

# The Protein Protocols Handbook

Third Edition

Edited by

John M. Walker



Humana Press

# Contents

Preface .....	v
Contributors .....	xxiii

## PART I QUANTITATION OF PROTEINS

1 Protein Determination by UV Absorption .....	3
<i>Alastair Aitken and Michèle P. Learmonth</i>	
2 The Lowry Method for Protein Quantitation .....	7
<i>Jakob H. Waterborg</i>	
3 The Bicinchoninic Acid (BCA) Assay for Protein Quantitation .....	11
<i>John M. Walker</i>	
4 The Bradford Method For Protein Quantitation .....	17
<i>Nicholas J. Kruger</i>	
5 Ultrafast Protein Determinations Using Microwave Enhancement.....	25
<i>Robert E. Akins and Rocky S. Tuan</i>	
6 The Nitric Acid Method for Protein Estimation in Biological Samples.....	35
<i>Scott A. Boerner, Crescent R. Isham, Yean Kit Lee, Jennifer D. Tibodeau, Scott H. Kaufmann, and Keith C. Bible</i>	
7 Quantitation of Tryptophan in Proteins .....	47
<i>Alastair Aitken and Michèle P. Learmonth</i>	
8 Kinetic Silver Staining of Proteins .....	51
<i>Douglas D. Root and Kuan Wang</i>	
9 Quantitation of Cellular Proteins by Flow Cytometry .....	55
<i>Thomas D. Friedrich, F. Andrew Ray, Ralph L. Smith, and John M. Lehman</i>	
10 Quantitation of Cellular Proteins by Laser Scanning Cytometry .....	63
<i>Thomas D. Friedrich, Ralph L. Smith, and John M. Lehman</i>	

PART II ELECTROPHORESIS OF PROTEINS AND PEPTIDES  
AND DETECTION IN GELS

11	Protein Solubility in Two-Dimensional Electrophoresis: <i>Basic Principles and Issues</i> .....	73
	<b><i>Thierry Rabilloud</i></b>	
12	Mouse and Human Tissue Sample Preparation for 2-D Electrophoresis .....	85
	<b><i>Claus Zabel and Joachim Klose</i></b>	
13	Plant Protein Sample Preparation for 2-DE .....	109
	<b><i>Sebastien Christian Carpentier, Rony Swennen, and Bart Panis</i></b>	
14	Preparation of Bacterial Samples for 2-D PAGE .....	121
	<b><i>Brian B. Vandahl, Gunna Christiansen, and Svend Birkeland</i></b>	
15	Preparation of Bodily Fluids for 2-D PAGE.....	131
	<b><i>Anthony G. Sullivan, Henry Brzeski, Jeban Ganesalingam, and Manuel Mayr</i></b>	
16	Immunoaffinity Depletion of High Abundance Plasma and Serum Proteins .....	139
	<b><i>Lynn A. Echan and David W. Speicher</i></b>	
17	Preparation of Yeast Samples for 2-D PAGE .....	155
	<b><i>Joakim Norbeck</i></b>	
18	Membrane Protein Preparation Using Aqueous Polymer Two-Phase Systems .....	159
	<b><i>Jens Schindler and Hans Gerd Nothwang</i></b>	
19	Subcellular Fractionation of Small Sample Amounts .....	165
	<b><i>Hans Gerd Nothwang, Isabelle Guillemin, and Jens Schindler</i></b>	
20	Nondenaturing Polyacrylamide Gel Electrophoresis of Proteins .....	171
	<b><i>John M. Walker</i></b>	
21	SDS Polyacrylamide Gel Electrophoresis of Proteins.....	177
	<b><i>John M. Walker</i></b>	
22	Gradient SDS Polyacrylamide Gel Electrophoresis of Proteins .....	187
	<b><i>John M. Walker</i></b>	
23	SDS-Polyacrylamide Gel Electrophoresis of Peptides .....	193
	<b><i>Ralph C. Judd</i></b>	
24	Separation of Proteins by Blue Native Electrophoresis (BN-PAGE) .....	201
	<b><i>Elke A. Dian, Joachim Rassow, and Christian Motz</i></b>	

