

The Protein Protocols Handbook

Edited by

John M. Walker



HUMANA PRESS

The Protein Protocols Handbook

Edited by

John M. Walker

University of Hertfordshire, Hatfield, UK

HUMANA PRESS  TOTOWA, NEW JERSEY

© 1996 Humana Press Inc.
999 Riverview Drive, Suite 208
Totowa, New Jersey 07512

For additional copies, pricing for bulk purchases, and/or other information about other Humana titles, contact Humana at the above address or at any of the following numbers: Tel.: 201-256-1699; Fax: 201-256-8341; E-mail: humana@interramp.com

All rights reserved. No part of this book may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, microfilming, recording, or otherwise without written permission from the Publisher. Methods in molecular biology™ is a trademark of the Humana Press Inc.

All authored papers, comments, opinions, conclusions, or recommendations are those of the author(s), and do not necessarily reflect the views of the publisher.

This publication is printed on acid-free paper.  ANSI Z39.48-1984 (American Standards Institute) Permanence of Paper for Printed Library Materials.

Photocopy Authorization Policy:

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by Humana Press Inc., provided that the base fee of US \$5.00 per copy, plus US \$0.25 per page, is paid directly to the Copyright Clearance Center at 222 Rosewood Drive, Danvers, MA 01923. For those organizations that have been granted a photocopy license from the CCC, a separate system of payment has been arranged and is acceptable to Humana Press Inc. The fee code for users of the Transactional Reporting Service is: [0-89603-338-4/96 \$5.00 + \$0.25].

Printed in the United States of America. 10 9 8 7 6 5 4 3 2

ISBN 0-89603-338-4 (hardcover)
ISBN 0-89603-339-2 (combbound)

The Protein Protocols Handbook

Preface

In *The Protein Protocols Handbook*, I have attempted to provide a cross-section of analytical techniques commonly used for proteins and peptides, thus providing a benchtop manual and guide both for those who are new to the protein chemistry laboratory and for those more established workers who wish to use a technique for the first time.

We each, of course, have our own favorite, commonly used gel system, gel-staining method, blotting method, and so on; I'm sure you will find yours here. However, I have also described a variety of alternatives for many of these techniques; though they may not be superior to the methods you commonly use, they may nevertheless be more appropriate in a particular situation. Only by knowing the range of techniques that are available to you, and the strengths and limitations of these techniques, will you be able to choose the method that best suits your purpose.

All chapters are written in the same format as that used in the *Methods in Molecular Biology* series. Each chapter opens with a description of the basic theory behind the method being described. The Materials section lists all the chemicals, reagents, buffers, and other materials necessary for carrying out the protocol. Since the principal goal of the book is to provide experimentalists with a full account of the practical steps necessary for carrying out each protocol successfully, the Methods section contains detailed step-by-step descriptions of every protocol that should result in the successful execution of each method. The Notes section complements the Methods material by indicating how best to deal with any problem or difficulty that may arise when using a given technique, and how to go about making the widest variety of modifications or alterations to the protocol.

In general, I have avoided analytical techniques that require expensive specialist hardware. Such techniques are described in specialist volumes in the *Methods in Molecular Biology* series (vol. 60, *Protein NMR Techniques*; vol. 56, *Crystallographic Methods and Protocols*; vol. 52, *Capillary Electrophoresis Guidebook*; vol. 40, *Protein Stability and Folding*; vol. 22, *Microscopy, Optical Microscopy, and Macroscopic Techniques*; vol. 17, *Spectroscopic Methods and Analyses*). The main exception has been the introduction of some techniques that involve the use of mass spectrometry. The recent availability of benchtop machines has made this technique available to a much wider range of workers than might previously have been possible, and thus mass spectrometry is fast becoming a routine analytical method for the protein chemist. However, for those who require a more detailed and in-depth description of the exciting new applications of this technique to the analysis of proteins and peptides, extensive coverage is provided in *Methods in Molecular Biology*, vol. 61, *Protein and Peptide Analysis by Mass Spectrometry*. For those of you who require guidance on protein and peptide purification, the subject is extensively covered in *Methods in Molecular Biology*, vol. 59, *Protein Purification Protocols*.

