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Edited by Keith Wilson and John Walker
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Principles and Techniques of

Biochemistry and Molecular Biology

Seventh edition

EDITED BY KEITH WILSON AND JOHN WALKER

This new edition of the bestselling textbook integrates the theoretical principles and experimental techniques common to all undergraduate courses in the bio- and medical sciences. Three of the 16 chapters have new authors and have been totally rewritten. The others have been updated and extended to reflect developments in their field exemplified by a new section on stem cells. Two new chapters have been added. One on clinical biochemistry discusses the principles underlying the diagnosis and management of common biochemical disorders. The second one on drug discovery and development illustrates how the principles and techniques covered in the book are fundamental to the design and development of new drugs. In-text worked examples are again used to enhance student understanding of each topic and case studies are selectively used to illustrate important examples. Experimental design, quality assurance and the statistical analysis of quantitative data are emphasised throughout the book.

- Motivates students by including cutting-edge topics and techniques, such as drug discovery, as well as the methods they will encounter in their own lab classes
- Promotes problem solving by setting students a challenge and then guiding them through the solution
- Integrates theory and practise to ensure students understand why and how each technique is used.

KEITH WILSON is Professor Emeritus of Pharmacological Biochemistry and former Head of the Department of Biosciences, Dean of the Faculty of Natural Sciences, and Director of Research at the University of Hertfordshire.

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Cover illustration

Main image Electrophoresis gel showing recombinant protein. Photographer: J.C. Revy. Courtesy of Science Photo Library.

Top inset Transcription factor and DNA molecule. Courtesy of: Laguna Design/Science Photo Library.

Second inset Microtubes, pipettor (pipette) tip & DNA sequence. Courtesy of Tek Image/Science Photo Library.

Third inset Stem cell culture, light micrograph. Photographer: Philippe Plailly. Courtesy of Science Photo Library.

Fourth inset Embryonic stem cells. Courtesy of Science Photo Library.

Bottom inset Herceptin breast cancer drug, molecular model. Photographer: Tim Evans. Courtesy of Science Photo Library.

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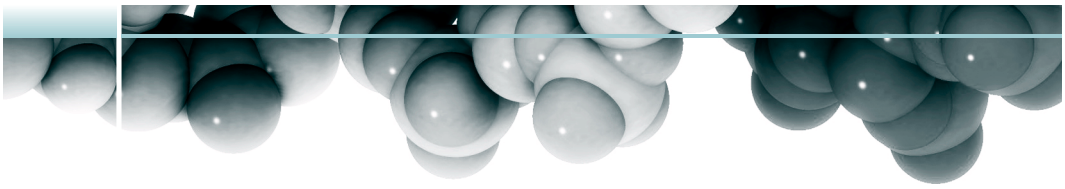

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PREFACE TO THE SEVENTH EDITION

In designing the content of this latest edition we continued our previous policy of placing emphasis on the recommendations we have received from colleagues and academics outside our university. Above all, we have attempted to respond to the invaluable feedback from student users of our book both in the UK and abroad. In this seventh edition we have retained all 16 chapters from the previous edition. All have been appropriately updated to reflect recent developments in their fields, as exemplified by the inclusion of a section on stem cells in the cell culture chapter. Three of these chapters have new authors and have been completely rewritten. Robert Burns, Scottish Agricultural Science Agency, Edinburgh has written the chapter on immunochemical techniques, and Andreas Hofmann, Eskitis Institute of Molecular Therapies, Griffith University, Brisbane, Australia has written the two chapters on spectroscopic techniques. We are delighted to welcome both authors to our team of contributors.

In addition to these changes of authors, two new chapters have been added to the book. Our decision taken for the sixth edition to include a section on the biochemical principles underlying clinical biochemistry has been well received and so we have extended our coverage of the subject and have devoted a whole chapter (16) to this subject. Written in collaboration with Dr John Fyffe, Consultant Biochemist, Royal Hospital for Sick Children, Yorkhill, Glasgow, new topics that are discussed in the chapter include the diagnosis and management of kidney disease, diabetes, endocrine disorders including thyroid dysfunction, conditions of the hypothalamus–pituitary–adrenal axis such as pregnancy, and pathologies of plasma proteins such as myeloma. Case studies are included to illustrate how the principles discussed apply to the diagnosis and treatment of individual patients with the conditions.

Our second major innovation for this new edition is the introduction of a new chapter on drug discovery and development. The strategic approaches to the discovery of new drugs has been revolutionised by developments in molecular biology. Pharmaceutical companies now rely on many of the principles and experimental techniques discussed in the chapters throughout the book to identify potential drug targets, screen chemical libraries and to evaluate the safety and efficacy of selected candidate drugs. The new chapter illustrates the principles of target selection by reference to current drugs used in the treatment of atherosclerosis and HIV/AIDS, emphasises the strategic decisions to be taken during the various stages of drug discovery and

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development and discusses the issues involved in clinical trials and the registration of new drugs.

We continue to welcome constructive comments from all students who use our book as part of their studies and academics who adopt the book to complement their teaching. Finally, we wish to express our gratitude to the authors and publishers who have granted us permission to reproduce their copyright figures and our thanks to Katrina Halliday and her colleagues at Cambridge University Press who have been so supportive in the production of this new edition.

KEITH WILSON AND JOHN WALKER

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ABBREVIATIONS

The following abbreviations have been used throughout this book.

AMP	adenosine 5′-monophosphate
ADP	adenosine 5′-diphosphate
ATP	adenosine 5′-triphosphate
bp	base-pairs
cAMP	cyclic AMP
CHAPS	3-[(3-chloroamidopropyl)dimethylamino]-1-propanesulphonic acid
c.p.m.	counts per minute
CTP	cytidine triphosphate
DDT	2,2-bis-(*p*-chlorophenyl)-1,1,1-trichloroethane
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
e^-	electron
EDTA	ethylenediaminetetra-acetate
ELISA	enzyme-linked immunosorbent assay
FAD	flavin adenine dinucleotide (oxidised)
$FADH_2$	flavin adenine dinucleotide (reduced)
FMN	flavin mononucleotide (oxidised)
$FMNH_2$	flavin mononucleotide (reduced)
GC	gas chromatography
GTP	guanosine triphosphate
HAT	hypoxanthine, aminopterin, thymidine medium
Hepes	4(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid
HPLC	high-performance liquid chromatography
kb	kilobase-pairs
M_r	relative molecular mass
min	minute
NAD^+	nicotinamide adenine dinucleotide (oxidised)
NADH	nicotinamide adenine dinucleotide (reduced)
$NADP^+$	nicotinamide adenine dinucleotide phosphate (oxidised)
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
Pipes	1,4-piperazinebis(ethanesulphonic acid)

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P_i	inorganic phosphate
p.p.m.	parts per million
p.p.b.	parts per billion
PP_i	inorganic pyrophosphate
RNA	ribonucleic acid
r.p.m.	revolutions per minute
SDS	sodium dodecyl sulphate
Tris	2-amino-2-hydroxymethylpropane-1,3-diol

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