



Contents

Preface	vii
Acknowledgments	ix
Chapter 1 An Overview of This Manual	1
Introduction	1
Kits, Cores, and Computers	3
How to Use This Book	3
Basic Laboratory Equipment	4
Laboratory Automation	5
Beyond Protein Analysis and Purification	6
Chapter 2 Protein Structure	8
Introduction	8
A. The Amino Acids	8
B. The Four Levels of Protein Structure	11
Primary Structure	11
Secondary Structure	13
Tertiary Structure	16
Quaternary Structure	17
C. Chemical Characteristics of Proteins	17
Hydrophobicity	19
Consensus Sequences	19
Proteomics	21

 **Protocol uses radioactive substances.** Make sure that all institutional rules for the safe handling of radioactivity are followed. Take precautions when disposing of radioactive waste. Refer to Appendix A for further safety tips.

 **Toxic substances are used.** Exercise caution when handling. Refer to Appendix A for further safety tips.

Chapter 3 Tracking the Target Protein	24
Introduction	24
A. Labeling Cells and Proteins	26
Metabolic Labeling Cells in Culture	27
☼ PROTOCOL 3.1 Metabolic Labeling Adherent Cells — 28	
☼ PROTOCOL 3.2 Metabolic Labeling Cells Growing in Suspension — 28	
☼ PROTOCOL 3.3 Pulse-Chase Labeling — 29	
Labeling Proteins Present at the Plasma Membrane	30
☼ PROTOCOL 3.4 Lactoperoxidase Labeling Cell Surface Proteins — 31	
☼ PROTOCOL 3.5 Labeling Surface Proteins with IODO-GEN® — 32	
PROTOCOL 3.6 Non-radioactive Biotinylation of Cell Surface Proteins — 33	
☼ PROTOCOL 3.7 Domain-Selective Biotinylation and Streptavidin-Agarose Precipitation — 34	
☼ PROTOCOL 3.8 Labeling Isolated Proteins with Chloramine T — 36	
B. Lysis—Preparation of the Cell Free Extract	37
Lysis Buffers	37
☼ PROTOCOL 3.9 Lysis of Cells in Suspension (Continuation of Protocol 3.2) — 38	
☼ PROTOCOL 3.10 Lysis of Adherent Cells (Continuation of Protocol 3.1) — 39	
C. Principles of Immunoprecipitation	39
Antibodies as Detection Tools	39
Polyclonal Antibodies	40
Monoclonal Antibodies	40
Antibody Based Analytical Techniques: Western Blotting and Immunoprecipitation	40
☼ PROTOCOL 3.11 Immunoprecipitation — 42	
Protein Interaction Analysis	44
☼ PROTOCOL 3.12 Sequential Immunoprecipitation: Dissociation and Reimmunoprecipitation of Immune Complexes — 44	
☼ PROTOCOL 3.13 Eliminating Interfering Immunoglobulin Bands During IP-Western Detection: Analysis of the Immunoprecipitate under Non-Reducing Conditions — 46	
☼ PROTOCOL 3.14 Nondenaturing Immunoprecipitation — 47	
D. Additional Methods to Identify Associated Proteins	47
Sucrose Gradients	48
PROTOCOL 3.15 Preparation of Sucrose Gradients — 48	
Fractionating a Sucrose Gradient	51
Chemical Cross-Linking	53
General Considerations for Cross-Linking	54
PROTOCOL 3.16 Cross-Linking Proteins Added to Cells: Analysis of Receptor-Ligand Interaction — 55	
PROTOCOL 3.17 Cross-Linking Proteins in Solution — 55	
☼ PROTOCOL 3.18 Cross-Linking Extraneously Added Ligand to Cells — 56	