25	Separation of Proteins by Gel Electrophoresis in the Tris-Taurine-HCl System.....	211
	<i>Sylvie Luche, Mireille Chevallat, Cécile Lelong, and Thierry Rabilloud</i>	
26	Cetyltrimethylammonium Bromide Discontinuous Gel Electrophoresis of Proteins: <i>M<sub>r</sub>-Based Separation of Proteins with Retained Native Activity</i> .....	221
	<i>Robert E. Akins and Rocky S. Tuan</i>	
27	Acetic Acid-Urea Polyacrylamide Gel Electrophoresis of Basic Proteins .....	239
	<i>Jakob H. Waterborg</i>	
28	Acid-Urea-Triton Polyacrylamide Gel Electrophoresis of Histones .....	251
	<i>Jakob H. Waterborg</i>	
29	Isoelectric Focusing of Proteins in Ultra-Thin Polyacrylamide Gels .....	263
	<i>John M. Walker</i>	
30	Serial Immobilized pH Gradient Isoelectric Focusing over pH 4-9 .....	269
	<i>Slobodan Poznанović, Wojciech Wozny, Helmut Zengerling, Gerhard P. Schwall, and Michael A. Cahill</i>	
31	Radiolabeling of Eukaryotic Cells and Subsequent Preparation for 2-Dimensional Electrophoresis.....	279
	<i>Nick Biziос</i>	
32	Two-Dimensional Polyacrylamide Gel Electrophoresis of Proteins Using Carrier Ampholyte pH Gradients in the First Dimension .....	285
	<i>Patricia Gravel</i>	
33	Vertical Agarose Electrophoresis and Electroblotting of High-Molecular-Weight Proteins .....	293
	<i>Marion L. Greaser and Chad M. Warren</i>	
34	Two-Dimensional PAGE of High-Molecular-Mass Proteins .....	299
	<i>Masamichi Oh-Ishi and Tadakazu Maeda</i>	
35	Casting Immobilized pH Gradients .....	305
	<i>Elisabetta Gianazza</i>	
36	A Modified Nonequilibrium pH Gradient Electrophoresis (NEPHGE) Technique for Protein Separation .....	323
	<i>James A. Carroll</i>	

37	Microchip Capillary Electrophoresis: <i>Application to Peptide Analysis</i> .....	329
	<b><i>Barbara A. Fogarty, Nathan A. Lacher, and Susan M. Lunte</i></b>	
38	Protein Separations in Microfluidic Chips .....	361
	<b><i>Andrea W. Chow and Bahram Fathollahi</i></b>	
39	Difference Gel Electrophoresis (DIGE) .....	379
	<b><i>David B. Friedman and Kathryn S. Lilley</i></b>	
40	Comparing 2D Electrophoretic Gels Across Internet Databases: An Open Source Application .....	409
	<b><i>Peter F. Lemkin, Gregory C. Thornwall, and Jai A. Evans</i></b>	
41	Quantification of Radiolabeled Proteins in Polyacrylamide Gels .....	443
	<b><i>Wayne R. Springer</i></b>	
42	Differential ProteoTope Radioactive Quantification of Protein Abundance Ratios .....	449
	<b><i>Wojciech Wozny, Gerhard P. Schwall, Chaturvedula S. Sastri, Slobodan Poznanović, Werner Stegmann, Christian Hunzinger<sup>†</sup>, Karlfried Groebe, and Michael A. Cahill</i></b>	
43	Quantification of Proteins on Polyacrylamide Gels .....	479
	<b><i>Bryan John Smith</i></b>	
44	Using SDS-PAGE and Scanning Laser Densitometry to Measure Yield and Degradation of Proteins.....	487
	<b><i>Aaron P. Miles and Allan Saul</i></b>	
45	Rapid and Sensitive Staining of Unfixed Proteins in Polyacrylamide Gels with Nile Red .....	497
	<b><i>Joan-Ramon Daban, Salvador Bartolomé, Antonio Bermúdez, and F. Javier Alba</i></b>	
46	Zn <sup>2+</sup> -Reverse Staining Technique .....	505
	<b><i>Carlos Fernandez-Patron</i></b>	
47	Protein Staining with Calconcarboxylic Acid in Polyacrylamide Gels.....	515
	<b><i>Wei-Tao Cong, Sun-Young Hwang, Li-Tai Jin, and Jung-Kap Choi</i></b>	
48	Detection of Proteins in Polyacrylamide Gels by Silver Staining .....	521
	<b><i>Michael J. Dunn</i></b>	
49	Background-Free Protein Detection in Polyacrylamide Gels and on Electroblots Using Transition Metal Chelate Stains.....	529
	<b><i>Wayne F. Patton</i></b>	