John M. Walker

EVERYTHING IS POSSIBLE...WITH THE RIGHT TOOLS.™



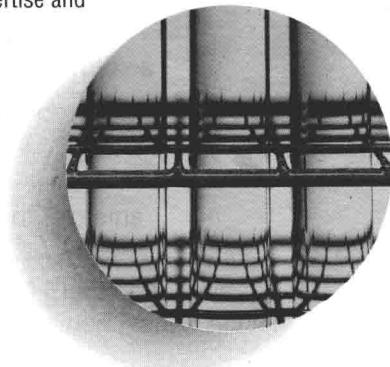
Protein Chemistry

Your source
of innovative,
dependable tools
for the
advanced
study of
proteins for
nearly 50 years.

From protein preparation,
quantitation and detection to
protein modification and conjugate

formation, Pierce is your resource for high-quality reagents and kits. For dependable, repeatable results, to make your work easier and for the best in technical expertise and guidance, there's only one name you need to know. Pierce.

- Protein Assay Reagents and Kits
- Cross-Linkers (Double-Agents™)
- Purified Detergents
- Protein Modification Products
 - Iodination
 - Reducing Agents
 - Proteases
 - Coupling Reagents
 - Buffers/Denaturants



Call 1-800-874-3723 for product information or 815-968-0747 for the name of your local distributor.



PIERCE

Contributors

- ALASTAIR AITKEN • *National Institute for Medical Research, Mill Hill, London, UK*
ROBERT E. AKINS • *Department of Medical Cell Biology, Nemours Research Program, AI duPont Institute, Wilmington, DE*
SALLY ANN AMERO • *Department of Biology, Washington University, St. Louis, MO*
GRAHAM S. BAILEY • *Department of Biological and Chemical Sciences, University of Essex, Colchester, UK*
SALVADOR BARTOLOMÉ • *Department de Bioquímica i Biología Molecular, Universitat Autònoma de Barcelona, Bellaterra (Barcelona), Spain*
DAVID J. BEGLEY • *Biomedical Sciences Division, King's College, London, UK*
ANTONIO BERMÚDEZ • *Department de Bioquímica i Biología Molecular, Universitat Autònoma de Barcelona, Bellaterra (Barcelona), Spain*
MAHESH K. BHALGAT • *Molecular Probes Inc., Eugene, OR*
SYLVIE BOURASSA • *Eastern Quebec Peptide Sequencing Facility, CHUL Research Center and Laval University, Ste-Foy, Québec, Canada*
J. MARK CARTER • *Cytogen, Princeton, NJ*
FRANCA CASAGRANDA • *CSIRO Division of Biomolecular Engineering, Victoria, Australia; Present address: European Molecular Biology Laboratory, Heidelberg, Germany*
JUNG-KAP CHOI • *College of Pharmacy, Chonnam National University, Kwangju, Korea*
ANTONELLA CIRCOLO • *Maxwell Finland Laboratory for Infectious Diseases, Boston City Hospital, Boston University School of Medicine, Boston, MA*
JOHN COLYER • *Department of Biochemistry and Molecular Biology, University of Leeds, UK*
JOAN-RAMON DABAN • *Department de Bioquímica i Biología Molecular, Universitat Autònoma de Barcelona, Bellaterra (Barcelona), Spain*
JAMES R. DAVIE • *Department of Biochemistry and Molecular Biology, University of Manitoba, Winnipeg, Manitoba, Canada*
MICHAEL J. DAVIES • *Department of Biochemistry and Molecular Biology, University College London, UK*
GENEVIÈVE P. DELCUVE • *Department of Biochemistry and Molecular Biology, University of Manitoba, Winnipeg, Manitoba, Canada*
SERGE DESNOYERS • *Eastern Quebec Peptide Sequencing Facility, CHUL Research Center and Laval University, Ste-Foy, Québec, Canada*
MONIQUE DIANO • *Lab de Génétique, CNRS, Marseille, France*
MICHAEL J. DUNN • *Department of Cardiothoracic Surgery, National Heart and Lung Institute, Heart Science Centre, Harefield Hospital, Middlesex, UK*
SARAH C. R. ELGIN • *Department of Biology, Washington University, St. Louis, MO*