<ul style="list-style-type: none"> ☢ PROTOCOL 3.19 Cross-Linking Proteins in Solution Using the Homobifunctional Reagent Dithiobis (succinimidyl propionate) (DSP) — 57 Analysis of Protein-Protein Interactions 58 Mapping Protein-Protein Contact Sites 58 Yeast Two-Hybrid Systems 59 Analyzing Protein Interactions by Fluorescence Resonance Energy Transfer (FRET) 60 	
Chapter 4 Electrophoretic Techniques	63
Introduction to Polyacrylamide Gel Electrophoresis (PAGE)	63
A. Preparation of SDS-Polyacrylamide Gels	66
PROTOCOL 4.1 Assembling the Plates — 66	
Choosing the Acrylamide Concentration	67
☢ PROTOCOL 4.2 Casting the Separating Gel — 67	
☢ PROTOCOL 4.3 Casting the Stacking Gel — 69	
☢ PROTOCOL 4.4 Gradient Gels — 70	
PROTOCOL 4.5 Sample Preparation — 72	
PROTOCOL 4.6 Running the Gel: Attaching the Gel Cassette to the Apparatus and Loading the Samples — 73	
PROTOCOL 4.7 Drying the Gel — 76	
☢ PROTOCOL 4.8 Separation of Low Molecular Weight Proteins by Tricine-SDS-PAGE (TSDS-PAGE) — 77	
Safety Considerations	79
B. 2-Dimensional (2-D) Gel Systems	79
Isoelectric Focusing	81
☢ PROTOCOL 4.9 Preparation of the Sample for Isoelectric Focusing — 83	
Single-Step Extraction/Solubilization Buffer	84
☢ PROTOCOL 4.10 Preparation and Running of Isoelectric Focusing Tube Gels — 84	
☢ PROTOCOL 4.11 Equilibration of the First-Dimension Gel or Strip — 86	
Flaws with 2-D Analysis	86
☢ PROTOCOL 4.12 Measuring the pH of the Gel Slices — 87	
☢ PROTOCOL 4.13 Nonequilibrium pH Gradient Electrophoresis (NEPHGE) — 89	
PROTOCOL 4.14 2-D Gels—The Second Dimension: SDS-PAGE — 90	
Fluorescence Two-Dimensional Difference Gel Electrophoresis (2-D DIGE)	91
PROTOCOL 4.15 Labeling Proteins with Cyanine Dyes (Cy3 and Cy5) — 91	
2-D PAGE Databases	92
PROTOCOL 4.16 Nonreducing-Reducing 2-D Gels — 92	

C. Detection of Protein Bands in Polyacrylamide Gels	95
PROTOCOL 4.17 Staining and Destaining the Gel with Coomassie Blue — 96	
PROTOCOL 4.18 Coomassie Staining Using GelCode® Blue — 98	
PROTOCOL 4.19 Staining Gels with SYPRO® Ruby — 98	
Viewing and Imaging a SYPRO Ruby-Stained 1-D or 2-D Gel	99
PROTOCOL 4.20 Silver Staining — 100	
PROTOCOL 4.21 Reversible Negative Staining of Proteins in Gels with Imidazole and Zinc Salts — 101	
PROTOCOL 4.22 Molecular Weight Determination by SDS-PAGE — 103	
D. Recovery of Proteins from the Gel	105
☢ PROTOCOL 4.23 Excising the Protein Band from the Dried Gel — 105	
PROTOCOL 4.24 Extracting the Target Protein from the Dried Gel — 106	
E. Identification of Enzyme Activity in Polyacrylamide Gels	106
General Considerations	107
☣ PROTOCOL 4.25 Localization of Proteases: Copolymerization of Substrate in the Separating Gel — 107	
☣ PROTOCOL 4.26 Identification of Protease Inhibitors: Reverse Zymography — 108	
PROTOCOL 4.27 Locating the Enzyme Activity: Reacting the Gel with Substrate Solution after Electrophoresis — 109	
PROTOCOL 4.28 Detection of β -glucuronidase Activity in Polyacrylamide Gels — 110	
Identification of DNA Binding Proteins—Gel Shift Assay	111
☢ PROTOCOL 4.29 Gel Shifts — 112	
 Chapter 5 Getting Started with Protein Purification	 118
Introduction	118
A. Making a Cell Free Extract	120
Cellular Disruption	121
Extraction Buffer Composition	122
Protease Inhibitors	122
Methods of Cell Disruption	123
Clarification of the Extract	125
PROTOCOL 5.1 Nuclear Extracts — 126	
PROTOCOL 5.2 Total Lymphocyte Extract — 127	
PROTOCOL 5.3 Subcellular Fractionation — 127	
Subcellular Markers	128
B. Protein Quantitation	128
The Bradford Method	130
☢ PROTOCOL 5.4 Bradford Standard Assay — 130	