50	Detection of Proteins in Polyacrylamide Gels by Fluorescent Staining .....	547
	<i>Michael J. Dunn</i>	
51	Detection of Glycoproteins in Gels and Blots.....	555
	<i>Nicolle H. Packer, Malcolm S. Ball, Peter L. Devine, and Wayne F. Patton</i>	
52	Staining of Glycoproteins/Proteoglycans on SDS-Gels .....	569
	<i>Holger J. Møller and Jørgen H. Poulsen</i>	
53	Detection of Proteins and Sialoglycoproteins in Polyacrylamide Gels Using Eosin Y Stain .....	575
	<i>Fan Lin and Gary E. Wise</i>	
54	A Modified Pro-Q Diamond Staining Protocol for Phosphoprotein Detection in Polyacrylamide Gels .....	579
	<i>Ganesh Kumar Agrawal and Jay J. Thelen</i>	
55	Electroelution of Proteins from Polyacrylamide Gels .....	587
	<i>Paul Jenö and Martin Horst</i>	
56	Autoradiography and Fluorography of Acrylamide Gels.....	595
	<i>Antonella Circolo and Sunita Gulati</i>	
57	Proteolytic Activity Detection by Two-Dimensional Zymography .....	605
	<i>Jeff Wilkesman</i>	

### PART III BLOTTING AND DETECTION METHODS

58	Protein Blotting by Electroblotting .....	617
	<i>Mark Page and Robin Thorpe</i>	
59	Protein Blotting by the Semidry Method.....	621
	<i>Patricia Gravel</i>	
60	Protein Blotting by the Capillary Method.....	631
	<i>John M. Walker</i>	
61	Western Blotting of Basic Proteins Electrophoretically Resolved on Acid-Urea-Triton-Polyacrylamide Gels .....	635
	<i>Geneviève P. Delcuve and James R. Davie</i>	
62	Immunoblotting of 2-DE Separated Proteins.....	641
	<i>Barbara Magi and Laura Bianchi</i>	
63	High-Efficiency Blotting of High-Molecular Weight Proteins.....	663
	<i>Marion L. Greaser and Darl R. Swartz</i>	
64	Alkaline Phosphatase Labeling of IgG Antibody .....	673
	<i>G. Brian Wisdom</i>	

65	β-Galactosidase Labeling of IgG Antibody..... <i>G. Brian Wisdom</i>	677
66	Horseradish Peroxidase Labeling of IgG Antibody ..... <i>G. Brian Wisdom</i>	681
67	Digoxigenin Labeling of IgG Antibody..... <i>G. Brian Wisdom</i>	685
68	Conjugation of Fluorochromes to Antibodies..... <i>Su-Yau Mao</i>	687
69	Coupling of Antibodies with Biotin..... <i>Rosaria P. Haugland and Wendy W. You</i>	693
70	Preparation of Avidin Conjugates..... <i>Rosaria P. Haugland and Mahesh K. Bhalgat</i>	705
71	MDPF Staining of Proteins on Western Blots ..... <i>F. Javier Alba and Joan-Ramon Daban</i>	717
72	Copper Iodide Staining of Proteins and Its Silver Enhancement..... <i>Douglas D. Root and Kuan Wang</i>	723
73	Detection of Proteins on Blots Using Direct Blue 71 ..... <i>Wei-Tao Cong, Sun-Young Hwang, Li-Tai Jin, and Jung-Kap Choi</i>	729
74	Detection of Proteins on Western Blots Using Colorimetric and Radiometric Visualization of Secondary Ligands ..... <i>Nicholas J. Kruger</i>	737
75	Identification of Glycoproteins on Nitrocellulose Membranes Using Lectin Blotting ..... <i>Patricia Gravel</i>	755
76	A Sensitive Method to Quantitatively Detect Total Protein on Membranes after Electrophoretic Transfer Using Avidin- or Streptavidin-Biotin..... <i>William J. LaRochelle</i>	771
77	Detection and Quantification of Proteins on Immunoblots using Enhanced Chemiluminescence..... <i>Jennifer J. Young</i>	779
78	Reutilization of Western Blots After Chemiluminescent or Autoradiographic Detection..... <i>Scott H. Kaufmann</i>	789
79	The Use of Quantum Dot Luminescent Probes for Western Blot Analysis ..... <i>Savvas C. Makrides, Christina Gasbarro, and Job M. Bello</i>	807