- JOSEPH FERNANDEZ • *Protein/DNA Technology Center, The Rockefeller University, New York*
- MERCEDES FERRERAS • *Departimiento de Bioquímica y Biología Molecular, Universidad Complutense, Madrid, Spain*
- SUSAN J. FOWLER • *Amersham International plc., Amersham, UK*
- THOMAS D. FRIEDRICH • *Department of Microbiology, Immunology, and Molecular Genetics, The Albany Medical College, Albany, NY*
- JUAN M. GARCÍA-SEGURA • *Departimiento de Bioquímica y Biología Molecular, Universidad Complutense, Madrid, Spain*
- OLIVIER GOLAZ • *Central Clinical Chemistry Laboratory, University Hospital of Geneva, Switzerland*
- MOHAMMAD T. GOODARZI • *Department of Clinical Biochemistry and Metabolic Medicine, The Medical School, University of Newcastle, UK*
- MORAG A. GRASSIE • *Division of Biochemistry and Molecular Biology, Institute of Biomedical and Life Sciences, University of Glasgow, Scotland, UK*
- PATRICIA GRAVEL • *Clinical Research Unit, Psychiatric Institutions of Geneva, Switzerland*
- SUNITA GULATI • *Maxwell Finland Laboratory for Infectious Diseases, Boston City Hospital, Boston University School of Medicine, Boston, MA*
- GRAEME R. GUY • *Institute of Molecular and Cell Biology, National University of Singapore*
- ROSARIA P. HAUGLAND • *Molecular Probes Inc., Eugene, OR*
- LUCY F. HENLEY • *Division of Life Sciences, King's College, University of London, UK*
- HEE-YOUN HONG • *College of Pharmacy, Chonnam National University, Kwangju, Korea*
- MARTIN HORST • *Department of Biochemistry, Biozentrum of the University of Basel, Switzerland*
- ELIZABETH F. HOUNSELL • *Department of Biochemistry and Molecular Biology, University College London, UK*
- G. BRENT IRVINE • *School of Biology and Biochemistry, Queen's University of Belfast, Ireland*
- THARAPPEL C. JAMES • *Department of Biology, Washington University, St. Louis, MO*
- PAUL JENÖ • *Department of Biochemistry, Biozentrum of the University of Basel, Switzerland*
- RALPH C. JUDD • *Division of Biological Sciences, University of Montana, Missoula, MT*
- NICHOLAS J. KRUGER • *Department of Plant Sciences, University of Oxford, UK*
- JUDITH LAFFIN • *Department of Microbiology, Immunology, and Molecular Genetics, The Albany Medical College, Albany, NY*
- WILLIAM J. LAROCHELLE • *Laboratory of Cellular and Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD*
- MICHELE LEARMONTH • *National Institute for Medical Research, Mill Hill, London, UK*
- ANDRÉ LE BIVIC • *Lab de Génétique, CNRS, Marseille, France*
- JOHN M. LEHMAN • *Department of Microbiology, Immunology, and Molecular Genetics, The Albany Medical College, Albany, NY*
- FAN LIN • *Department of Anatomy and Cell Biology, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA*

