

Contents

Preface	vii
List of Contributors	ix

I

LABELING AND DETECTING PROTEINS

1. Protein Determination 3
2. Phosphopeptide Mapping:
A Basic Protocol 13
3. Radioiodination of Proteins and
Peptides 21
4. Protein Detection in Gels by Silver Staining:
A Procedure Compatible with Mass
Spectrometry 29
5. Fluorescence Detection of Proteins in Gels
Using SYPRO Dyes 35
6. Autoradiography and Fluorography:
Film-Based Techniques for Imaging
Radioactivity in Flat Samples 47

II

PROTEOMIC ANALYSES BASED ON PROTEIN SEPARATION

7. Free-Flow Electrophoresis 57
8. Gel-Based Proteomics: High-Resolution
Two-Dimensional Gel Electrophoresis of
Proteins. Isoelectric Focusing and

Nonequilibrium pH Gradient
Electrophoresis 65

9. High-Resolution Two-Dimensional
Electrophoresis with Immobilized pH
Gradients for Proteome Analysis 77
10. Two-Dimensional Difference Gel
Electrophoresis: Application for the
Analysis of Differential Protein Expression
in Multiple Biological Samples 97
11. Image Analysis and Quantitation 109
12. Two-Dimensional Gel Profiling of
Posttranslationally Modified Proteins by
in vivo Isotope Labeling 121

III

PROTEIN-PROTEIN AND PROTEIN-SMALL MOLECULE INTERACTIONS

13. Immunoprecipitation of Proteins under
Nondenaturing Conditions 133
14. Nondenaturing Polyacrylamide Gel
Electrophoresis as a Method for Studying
Protein Interactions: Applications in the
Analysis of Mitochondrial Oxidative
Phosphorylation Complexes 141
15. Analysis of Protein-Protein Interactions by
Chemical Cross-Linking 151
16. Peroxisomal Targeting as a Tool to Assess
Protein-Protein Interactions 157

17. Biomolecular Interaction Analysis Mass Spectrometry 163
18. Ligand Blot Overlay Assay: Detection of Ca^{2+} - and Small GTP-Binding Proteins 171
19. Modular Scale Yeast Two-Hybrid Screening 179
20. Affinity Electrophoresis for Studies of Biospecific Interactions: High-Resolution Two-Dimensional Affinity Electrophoresis for Separation of Hapten-Specific Polyclonal Antibodies into Monoclonal Antibodies in Murine Blood Plasma 191
21. Methods in Protein Ubiquitination 203
25. Proteome Specific Sample Preparation Methods for Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry 253
26. In-Gel Digestion of Protein Spots for Mass Spectrometry 263
27. Peptide Sequencing by Tandem Mass Spectrometry 269
28. Direct Database Searching Using Tandem Mass Spectra of Peptides 279
29. Identification of Proteins from Organisms with Unsequenced Genomes by Tandem Mass Spectrometry and Sequence-Similarity Database Searching Tools 289
30. Identification of Protein Phosphorylation Sites by Mass Spectrometry 301
31. Analysis of Carbohydrates/Glycoproteins by Mass Spectrometry 309
32. Stable Isotope Labeling by Amino Acids in Cell Culture for Quantitative Proteomics 325
33. Site-Specific, Stable Isotope Labeling of Cysteinyll Peptides in Complex Peptide Mixtures 337
34. Protein Hydrogen Exchange Measured by Electrospray Ionization Mass Spectrometry 345
35. Nongel-Based Proteomics: Selective Reversed-Phase Chromatographic Isolation of Methionine-Containing Peptides from Complex Peptide Mixtures 355
36. Mass Spectrometry in Noncovalent Protein Interactions and Protein Assemblies 361

IV

PROTEIN-DNA INTERACTIONS

22. Gel Mobility Shift Assay 219
23. DNA Affinity Chromatography of Transcription Factors: The Oligonucleotide Trapping Approach 231

V

MASS SPECTROMETRY METHODS FOR DETERMINATION OF PROTEIN IDENTITY AND PROTEIN MODIFICATIONS

24. Protein Identification and Sequencing by Mass Spectrometry 243

Index