PROTOCOL 5.5	Bradford Microassay — 132	
PROTOCOL 5.6	Protein Determination Using Bicinchoninic Acid (BCA) — 133	
Compatible Substances for the BCA Protein Assay	134
Incompatible Substances	134
PROTOCOL 5.7	NanoOrange® Protein Quantitation Assay: A Fluorescence-Based Assay of Proteins in Solution — 135	
C. Manipulating Proteins in Solution	136
Stabilization and Storage of Proteins	136
Concentrating Proteins from Dilute Solutions	137
PROTOCOL 5.8	Recovery of Protein by Ammonium Sulfate Precipitation — 138	
Ultrafiltration	138
Lyophilization	140
Dialysis	140
PROTOCOL 5.9	Preparation of Dialysis Tubing — 141	
Changing the Buffer by Gel Filtration	142
D. Precipitation Techniques	143
PROTOCOL 5.10	Salting Out with Ammonium Sulfate — 143	
PROTOCOL 5.11	Precipitation with Acetone — 145	
Precipitation with Polyethylene Glycol (PEG)	146
PROTOCOL 5.12	PEG Precipitation — 146	
PROTOCOL 5.13	Removal of PEG from Precipitated Proteins — 147	
Precipitation by Selective Denaturation	148
PROTOCOL 5.14	Recovery of Protein from Dilute Solutions by Methanol Chloroform Precipitation — 148	
PROTOCOL 5.15	Recovery of Protein by Trichloroacetic Acid (TCA) Precipitation — 149	
PROTOCOL 5.16	Concentration of Proteins by Acetone Precipitation — 150	
What to Do When All Activity Is Lost	150
Chapter 6 Membrane Proteins	153
Introduction	153
A. Peripheral Membrane Proteins	154
PROTOCOL 6.1	Alkali Extraction — 156	
PROTOCOL 6.2	High pH Membrane Fractionation — 157	
B. Integral Membrane Proteins	158
Organic Alcohol Extraction of Peripheral Membrane Proteins	158

PROTOCOL 6.3	Butanol Extraction — 159	
PROTOCOL 6.4	Single-Phase Butanol Extraction — 159	
C. Detergents		160
Properties of Detergents		165
Critical Micelle Concentration (CMC)		165
Micelle Molecular Weight		165
Hydrophile-Lipophile Balance (HLB)		166
Classification of Detergents		166
Ionic Detergents		166
Nonionic Detergents		166
Bile Salts		166
Detergent Solubilization		167
Choosing a Detergent		168
Choice of Initial Conditions		169
PROTOCOL 6.5	Differential Detergent Solubilization — 169	
☢ PROTOCOL 6.6	Solubilization Trial — 171	
Protein-to-Detergent Ratio		172
Detergent Removal		173
Removal of Ionic Detergents		173
Removal of Nonionic Detergents		173
Extracti-Gel® D		173
Chapter 7	Transfer and Detection of Proteins on	
	Membrane Supports	177
Introduction		177
A. Transfer of Proteins to Membrane Supports		177
PROTOCOL 7.1	Transfer of Proteins to Nitrocellulose or Polyvinylidene Difluoride — 178	
Troubleshooting Western Blots		181
PROTOCOL 7.2	Enhanced Capture of Small Histidine Containing Polypeptides on Membranes in the Presence of ZnCl ₂ — 181	
PROTOCOL 7.3	Dot Blots — 182	
PROTOCOL 7.4	Thin-Layer Chromatography Blotting — 182	
B. Staining the Blot		183
PROTOCOL 7.5	Total Protein Staining with India Ink — 183	
PROTOCOL 7.6	Reversible Staining with Ponceau S — 184	
PROTOCOL 7.7	Irreversible, Rapid Staining with Coomassie Brilliant Blue — 184	
PROTOCOL 7.8	Staining Immobilized Glycoproteins by Periodic Acid/Schiff (PAS) — 185	