- PHILIP S. LOW • *Department of Chemistry, Purdue University, West Lafayette, IN*
- HARRY R. MATTHEWS • *Department of Biological Chemistry, University of California, Davis, CA*
- PHILIP N. MCFADDEN • *Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR*
- GRAEME MILLIGAN • *Division of Biochemistry and Molecular Biology, Institute of Biomedical and Life Sciences, University of Glasgow, Scotland, UK*
- SHEENAH M. MISCHE • *Protein/DNA Technology Center, The Rockefeller University, New York*
- TSUGUO MIZUOCHI • *Department of Biochemistry and Molecular Biology, University College London, UK*
- HOLGER J. MØLLER • *Department of Clinical Biochemistry, KH University Hospital, Nørrebrogade, Århus, Denmark*
- GLENN E. MORRIS • *MRIC, NE Wales Institute, Deeside, Clwyd, UK*
- ULF NEUMANN • *Medizinische Hochschule Hannover, Zentrum Biochem/Physiologische Chem, Hannover, Germany*
- NGUYEN THI MAN • *MRIC, NE Wales Institute, Deeside, Clwyd, UK*
- MARK PAGE • *MEDEVA, Vaccine Research Unit, Department of Biochemistry, Imperial College of Science, Technology, and Medicine, London, UK*
- ROBIN J. PHILP • *Institute of Molecular and Cell Biology, National University of Singapore*
- GUY G. POIRIER • *Eastern Quebec Peptide Sequencing Facility, CHUL Research Center and Laval University, Ste-Foy, Québec, Canada*
- JEFFREY W. POLLARD • *Departments of Developmental and Molecular Biology/Obstetrics and Gynecology, Albert Einstein College of Medicine, Bronx, NY*
- JORGEN H. POULSEN • *Department of Clinical Biochemistry, KH University Hospital, Nørrebrogade, Århus, Denmark*
- THOMAS J. PRITCHETT • *Beckman Instruments, Fullerton, CA*
- F. ANDREW RAY • *Department of Microbiology, Immunology, and Molecular Genetics, The Albany Medical College, Albany, NY*
- DOUGLAS D. ROOT • *Department of Chemistry and Biochemistry, University of Texas at Austin, TX*
- JOHN RUSH • *Biotechnology Resource Laboratory, Yale University, New Haven, CT*
- SUZY M. SAMANDAR • *Biotechnology Resource Laboratory, Yale University, New Haven, CT*
- MELISSA SAYLOR • *Biotechnology Resource Laboratory, Yale University, New Haven, CT*
- BRYAN JOHN SMITH • *Celltech Therapeutics Ltd., Slough, UK*
- KEVIN D. SMITH • *Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow, Scotland, UK*
- WAYNE R. SPRINGER • *Medical Center, Department of Veterans Affairs, San Diego, CA*
- KATHRYN L. STONE • *Biotechnology Resource Laboratory, Yale University, New Haven, CT*
- DONALD F. SUMMERS • *Department of Microbiology and Molecular Genetics, University of California, College of Medicine, Irvine, CA*
- PATRICIA J. SWEENEY • *Division of Biosciences, University of Hertfordshire, Hatfield, UK*

- BOGUSLAW SZEWczyk • *Department of Microbiology and Molecular Genetics, College of Medicine, University of California, Irvine, CA*
- DAN S. TAWFIK • *Department of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel; Present address: Centre for Protein Engineering, Medical Research Council Centre, Cambridge, UK*
- ROBIN THORPE • *National Institute for Biological Standards and Control, Potters Bar, UK*
- CHRISTOPHER F. THURSTON • *Division of Life Sciences, King's College, University of London, UK*
- ROCKY S. TUAN • *Department of Orthopedic Surgery, Thomas Jefferson University, Philadelphia, PA*
- GRAHAM A. TURNER • *Department of Clinical Biochemistry and Metabolic Medicine, The Medical School, University of Newcastle, UK*
- JOHN M. WALKER • *Division of Biosciences, University of Hertfordshire, Hatfield, UK*
- KUAN WANG • *Department of Chemistry and Biochemistry, University of Texas at Austin, TX*
- MALCOM WARD • *Structural Chemistry Department, Glaxo Research and Development, Hertfordshire, UK*
- JAKOB H. WATERBORG • *School of Biological Sciences, University of Missouri-Kansas City, Kansas City, MO*
- DARIN J. WEBER • *Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR*
- KENNETH R. WILLIAMS • *Biotechnology Resource Laboratory, Yale University, New Haven, CT*
- JOHN F. K. WILSHIRE • *CSIRO Division of Biomolecular Engineering, Victoria, Australia*
- JULIA S. WINDER • *Division of Biosciences, University of Hertfordshire, Hatfield, UK*
- G. BRIAN WISDOM • *Division of Biochemistry, School of Biology and Biochemistry, The Queen's University, Medical Biology Centre, Belfast, UK*
- GARY E. WISE • *Department of Anatomy and Cell Biology, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA*
- GYURNG-SOO YOO • *College of Pharmacy, Chonnam National University, Kwangju, Korea*
- WENDY W. YOU • *Molecular Probes Inc., Eugene, OR*
- JIE YUAN • *Department of Chemistry, Purdue University, West Lafayette, IN*