80	The Use of Infrared Fluorescent Dyes in Quantitative Immunoblotting.....	819
	<i>Ching-Hui Yang, Christopher Kasbek, and Harold A. Fisk</i>	
81	The Use of Infrared Fluorescent Dyes in Immunofluorescence Microscopy.....	827
	<i>Christopher Kasbek, Ching-Hui Yang, and Harold A. Fisk</i>	
<b>PART IV CHEMICAL MODIFICATION OF PROTEINS AND PEPTIDE PRODUCTION AND PURIFICATION</b>		
82	Carboxymethylation of Cysteine Using Iodoacetamide/Iodoacetic Acid .....	837
	<i>Alastair Aitken and Michèle Learmonth</i>	
83	Performic Acid Oxidation.....	841
	<i>Alastair Aitken and Michèle Learmonth</i>	
84	Succinylation of Proteins.....	845
	<i>Alastair Aitken and Michèle Learmonth</i>	
85	Pyridylethylation of Cysteine Residues.....	847
	<i>Malcolm Ward</i>	
86	Side Chain Selective Chemical Modifications of Proteins.....	851
	<i>Dan S. Tawfik</i>	
87	Nitration of Tyrosines.....	855
	<i>Dan S. Tawfik</i>	
88	Ethoxyformylation of Histidine.....	859
	<i>Dan S. Tawfik</i>	
89	Modification of Arginine Side Chains with <i>p</i> -Hydroxyphenylglyoxal.....	863
	<i>Dan S. Tawfik</i>	
90	Amidation of Carboxyl Groups.....	865
	<i>Dan S. Tawfik</i>	
91	Amidination of Lysine Side Chains .....	867
	<i>Dan S. Tawfik</i>	
92	Modification of Tryptophan with 2-Hydroxy- 5-Nitrobenzylbromide.....	871
	<i>Dan S. Tawfik</i>	
93	Modification of Sulfhydryl Groups with DTNB .....	873
	<i>Dan S. Tawfik</i>	
94	Chemical Cleavage of Proteins at Methionyl-X Peptide Bonds.....	875
	<i>Bryan John Smith</i>	

95	Chemical Cleavage of Proteins at Tryptophanyl-X Peptide Bonds..... <i>Bryan John Smith</i>	883
96	Chemical Cleavage of Proteins at Aspartyl-X Peptide Bonds..... <i>Bryan John Smith</i>	891
97	Chemical Cleavage of Proteins at Cysteinyl-X Peptide Bonds..... <i>Bryan John Smith</i>	895
98	Chemical Cleavage of Proteins at Asparaginyl-Glycyl Peptide Bonds..... <i>Bryan John Smith</i>	899
99	Enzymatic Digestion of Proteins in Solution and in SDS Polyacrylamide Gels .....	905
	<i>Kathryn L. Stone, Erol E. Gulcicek, and Kenneth R. Williams</i>	
100	On-PVDF Protein Digestions for N-terminal Sequencing and Peptide Mass Fingerprinting .....	919
	<i>Victoria Pham, William Henzel, and Jennie R. Lill</i>	
101	Enzymatic Digestion of Membrane-Bound Proteins for Peptide Mapping and Internal Sequence Analysis .....	927
	<i>Joseph Fernandez and Sheenah M. Mische</i>	
102	Reverse-Phase HPLC Separation of Enzymatic Digests of Proteins..... <i>Kathryn L. Stone and Kenneth R. Williams</i>	941
103	Peptide Mapping by Two-Dimensional Thin-Layer Electrophoresis-Thin-Layer Chromatography .....	951
	<i>Ralph C. Judd</i>	
104	Peptide Mapping by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis .....	963
	<i>Ralph C. Judd</i>	
105	Peptide Mapping for Protein Characterization .....	969
	<i>Peter Højrup</i>	
106	Production of Protein Hydrolysates Using Enzymes..... <i>John M. Walker and Patricia J. Sweeney</i>	989
107	Amino Acid Analysis by Precolumn Derivatization with 1-Fluoro-2,4-Dinitrophenyl-5-i-Alanine Amide (Marfey's Reagent)..... <i>Sunil Kochhar and Philipp Christen</i>	995
108	Amino Acid Analysis of Protein Hydrolysates Using Anion Exchange Chromatography and IPAD Detection .....	1001
	<i>Petr Jandik, Jun Cheng, and Nebojsa Avdalovic</i>	