C. Recovery of Proteins from the Blot	186
PROTOCOL 7.9 Recovery of Proteins Using an Organic Solvent System — 186	
PROTOCOL 7.10 Recovery of Proteins from the Blot Using a Detergent-Based Solvent System — 187	
PROTOCOL 7.11 Blocking the Blot — 188	
PROTOCOL 7.12 Exposing the Blot to Primary Antibody — 188	
Ligand Blotting	190
Southwestern Blotting	191
Far Western Blotting	191
⊕ PROTOCOL 7.13 Ligand Binding — 192	
PROTOCOL 7.14 Lectin Blotting — 193	
PROTOCOL 7.15 Bacterial Protein Overlay Analysis — 195	
D. Detection of the Target Protein	196
Protein A	196
Second Antibody Conjugate	197
Biotin Avidin System	197
PROTOCOL 7.16 Biotinylation of Proteins — 197	
PROTOCOL 7.17 Purifying and Biotinylating Antibodies from Immunoblots — 198	
Enzymatic Detection Methods	199
Horseradish Peroxidase	199
⊕ PROTOCOL 7.18 Colorimetric Detection with Diaminobenzidine, 3,3',4,4'-tetraaminobiphenyl) (DAB) — 199	
PROTOCOL 7.19 Colorimetric Detection Using Alkaline Phosphatase — 200	
PROTOCOL 7.20 Enhanced Chemiluminescence — 201	
PROTOCOL 7.21 Stripping and Reprobing the Blot — 203	
Detection of Radiolabeled Proteins	203
⊕ PROTOCOL 7.22 Direct Autoradiography — 204	
Removing Unwanted Background Signal from X-ray Film	205
⊕ PROTOCOL 7.23 Phosphorimaging — 207	
Chapter 8 Identification of the Target Protein	211
Introduction	211
A. Peptide Mapping	211
PROTOCOL 8.1 Thermal Denaturation — 212	
Preparing the Target Protein for Digestion	213
B. Enzymatic Cleavage of Proteins	214
⊕ PROTOCOL 8.2 Peptide Mapping by Proteolysis and Analysis by Electrophoresis — 215	
Cleavage of Proteins Transferred to PVDF or NC Membranes	217
PROTOCOL 8.3 Cleavage of Proteins Immobilized on the Membrane — 217	

PROTOCOL 8.4 Tryptic Cleavage of Protein Eluted from PVDF Membrane — 218	
C. Chemical Cleavage	219
☉ PROTOCOL 8.5 Cyanogen Bromide Cleavage of Proteins on PVDF Membrane — 219	
☉ PROTOCOL 8.6 N-Chlorosuccinimide (NCS) Mapping — 221	
PROTOCOL 8.7 Hydroxylamine Cleavage of Proteins in Polyacrylamide Gels — 222	
PROTOCOL 8.8 Formic Acid Cleavage — 223	
☉ PROTOCOL 8.9 Chemical Cleavage at Cysteine Residues with DTNB — 224	
D. Microsequencing from PVDF Membranes	225
When, and In What Form Do You Submit the Target Protein to the Protein Sequencing Core?	227
☉ PROTOCOL 8.10 Transferring Spots from 2-D Gels to PVDF Membranes — 228	
Sequencing Glycopeptides	228
☉ PROTOCOL 8.11 Protein Hydrolysis: Total Amino Acid Composition of the Target Protein — 229	
Identifying Proteins Using Mass Spectrometry	230
Preparation of Proteins for MS Analysis	231
Partial Proteolysis	231
☉ PROTOCOL 8.12 In-gel Tryptic Digestion — 232	
☉ PROTOCOL 8.13 Extraction of Peptides from Gel Pieces Containing Integral Membrane Proteins — 233	
Elution of Target Protein from SDS-PAGE	233
Protein Transfer to a Membrane	234
Considerations	234
MS Basics	234
Electrospray and Tandem Mass Spectrometry	235
MALDI and Peptide-Mass Mapping	237
Post-Source Decay (PSD) MALDI-MS	237
Protein Identification by MS	238
Peptide Mass Fingerprint Analysis	238
Peptide Fragmentation	239
Peptide Ladder Sequencing	240
Peptide Sequence Tag	240
Identification of a Gene Product	240
Posttranslational Modifications and MS	241
Caveats When Using MS	243
Protein Database Searches	244
Bioinformatics	244