Contents

Preface	v
Contributors	xv
PART I: QUANTITATION OF PROTEINS	
1 Protein Determination by UV Absorption <i>Alastair Aitken and Michèle Learmonth</i>	3
2 The Lowry Method for Protein Quantitation <i>Jakob H. Waterborg and Harry R. Matthews</i>	7
3 The Bicinchoninic Acid (BCA) Assay for Protein Quantitation <i>John M. Walker</i>	11
4 The Bradford Method for Protein Quantitation <i>Nicholas J. Kruger</i>	15
5 Ultrafast Protein Determinations Using Microwave Enhancement <i>Robert E. Akins and Rocky S. Tuan</i>	21
6 Quantitation of Tryptophan in Proteins <i>Alastair Aitken and Michèle Learmonth</i>	29
7 Protein Quantitation Using Flow Cytometry <i>F. Andrew Ray, Thomas D. Friedrich, Judith Laffin, and John M. Lehman</i>	33
8 Copper Iodide Staining of Proteins on Solid Phases <i>Douglas D. Root and Kuan Wang</i>	39
9 Kinetic Silver Staining of Proteins Adsorbed to Microtiter Plates <i>Douglas D. Root and Kuan Wang</i>	45
PART II: ELECTROPHORESIS OF PROTEINS AND PEPTIDES AND DETECTION IN GELS	
10 Nondenaturing Polyacrylamide Gel Electrophoresis of Proteins <i>John M. Walker</i>	51
11 SDS Polyacrylamide Gel Electrophoresis of Proteins <i>John M. Walker</i>	55
12 Gradient SDS Polyacrylamide Gel Electrophoresis of Proteins <i>John M. Walker</i>	63
13 Cetyltrimethylammonium Bromide Discontinuous Gel Electrophoresis of Proteins: M_r -Based Separation of Proteins with Retained Native Activity <i>Robert E. Akins and Rocky S. Tuan</i>	67
14 Acetic Acid-Urea Polyacrylamide Gel Electrophoresis of Basic Proteins <i>Jakob H. Waterborg</i>	83

15	Acid-Urea-Triton Polyacrylamide Gels for Histones <i>Jakob H. Waterborg</i>	91
16	SDS-Polyacrylamide Gel Electrophoresis of Peptides <i>Ralph C. Judd</i>	101
17	Isoelectric Focusing of Proteins in Ultra-Thin Polyacrylamide Gels <i>John M. Walker</i>	109
18	Isoelectric Focusing Under Denaturing Conditions <i>Christopher F. Thurston and Lucy F. Henley</i>	115
19	Radioisotopic Labeling of Proteins for Polyacrylamide Gel Electrophoresis <i>Jeffrey W. Pollard</i>	121
20	Two-Dimensional PAGE Using Carrier Ampholyte pH Gradients in the First Dimension <i>Patricia Gravel and Olivier Golaz</i>	127
21	Two-Dimensional PAGE Using Immobilized pH Gradients <i>Graeme R. Guy and Robin J. Philp</i>	133
22	Two-Dimensional PAGE Using Flat-Bed IEF in the First Dimension <i>Robin J. Philp</i>	139
23	Free Zone Capillary Electrophoresis <i>David J. Begley</i>	143
24	Capillary Isoelectric Focusing with Electro-Osmotic Flow Mobilization <i>Thomas J. Pritchett</i>	155
25	Quantification of Radiolabeled Proteins in Polyacrylamide Gels <i>Wayne R. Springer</i>	161
26	Quantification of Proteins by Staining in Polyacrylamide Gels <i>Bryan John Smith</i>	167
27	Quantitation of Proteins Separated by Electrophoresis Using Coomassie Brilliant Blue <i>Ulf Neumann</i>	173
28	Rapid Staining of Proteins in Polyacrylamide Gels with Nile Red <i>Joan-Ramon Daban, Salvador Bartolomé, and Antonio Bermúdez</i>	179
29	Zn ²⁺ -Reverse Staining of Proteins in Polyacrylamide Gels <i>Juan M. García-Segura and Mercedes Ferreras</i>	187
30	Protein Staining with Calconcarboxylic Acid in Polyacrylamide Gels <i>Jung-Kap Choi, Hee-Youn Hong, and Gyurng-Soo Yoo</i>	197
31	Detection of Proteins and Sialoglycoproteins in Polyacrylamide Gels Using Eosin Y Stain <i>Fan Lin and Gary E. Wise</i>	203
32	Electroelution of Proteins from Polyacrylamide Gels <i>Paul Jenö and Martin Horst</i>	207
33	High-Performance Electrophoresis Chromatography <i>Serge Desnoyers, Sylvie Bourassa, and Guy G. Poirier</i>	215
34	Drying Polyacrylamide Gels <i>Bryan John Smith</i>	223