109	Validation of Amino Acid Analysis Methods .....	1015
	<i>Andrew J. Reason</i>	
110	Molecular Weight Estimation for Native Proteins Using High-Performance Size-Exclusion Chromatography .....	1029
	<i>G. Brent Irvine</i>	
111	Detection of Disulfide-Linked Peptides by HPLC .....	1039
	<i>Alastair Aitken and Michèle Learmonth</i>	
112	Detection of Disulfide-Linked Peptides by Mass Spectrometry .....	1043
	<i>Alastair Aitken and Michèle Learmonth</i>	
113	Diagonal Electrophoresis for Detecting Disulfide Bridges.....	1047
	<i>Alastair Aitken and Michèle Learmonth</i>	
114	Estimation of Disulfide Bonds Using Ellman's Reagent.....	1053
	<i>Alastair Aitken and Michèle Learmonth</i>	
115	Quantitation of Cysteine Residues and Disulfide Bonds by Electrophoresis .....	1057
	<i>Alastair Aitken and Michèle Learmonth</i>	
116	N-Terminal Sequencing of N-Terminally Modified Proteins .....	1063
	<i>Roza Maria Kamp and Hisashi Hirano</i>	
117	Deblocking of Proteins Containing N-Terminal Pyroglutamic Acid .....	1075
	<i>Jacek Mozdzanowski</i>	
118	Detection and Characterization of Protein Mutations by Mass Spectrometry.....	1081
	<i>Yoshinao Wada</i>	
119	Peptide Sequencing by Nanoelectrospray Tandem Mass Spectrometry .....	1095
	<i>Ole Nørregaard Jensen and Matthias Wilm</i>	
120	Protein Identification by Peptide Mass Fingerprinting using MALDI-TOF Mass Spectrometry .....	1117
	<i>Judith Webster and David Oxley</i>	
121	Protein Ladder Sequencing .....	1131
	<i>Rong Wang and Brian T. Chait</i>	
122	Sequence Analysis with WinGene/WinPep.....	1141
	<i>Lars Hennig</i>	
123	HPLC and Mass Spectrometry of Integral Membrane Proteins.....	1149
	<i>Julian P. Whitelegge</i>	

124	Enrichment of Serum Peptides and Analysis by MALDI-TOF Mass Spectrometry .....	1167
	<i>Yanming An, Habtom W. Ressom, and Radoslav Goldman</i>	
125	Computational Methods for Analysis of MALDI-TOF Spectra to Discover Peptide Serum Biomarkers .....	1175
	<i>Habtom W. Ressom, Rency S. Varghese, and Radoslav Goldman</i>	
<b>PART V POSTTRANSLATIONAL MODIFICATIONS</b>		
126	Simple Tools for Complex N-Glycan Analysis .....	1187
	<i>Anne-Catherine Fitchette, Meriem Benchabane, Thomas Paccalet, Loïc Faye, and Véronique Gomord</i>	
127	A Lectin-Binding Assay for the Rapid Characterization of the Glycosylation of Purified Glycoproteins .....	1205
	<i>Mohammad T. Goodarzi, Angeliki Fotinopoulou, and Graham A. Turner</i>	
128	Chemical Methods of Analysis of Glycoproteins.....	1215
	<i>Elizabeth F. Hounsell, Michael J. Davies, and Kevin D. Smith</i>	
129	Monosaccharide Analysis by HPAEC .....	1219
	<i>Elizabeth F. Hounsell, Michael J. Davies, and Kevin D. Smith</i>	
130	Monosaccharide Analysis by Gas Chromatography (GC) .....	1223
	<i>Elizabeth F. Hounsell, Michael J. Davies, and Kevin D. Smith</i>	
131	Determination of Monosaccharide Linkage and Substitution Patterns by GC-MS Methylation Analysis .....	1227
	<i>Elizabeth F. Hounsell, Michael J. Davies, and Kevin D. Smith</i>	
132	Sialic Acid Analysis by HPAEC-PAD .....	1231
	<i>Elizabeth F. Hounsell, Michael J. Davies, and Kevin D. Smith</i>	
133	Chemical Release of O-Linked Oligosaccharide Chains .....	1233
	<i>Elizabeth F. Hounsell, Michael J. Davies, and Kevin D. Smith</i>	
134	O-Linked Oligosaccharide Profiling by HPLC .....	1235
	<i>Elizabeth F. Hounsell, Michael J. Davies, and Kevin D. Smith</i>	
135	O-Linked Oligosaccharide Profiling by HPAEC-PAD.....	1237
	<i>Elizabeth F. Hounsell, Michael J. Davies, and Kevin D. Smith</i>	

