139 | 8 Protein structure, purification, characterisation and function analysis | 300 |
| 145 | J. WALKER | |
| 152 | 8.1 Ionic properties of amino acids and proteins | 300 |
| 162 | 8.2 Protein structure | 304 |
| 164 | 8.3 Protein purification | 307 |
| 170 | 8.4 Protein structure determination | 328 |
| 171 | 8.5 Proteomics and protein function | 340 |
| 178 | 8.6 Suggestions for further reading | 351 |
| 187 | | |
| 194 | 9 Mass spectrometric techniques | 352 |
| 195 | A. AITKEN | |
| 195 | 9.1 Introduction | 352 |
| 196 | 9.2 Ionisation | 354 |
| 206 | 9.3 Mass analysers | 359 |
| 223 | 9.4 Detectors | 377 |
| 225 | 9.5 Structural information by tandem mass spectrometry | 379 |
| 229 | 9.6 Analysing protein complexes | 390 |
| 234 | 9.7 Computing and database analysis | 394 |
| 240 | 9.8 Suggestions for further reading | 397 |
| 254 | 10 Electrophoretic techniques | 399 |
| 259 | J. WALKER | |
| 260 | 10.1 General principles | 399 |
| 262 | 10.2 Support media | 403 |
| 263 | 10.3 Electrophoresis of proteins | 407 |
| 263 | 10.4 Electrophoresis of nucleic acids | 422 |
| 273 | 10.5 Capillary electrophoresis | 427 |
| 273 | 10.6 Microchip electrophoresis | 431 |
| 273 | 10.7 Suggestions for further reading | 432 |

| | | | |
|--------------------------------------------------------------------------------|-----|--------------------------------------------|-----------------|
| 11 Chromatographic techniques | 433 | 15 Enzyme kinetics | K. WILSON |
| K. WILSON | | 15.1 Characteristics of enzymes | 15.1 Charact... |
| 11.1 Principles of chromatography | 433 | 15.2 Enzyme inhibition | 15.2 Enzym... |
| 11.2 Chromatographic performance parameters | 435 | 15.3 Analytical applications | 15.3 Analyt... |
| 11.3 High-performance liquid chromatography | 446 | 15.4 Enzyme regulation | 15.4 Enzym... |
| 11.4 Adsorption chromatography | 453 | 15.5 Control of enzyme activity | 15.5 Contro... |
| 11.5 Partition chromatography | 455 | 15.6 Suggestions for further reading | 15.6 Sugges... |
| 11.6 Ion-exchange chromatography | 459 | | |
| 11.7 Molecular (size) exclusion chromatography | 462 | | |
| 11.8 Affinity chromatography | 465 | | |
| 11.9 Gas chromatography | 470 | | |
| 11.10 Suggestions for further reading | 476 | | |
| 12 Spectroscopic techniques: I Spectrophotometric techniques | 477 | 16 Principles of spectroscopy | J. FYFE |
| A. HOFMANN | | 16.1 Principles of spectroscopy | 16.1 Princi... |
| 12.1 Introduction | 477 | 16.2 Clinical applications | 16.2 Clinic... |
| 12.2 Ultraviolet and visible light spectroscopy | 482 | 16.3 Examination of biological samples | 16.3 Exam... |
| 12.3 Fluorescence spectroscopy | 493 | 16.4 Suggestions for further reading | 16.4 Sugges... |
| 12.4 Luminometry | 507 | | |
| 12.5 Circular dichroism spectroscopy | 509 | | |
| 12.6 Light scattering | 514 | | |
| 12.7 Atomic spectroscopy | 516 | | |
| 12.8 Suggestions for further reading | 519 | | |
| 13 Spectroscopic techniques: II Structure and interactions | 522 | 17 Cell biology | K. WILLIAMS |
| A. HOFMANN | | 17.1 Reception of signals | 17.1 Recep... |
| 13.1 Introduction | 522 | 17.2 Quantification of cellular components | 17.2 Quan... |
| 13.2 Infrared and Raman spectroscopy | 523 | 17.3 Ligand-receptor interactions | 17.3 Ligan... |
| 13.3 Surface plasmon resonance | 527 | 17.4 Mechanisms of signal transduction | 17.4 Mech... |
| 13.4 Electron paramagnetic resonance | 530 | 17.5 Receptor-gated ion channels | 17.5 Rece... |
| 13.5 Nuclear magnetic resonance | 536 | 17.6 Suggestions for further reading | 17.6 Sugges... |
| 13.6 X-ray diffraction | 546 | | |
| 13.7 Small-angle scattering | 549 | | |
| 13.8 Suggestions for further reading | 551 | | |
| 14 Radioisotope techniques | 553 | 18 Drugs and their actions | K. WILLIAMS |
| R.J. SLATER | | 18.1 Human pharmacology | 18.1 Hun... |
| 14.1 Why use a radioisotope? | 553 | 18.2 Drug delivery systems | 18.2 Drug... |
| 14.2 The nature of radioactivity | 554 | 18.3 Drug targets | 18.3 Drug... |
| 14.3 Detection and measurement of radioactivity | 561 | 18.4 Suggestions for further reading | 18.4 Sugges... |
| 14.4 Other practical aspects of counting of radioactivity and analysis of data | 573 | | |
| 14.5 Safety aspects | 577 | | |
| 14.6 Suggestions for further reading | 580 | | |

| | | |
|-----|---------------------------------------------------------------|-----|
| 433 | 15 Enzymes | 581 |
| | K. WILSON | |
| 433 | 15.1 Characteristics and nomenclature | 581 |
| 435 | 15.2 Enzyme steady-state kinetics | 584 |
| 446 | 15.3 Analytical methods for the study of enzyme reactions | 602 |
| 453 | 15.4 Enzyme active sites and catalytic mechanisms | 611 |
| 455 | 15.5 Control of enzyme activity | 615 |
| 459 | 15.6 Suggestions for further reading | 624 |
| 462 | | |
| 465 | 16 Principles of clinical biochemistry | 625 |
| | J. FYFFE AND K. WILSON | |
| 470 | 16.1 Principles of clinical biochemical analysis | 625 |
| 476 | 16.2 Clinical measurements and quality control | 629 |
| 477 | 16.3 Examples of biochemical aids to clinical diagnosis | 640 |
| 477 | 16.4 Suggestions for further reading | 658 |
| 482 | 16.5 Acknowledgements | 659 |
| 493 | | |
| 507 | 17 Cell membrane receptors and cell signalling | 660 |
| | K. WILSON | |
| 509 | 17.1 Receptors for cell signalling | 660 |
| 514 | 17.2 Quantitative aspects of receptor-ligand binding | 663 |
| 516 | 17.3 Ligand-binding and cell-signalling studies | 680 |
| 519 | 17.4 Mechanisms of signal transduction | 685 |
| 522 | 17.5 Receptor trafficking | 703 |
| | 17.6 Suggestions for further reading | 707 |
| 522 | | |
| 523 | 18 Drug discovery and development | 709 |
| | K. WILSON | |
| 527 | 18.1 Human disease and drug therapy | 709 |
| 530 | 18.2 Drug discovery | 718 |
| 536 | 18.3 Drug development | 727 |
| 546 | 18.4 Suggestions for further reading | 734 |
| 549 | | |
| 551 | <i>Index</i> | 736 |
| 553 | <i>The colour figure section is between pages 128 and 129</i> | |
| 553 | | |
| 554 | | |
| 561 | | |
| 573 | | |
| 577 | | |
| 580 | | |