35	Detection of Proteins in Polyacrylamide Gels by Silver Staining <i>Michael J. Dunn</i>	229
36	Autoradiography and Fluorography of Acrylamide Gels <i>Antonella Circolo and Sunita Gulati</i>	235
PART III: BLOTTING AND DETECTION METHODS		
37	Protein Blotting by Electroblotting <i>Mark Page and Robin Thorpe</i>	245
38	Protein Blotting by the Semidry Method <i>Patricia Gravel and Olivier Golaz</i>	249
39	Protein Blotting by the Capillary Method <i>John M. Walker</i>	261
40	Protein Blotting of Basic Proteins Resolved on Acid-Urea-Triton-Polyacrylamide Gels <i>Geneviève P. Delcuve and James R. Davie</i>	263
41	Alkaline Phosphatase Labeling of Antibody Using Glutaraldehyde <i>G. Brian Wisdom</i>	269
42	β -Galactosidase Labeling of Antibody Using MBS <i>G. Brian Wisdom</i>	271
43	Horseradish Peroxidase Labeling of Antibody Using Periodate Oxidation <i>G. Brian Wisdom</i>	273
44	Protein Staining and Immunodetection Using Colloidal Gold <i>Susan J. Fowler</i>	275
45	Fluorescent Protein Staining on Nitrocellulose with Subsequent Immunodetection of Antigen <i>Donald F. Summers and Boguslaw Szewczyk</i>	289
46	Coupling of Antibodies with Biotin <i>Rosaria P. Haugland and Wendy W. You</i>	293
47	Preparation of Avidin Conjugates <i>Rosaria P. Haugland and Mahesh K. Bhalgat</i>	303
48	Detection of Polypeptides on Blots Using Secondary Antibodies or Protein A <i>Nicholas J. Kruger</i>	313
49	Detection of Proteins on Blots Using Avidin- or Streptavidin-Biotin <i>William J. LaRochelle</i>	323
50	Detection of Proteins on Blots Using Chemiluminescent Systems <i>Graeme R. Guy</i>	329
PART IV: CHEMICAL MODIFICATION OF PROTEINS AND PEPTIDE PRODUCTION AND PURIFICATION		
51	Carboxymethylation of Cysteine Using Iodoacetamide/Iodoacetic Acid <i>Alastair Aitken and Michèle Learmonth</i>	339
52	Performic Acid Oxidation <i>Alastair Aitken and Michèle Learmonth</i>	341

53	Succinylation of Proteins <i>Alastair Aitken and Michèle Learmonth</i>	343
54	Pyridylethylation of Cysteine Residues <i>Malcolm Ward</i>	345
55	Side-Chain Selective Chemical Modifications of Proteins <i>Dan S. Tawfik</i>	349
56	Nitration of Tyrosines <i>Dan S. Tawfik</i>	353
57	Ethoxyformylation of Histidine <i>Dan S. Tawfik</i>	357
58	Modification of Arginine Side Chains with <i>p</i> -Hydroxyphenylglyoxal <i>Dan S. Tawfik</i>	359
59	Amidation of Carboxyl Groups <i>Dan S. Tawfik</i>	361
60	Amidination of Lysine Side Chains <i>Dan S. Tawfik</i>	363
61	Modification of Tryptophan with 2-Hydroxy-5-Nitrobenzylbromide <i>Dan S. Tawfik</i>	365
62	Modification of Sulphydryl Groups with DTNB <i>Dan S. Tawfik</i>	367
63	Chemical Cleavage of Proteins at Methionyl Residues <i>Bryan John Smith</i>	369
64	Chemical Cleavage of Proteins at Tryptophan Residues <i>Bryan John Smith</i>	375
65	Chemical Cleavage of Proteins at Aspartyl Residues <i>Bryan John Smith</i>	381
66	Chemical Cleavage of Proteins at Cysteinyl Residues <i>Bryan John Smith</i>	385
67	Chemical Cleavage of Proteins at Asparaginyl-Glycyl Peptide Bonds <i>Bryan John Smith</i>	389
68	Preparation of Peptides for Microsequencing from Proteins in Polyacrylamide Gels <i>Robin J. Philp</i>	393
69	<i>In Situ</i> Chemical and Enzymatic Digestions of Proteins Immobilized on Miniature Hydrophobic Columns <i>Malcolm Ward</i>	399
70	Enzymatic Digestion of Membrane-Bound Proteins for Peptide Mapping and Internal Sequence Analysis <i>Joseph Fernandez and Sheenah M. Mische</i>	405
71	Enzymatic Digestion of Proteins in Solution and in SDS Polyacrylamide Gels <i>Kathryn L. Stone and Kenneth R. Williams</i>	415
72	Reverse-Phase HPLC Separation of Enzymatic Digests of Proteins <i>Kathryn L. Stone and Kenneth R. Williams</i>	427