136	Release of N-Linked Oligosaccharide Chains by Hydrazinolysis .....	1239
	<i>Tsuguo Mizuochi and Elizabeth F. Hounsell</i>	
137	Enzymatic Release of O-and N-Linked Oligosaccharide Chains .....	1243
	<i>Elizabeth F. Hounsell, Michael J. Davies, and Kevin D. Smith</i>	
138	N-Linked Oligosaccharide Profiling by HPLC on Porous Graphitized Carbon (PGC) .....	1247
	<i>Elizabeth F. Hounsell, Michael J. Davies, and Kevin D. Smith</i>	
139	N-Linked Oligosaccharide Profiling by HPAEC-PAD .....	1249
	<i>Elizabeth F. Hounsell, Michael J. Davies, and Kevin D. Smith</i>	
140	HPAEC-PAD Analysis of Monosaccharides Released by Exoglycosidase Digestion Using the CarboPac MA1 Column .....	1253
	<i>Michael Weitzhandler, Jeffrey Rohrer, James R. Thayer, and Nebojsa Avdalovic</i>	
141	Microassay Analyses of Protein Glycosylation .....	1261
	<i>Nicky K. C. Wong, Nnennaya Kanu, Natasha Thandrayen, Geert Jan Rademaker, Christopher I. Baldwin, David V. Renouf, and Elizabeth F. Hounsell</i>	
142	Polyacrylamide Gel Electrophoresis of Fluorophore-Labeled Carbohydrates from Glycoproteins .....	1273
	<i>Brian K. Brandley, John C. Klock, and Christopher M. Starr</i>	
143	HPLC Analysis of Fluorescently Labeled Glycans .....	1289
	<i>Tony Merry and Sviatlana Astrautsova</i>	
144	Glycoprofiling Purified Glycoproteins Using Surface Plasmon Resonance .....	1313
	<i>Angeliki Fotinopoulou and Graham A. Turner</i>	
145	Sequencing Heparan Sulfate Saccharides .....	1321
	<i>Jeremy E. Turnbull</i>	
146	Analysis of Glycoprotein Heterogeneity by Capillary Electrophoresis and Mass Spectrometry .....	1335
	<i>Andrew D. Hooker and David C. James</i>	
147	Affinity Chromatography of Oligosaccharides and Glycopeptides with Immobilized Lectins .....	1347
	<i>Kazuo Yamamoto</i>	
148	In-Gel Enzymatic Release of N-Glycans.....	1363
	<i>David J. Harvey</i>	