The Rise of Biological Databases	245
Search Engines	245
Databases	246
Identifying a Target Protein	248
Gene Analysis Tools	248
Subcellular Localization	249
Protein Domain Families	250
Structural Classification of Proteins	251
Genomic Organization	252
Additional Useful Sites on the Internet	252
PCR Primer Design Programs	252
Microarrays and Data Mining—the Challenge of Data Analysis	253

Chapter 9 Identifying and Analyzing Posttranslational Modifications	259
Introduction	259
A. Glycosylation	261
PROTOCOL 9.1 Chemical Deglycosylation Using Trifluoromethanesulfonic Acid (TFMS) — 265	
N-Glycosylation	267
PROTOCOL 9.2 Removal of the Oligosaccharide from the Glycoprotein with N-Glycanase — 267	
PROTOCOL 9.3 N-glycosidase F (GPase F) Treatment of Glycoproteins in Immunoprecipitates — 268	
☣ ☣ PROTOCOL 9.4 Tunicamycin — 269	
O-Glycosylation	270
PROTOCOL 9.5 Identification of O-Glycosylated Amino Acids by Alkaline β -Elimination — 270	
PROTOCOL 9.6 β -Elimination of O-Glycans from Glycoproteins Immobilized on Blots — 271	
PROTOCOL 9.7 O-Glycosidase — 271	
PROTOCOL 9.8 O-Glycanase — 272	
Combined Use of N-Glycanase and O-Glycanase	272
PROTOCOL 9.9 Endoglycosidase H — 272	
Neuraminidase (NA)	273
PROTOCOL 9.10 Desialylation with <i>Clostridium perfringens</i> Neuraminidase — 274	
☣ PROTOCOL 9.11 Desialylation with <i>Arthrobacter ureafaciens</i> Neuraminidase — 275	
Lectins as Tools for Carbohydrate Analysis	276
Proteoglycans	277
☣ PROTOCOL 9.12 Is the Target Protein a Proteoglycan? — 277	

B. Phosphorylation	278
☢ PROTOCOL 9.13 Metabolic Labeling of Cells with [³² P]orthophosphate —	280
☢ PROTOCOL 9.14 Can the Target Protein Be Phosphorylated? —	281
PROTOCOL 9.15 Determination of the Type of Phosphorylated Amino Acid- Immunoblotting with Anti-Phosphoamino Acid Antibodies —	282
☢ PROTOCOL 9.16 Phosphorylation of Membrane Proteins with [γ- ³² P]GTP —	283
Enzymatic Dephosphorylation	283
PROTOCOL 9.17 Potato Acid Phosphatase —	283
PROTOCOL 9.18 Alkaline Phosphatase —	285
☢ PROTOCOL 9.19 Immune Complex Kinase —	285
☢ PROTOCOL 9.20 Renaturation of Immobilized Kinases on PVDF Membranes —	287
☢ PROTOCOL 9.21 Phosphorylation of Substrates in SDS-Gels —	289
Phosphopeptide and Phosphoamino Acid Analysis	290
☢ PROTOCOL 9.22 One-Dimensional Phosphopeptide Mapping —	291
☢ Two-Dimensional Phosphopeptide Mapping —	291
☢ PROTOCOL 9.23 Isolation of Phospho-Proteins from SDS Gels: Preparation for Phosphopeptide Mapping —	292
PROTOCOL 9.24 Tryptic Digestion of Isolated Phosphoproteins —	293
PROTOCOL 9.25 Applying the Sample to the TLC Plate and Electrophoresis in the First Dimension —	294
PROTOCOL 9.26 Second Dimension: Thin-Layer Chromatography —	295
PROTOCOL 9.27 Isolation of Individual Phosphopeptides from TLC Plates —	296
☢ PROTOCOL 9.28 Phosphoamino Acid Analysis —	296
☢ PROTOCOL 9.29 Phosphoamino Acid Analysis of Phosphoproteins Isolated from PVDF Membranes —	298
☢ PROTOCOL 9.30 Identification of Phosphohistidine Residues Following Heat Treatment —	299
☢ PROTOCOL 9.31 Treatment with Diethyl Pyrocarbonate —	300
☢ PROTOCOL 9.32 Treatment of Phosphorylated Membranes with HCl and NaOH —	300
☢ PROTOCOL 9.33 Sulfation —	301
C. Lipid Modification of Proteins	302
Palmitoylation and N-Myristoylation of Proteins	303
Analysis of Bound Fatty Acids	304
☢ PROTOCOL 9.34 Identification of Palmitoylated and Myristoylated Proteins —	304
Isoprenylation	305
☢ PROTOCOL 9.35 Metabolic Labeling with [³ H]Mevalonic Acid Derivatives —	306
☢ PROTOCOL 9.36 Enzymatic Prenylation of Recombinant Proteins —	308
Glypiation	308
Is the Target Protein Glycosyl Phosphatidylinositol Anchored?	310
Use of Triton X-114	310