PART V: PROTEIN/PEPTIDE CHARACTERIZATION

73	Peptide Mapping by Two-Dimensional Thin-Layer Electrophoresis—Thin-Layer Chromatography <i>Ralph C. Judd</i>	437
74	Peptide Mapping by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis <i>Ralph C. Judd</i>	447
75	Peptide Mapping by High-Performance Liquid Chromatography <i>Ralph C. Judd</i>	453
76	Production of Protein Hydrolysates Using Enzymes <i>John M. Walker and Patricia J. Sweeney</i>	457
77	Amino Acid Analysis Using Precolumn Derivatization with 6-Aminoquinolyl- <i>n</i> -Hydroxysuccinimidyl Carbamate <i>Malcolm Ward</i>	461
78	Amino Acid Analysis Using Precolumn Derivatization with Phenylisothiocyanate <i>G. Brent Irvine</i>	467
79	Molecular-Weight Estimation for Native Proteins Using Size-Exclusion High-Performance Liquid Chromatography <i>G. Brent Irvine</i>	473
80	Detection of Disulfide-Linked Peptides by HPLC <i>Alastair Aitken and Michèle Learmonth</i>	479
81	Diagonal Electrophoresis for Detecting Disulfide Bridges <i>Alastair Aitken and Michèle Learmonth</i>	481
82	Estimation of Disulfide Bonds Using Ellman's Reagent <i>Alastair Aitken and Michèle Learmonth</i>	487
83	Quantitation of Cysteine Residues and Disulfide Bonds by Electrophoresis <i>Alastair Aitken and Michèle Learmonth</i>	489
84	Detection of Disulfide-Linked Peptides by Mass Spectrometry <i>Alastair Aitken and Michèle Learmonth</i>	495
85	Analyzing Protein Phosphorylation <i>John Colyer</i>	501
86	Identification of Proteins Modified by Protein (D-Aspartyl/L-Isoaspartyl) Carboxyl Methyltransferase <i>Darin J. Weber and Philip N. McFadden</i>	507
87	Analysis of Protein Palmitoylation <i>Morag A. Grassie and Graeme Milligan</i>	517
88	Removal of Pyroglutamic Acid Residues from the N-Terminus of Peptides and Proteins <i>John M. Walker and Patricia J. Sweeney</i>	525
89	The Dansyl Method for Identifying N-Terminal Amino Acids <i>John M. Walker</i>	529
90	The Dansyl-Edman Method for Peptide Sequencing <i>John M. Walker</i>	535

91	Matrix-Assisted Laser Desorption Ionization Mass Spectrometry as a Complement to Internal Protein Sequencing <i>Kenneth R. Williams, Suzy M. Samandar, Kathryn L. Stone, Melissa Saylor, and John Rush</i>	541
92	A Manual C-Terminal Sequencing Procedure for Peptides: <i>The Thiocyanate Degradation Method</i> <i>Franca Casagranda and John F. K. Wilshire</i>	557
93	C-Terminal Sequence Analysis with Carboxypeptidase Y <i>John M. Walker and Julia S. Winder</i>	569
94	Rapid Epitope Mapping by Carboxypeptidase Digestion and Immunoblotting <i>Philip S. Low and Jie Yuan</i>	573
95	Epitope Mapping of a Protein Using the Geysen (PEPSCAN) Procedure <i>J. Mark Carter</i>	581
96	Epitope Mapping of Protein Antigens by Competition ELISA <i>Glenn E. Morris</i>	595