149	Analysis of N-Linked Glycans by Mass Spectrometry .....	1371
	<i>David J. Harvey</i>	
150	MS Analysis of Protein Glycosylation .....	1387
	<i>Nobuaki Takemori, Naoka Komori, and Hiroyuki Matsumoto</i>	
151	Mapping protein N-Glycosylation by COFRADIC .....	1395
	<i>Bart Ghesquière, Joël Vandekerckhove, and Kris Gevaert</i>	
152	Mass Spectrometric Analysis of O-Linked Glycans Released Directly from Glycoproteins in Gels Using $\beta$ -Elimination.....	1403
	<i>Adrian M. Taylor and Jane Thomas-Oates</i>	
153	Glycopeptide Analysis Using LC/MS and LC/MS <sup>2</sup> : <i>Site-Specific Glycosylation Analysis of a Glycoprotein.....</i>	1419
	<i>Satsuki Itoh, Daisuke Takakura, Nana Kawasaki, and Teruhide Yamaguchi</i>	
154	Identification of Vitamin K-Dependent Proteins Using a Gla-Specific Monoclonal Antibody .....	1431
	<i>Karin Hansson</i>	
155	The Identification of Protein S-Nitrosocysteine .....	1451
	<i>Todd M. Greco, Sheryl L. Stamer, Daniel C. Liebler, and Harry Ischiropoulos</i>	
156	Detection of Nitrotyrosine-Containing Proteins .....	1467
	<i>Xianquan Zhan and Dominic M. Desiderio</i>	
157	Mass Spectrometric Determination of Protein Ubiquitination .....	1491
	<i>Carol E. Parker, Maria Warren Hines, Viorel Mocanu, Susanna F. Greer, and Christoph H. Borchers</i>	
158	Detection of Sumoylated Proteins.....	1519
	<i>Ok-Kyong Park-Sarge and Kevin D. Sarge</i>	
159	Efficient Enrichment of Intact Phosphorylated Proteins by Modified Immobilized Metal-Affinity Chromatography .....	1531
	<i>Anna Dubrovska</i>	
160	Analyzing Protein Phosphorylation .....	1547
	<i>John Colyer</i>	
161	Mass Spectrometric Analysis of Protein Phosphorylation .....	1555
	<i>Stefan Gander, Alessio Cremonesi, Johana Chicher, Suzette Moes, and Paul Jenő</i>	

162	Protein Microarrays for Phosphorylation Studies .....	1567
	<i>Birgit Kersten and Tanja Feilner</i>	
163	Two-Dimensional Phosphopeptide Mapping .....	1579
	<i>Hikaru Nagahara, Robert R. Latek, Sergei A. Ezhevsky, and Steven F. Dowdy</i>	
164	Identification of Proteins Modified by Protein (D-Aspartyl/L-Isoaspartyl) Carboxyl Methyltransferase .....	1589
	<i>Darin J. Weber and Philip N. McFadden</i>	
165	Analysis of Tyrosine-O-Sulfation .....	1601
	<i>Jens R. Bundgaard, Jette W. Sen, Anders H. Johnsen, and Jens F. Rehfeld</i>	
166	Analysis of Protein Palmitoylation by Metabolic Radiolabeling Methods .....	1623
	<i>Katherine H. Pedone, Leah S. Bernstein, Maurine E. Linder, and John R. Hepler</i>	
167	Incorporation of Radiolabeled Prenyl Alcohols and Their Analogs into Mammalian Cell Proteins: <i>A Useful Tool for Studying Proteins Prenylation</i> .....	1637
	<i>Alberto Corsini, Christopher C. Farnsworth, Paul McGeady, Michael H. Gelb, and John A. Glomset</i>	
168	The Metabolic Labeling and Analysis of Isoprenylated Proteins .....	1657
	<i>Douglas A. Andres, Dean C. Crick, H. Peter Spielmann, and Charles J. Waechter</i>	

## PART VI ANTIBODY TECHNIQUES

169	Antibody Production .....	1679
	<i>Robert Burns</i>	
170	Production of Antibodies Using Proteins in Gel Bands .....	1687
	<i>Sally Ann Amero, Tharappel C. James, and Sarah C. R. Elgin</i>	
171	Raising Highly Specific Polyclonal Antibodies Using Biocompatible Support-Bound Antigens .....	1693
	<i>Monique Diano and André Le Bivic</i>	
172	Production of Antisera Using Peptide Conjugates .....	1705
	<i>Thomas E. Adrian</i>	
173	Small-Molecule-Protein Conjugation Procedures .....	1715
	<i>Stephen Thompson</i>	

174	The Chloramine T Method for Radiolabeling Protein .....	1727
	<i>Graham S. Bailey</i>	
175	The Lactoperoxidase Method for Radiolabeling Protein.....	1731
	<i>Graham S. Bailey</i>	
176	The Bolton and Hunter Method for Radiolabeling Protein.....	1733
	<i>Graham S. Bailey</i>	
177	Preparation of $^{125}\text{I}$ -Labeled Peptides and Proteins with High Specific Activity Using IODO-GEN.....	1735
	<i>J. Michael Conlon</i>	
178	Purification and Assessment of Quality of Radioiodinated Protein.....	1743
	<i>Graham S. Bailey</i>	
179	Purification of IgG by Precipitation with Sodium Sulfate or Ammonium Sulfate .....	1749
	<i>Mark Page and Robin Thorpe</i>	
180	Purification of IgG Using Caprylic Acid.....	1753
	<i>Mark Page and Robin Thorpe</i>	
181	Purification of IgG Using DEAE-Sepharose Chromatography .....	1755
	<i>Mark Page and Robin Thorpe</i>	
182	Purification of IgG Using Ion-Exchange HPLC .....	1757
	<i>Carl Dolman, Mark Page, and Robin Thorpe</i>	
183	Purification of IgG by Precipitation with Polyethylene Glycol (PEG) .....	1759
	<i>Mark Page and Robin Thorpe</i>	
184	Purification of IgG Using Protein A or Protein G .....	1761
	<i>Mark Page and Robin Thorpe</i>	
185	Analysis and Purification of IgG Using Size-Exclusion High Performance Liquid Chromatography (SE-HPLC) .....	1765
	<i>Carl Dolman and Robin Thorpe</i>	
186	Purification of IgG Using Affinity Chromatography on Antigen-Ligand Columns .....	1769
	<i>Mark Page and Robin Thorpe</i>	
187	Purification of IgG Using Thiophilic Chromatography .....	1773
	<i>Mark Page and Robin Thorpe</i>	
188	Analysis of IgG Fractions by Electrophoresis.....	1775
	<i>Mark Page and Robin Thorpe</i>	

189	Purification of Immunoglobulin Y (IgY) from Chicken Eggs.....	1779
	<i>Christopher R. Bird and Robin Thorpe</i>	
190	Affinity Purification of Immunoglobulins Using Protein A Mimetic (PAM) .....	1783
	<i>Giorgio Fassina, Giovanna Palombo, Antonio Verdoliva, and Menotti Ruvo</i>	
191	Detection of Serological Cross-Reactions by Western Cross-Blotting .....	1799
	<i>Peter Hammerl, Arnulf Hartl, Johannes Freund, and Josef Thalhamer</i>	
192	Enzymatic Digestion of Monoclonal Antibodies .....	1811
	<i>Sarah M. Andrew</i>	
193	How to Make Bispecific Antibodies .....	1819
	<i>Ruth R. French</i>	
194	Antigen Measurement Using ELISA .....	1827
	<i>William Jordan</i>	
195	Enhanced Chemiluminescence Immunoassay .....	1835
	<i>Richard A. W. Stott</i>	
196	Immunoprecipitation and Blotting: <i>The Visualization of Small Amounts of Antigens Using Antibodies and Lectins.</i> .....	1845
	<i>Stephen Thompson</i>	
197	Determination of Epitopes by Mass Spectrometry.....	1859
	<i>Christine Hager-Braun and Kenneth B. Tomer</i>	
198	Immogen Preparation and Immunization Procedures for Rats and Mice .....	1873
	<i>Mark Page and Robin Thorpe</i>	
199	Making Hybridomas .....	1877
	<i>Robert Burns</i>	
200	Growing Hybridomas .....	1887
	<i>Gary Entrican, Catherine Jepson, and David Deane</i>	
201	Mouse Hybridomas as an Entryway to Monoclonal Antibody Design and Production.....	1901
	<i>Eugene Mechetner</i>	
202	Potential Pitfalls in Monoclonal Antibody Development and Applications.....	1911
	<i>Eugene Mechetner</i>	

203	Recombinant Antibody Expression and Purification .....	1929
	<i>Achim Knappik and Ralf Brundiers</i>	
204	Screening Hybridoma Culture Supernatants Using Solid-Phase Radiobinding Assay.....	1945
	<i>Mark Page and Robin Thorpe</i>	
205	Screening Hybridoma Culture Supernatants Using ELISA.....	1947
	<i>Mark Page and Robin Thorpe</i>	
206	Growth and Purification of Murine Monoclonal Antibodies.....	1949
	<i>Mark Page and Robin Thorpe</i>	
207	Affinity Purification Techniques for Monoclonal Antibodies .....	1951
	<i>Alexander Schwarz</i>	
208	A Rapid Method for Generating Large Numbers of High-Affinity Monoclonal Antibodies from a Single Mouse .....	1961
	<i>Nguyen Thi Man and Glenn E. Morris</i>	
	Index .....	1975