PART VI: GLYCOPROTEINS

97	Identification of Glycoproteins on Nitrocellulose Membranes Using Lectin Blotting <i>Patricia Gravel and Olivier Golaz</i>	603
98	A Lectin-Binding Assay for the Rapid Characterization of the Glycosylation of Purified Glycoproteins <i>Mohammad T. Goodarzi and Graham A. Turner</i>	619
99	Staining of Glycoproteins/Proteoglycans on SDS-Gels <i>Holger J. Møller and Jørgen H. Poulsen</i>	627
100	Chemical Methods of Analysis of Glycoproteins <i>Elizabeth F. Hounsell, Michael J. Davies, and Kevin D. Smith</i>	633
101	Monosaccharide Analysis by HPAEC <i>Elizabeth F. Hounsell, Michael J. Davies, and Kevin D. Smith</i>	635
102	Monosaccharide Analysis by GC <i>Elizabeth F. Hounsell, Michael J. Davies, and Kevin D. Smith</i>	639
103	Determination of Monosaccharide Linkage and Substitution Patterns by GC-MS Methylation Analysis <i>Elizabeth F. Hounsell, Michael J. Davies, and Kevin D. Smith</i>	641
104	Sialic Acid Analysis by HPAEC-PAD <i>Elizabeth F. Hounsell, Michael J. Davies, and Kevin D. Smith</i>	645
105	Chemical Release of O-Linked Oligosaccharide Chains <i>Elizabeth F. Hounsell, Michael J. Davies, and Kevin D. Smith</i>	647
106	O-Linked Oligosaccharide Profiling by HPLC <i>Elizabeth F. Hounsell, Michael J. Davies, and Kevin D. Smith</i>	649
107	O-Linked Oligosaccharide Profiling by HPAEC-PAD <i>Elizabeth F. Hounsell, Michael J. Davies, and Kevin D. Smith</i>	651
108	Release of N-Linked Oligosaccharide Chains by Hydrazinolysis <i>Tsuguo Mizuochi and Elizabeth F. Hounsell</i>	653

109 Enzymatic Release of O- and N-Linked Oligosaccharide Chains <i>Elizabeth F. Hounsell, Michael J. Davies, and Kevin D. Smith</i>	657
110 N-Linked Oligosaccharide Profiling by HPLC on Porous Graphitized Carbon (PGC) <i>Elizabeth F. Hounsell, Michael J. Davies, and Kevin D. Smith</i>	659
111 N-Linked Oligosaccharide Profiling by HPAEC-PAD <i>Elizabeth F. Hounsell, Michael J. Davies, and Kevin D. Smith</i>	661
PART VII: IMMUNOCHEMICAL TECHNIQUES	
112 The Chloramine T Method for Radiolabeling Protein <i>Graham S. Bailey</i>	665
113 The Lactoperoxidase Method for Radiolabeling Protein <i>Graham S. Bailey</i>	669
114 The Bolton and Hunter Method for Radiolabeling Protein <i>Graham S. Bailey</i>	671
115 The Iodogen Method for Radiolabeling Protein <i>Graham S. Bailey</i>	673
116 Purification and Assessment of Quality of Radioiodinated Protein <i>Graham S. Bailey</i>	675
117 Conjugation of Peptides to Carrier Proteins via Glutaraldehyde <i>J. Mark Carter</i>	679
118 Conjugation of Peptide to Carrier Proteins via <i>m</i> -Maleimidobenzoyl-N-Hydroxysuccinimide Ester (MBS) <i>J. Mark Carter</i>	689
119 Conjugation of Peptides to Carrier Protein via Carbodiimide <i>J. Mark Carter</i>	693
120 Raising of Polyclonal Antisera <i>Graham S. Bailey</i>	695
121 Elution of SDS-PAGE Separated Proteins from Immobilon Membranes for Use as Antigens <i>Donald F. Summers and Boguslaw Szewczyk</i>	699
122 Production of Highly Specific Polyclonal Antibodies Using a Combination of 2D Electrophoresis and Nitrocellulose-Bound Antigen <i>Monique Diano and André Le Bivic</i>	703
123 Production and Characterization of Antibodies Against Peptides <i>J. Mark Carter</i>	711
124 Production of Antibodies Using Proteins in Gel Bands <i>Sally Ann Amero, Tharappel C. James, and Sarah C. R. Elgin</i>	717
125 Purification of IgG by Precipitation with Sodium Sulfate or Ammonium Sulfate <i>Mark Page and Robin Thorpe</i>	721
126 Purification of IgG Using Caprylic Acid <i>Mark Page and Robin Thorpe</i>	723