PROTOCOL 9.37	Preparation of Triton X-114 — 310	
PROTOCOL 9.38	Fractionation of Integral Membrane Proteins with Triton X-114 — 311	
PROTOCOL 9.39	Digestion with Phosphatidylinositol Specific Phospholipase C (PI-PLC) — 312	
	Metabolic Labeling with Precursors of the GPI Structure	313
	Use of Anti-CRD	313
D.	Selected Modifications	314
	Transamidation	314
	Acetylation	314
	Methylation	314
	Hydroxylation of Proline and Lysine	315
	Degradation	315
	Ubiquitination	315
	Proteolytic Processing	316
Chapter 10	Chromatography	324
	Introduction	324
A.	Important Terminology Used in Chromatography	325
B.	Gel Filtration Chromatography	326
	Choice of Buffer	329
	Choice of Column Size	329
	PROTOCOL 10.1 Preparation of the Gel: Hydrating and Degassing — 330	
	PROTOCOL 10.2 Packing the Column — 331	
	Flow Rate	332
	Hydrostatic Pressure	333
	Sample Application	334
	PROTOCOL 10.3 Loading Sample onto a Drained Bed — 334	
	Loading Sample Under the Eluent	335
	Making Sure the Column Does Not Run Dry	335
	Molecular Weight Determination	335
	Spin Columns Used in Gel Filtration	336
	PROTOCOL 10.4 Spin Columns — 337	
	PROTOCOL 10.5 Testing Fractions to Locate Protein: Bradford Spot Test — 338	
C.	Introduction to HPLC	338
	Packing Materials	339
	Column Designs	340
	Column Guards	340
	Detectors	340

Choosing the Right Conditions—Some Helpful Tips	341
HPLC—Size Exclusion	342
D. Ion Exchange Chromatography: Separation on the Basis of Charge	342
Simplified Theory of Ion Exchange	343
Functional Groups on Exchange Columns	344
Choice of Exchanger Matrix	345
Preparation of the Exchanger	346
Choice of Buffer	346
Batch Adsorption	347
PROTOCOL 10.6 Selecting the Starting pH — 348	
PROTOCOL 10.7 Packing an Ion Exchange Column — 348	
Experimental Tips	349
Elution-Step or Linear Gradient?	349
PROTOCOL 10.8 Regeneration of Sephadex Ion Exchangers — 351	
PROTOCOL 10.9 Regeneration of Sepharose Ion Exchangers — 351	
PROTOCOL 10.10 Chromatofocusing — 351	
Removing the Polybuffer	353
HPLC-Ion Exchange Chromatography	353
Membrane Adsorbers	354
Perfusion Chromatography	355
E. Hydrophobic Interaction Chromatography (HIC)	356
Simplified Theory of HIC	356
PROTOCOL 10.11 Protein Fractionation by HIC — 357	
PROTOCOL 10.12 Solid Phase Extraction Cartridges — 358	
Reversed Phase HPLC	359
Reversed Phase HPLC for the Isolation of Peptides	361
Multidimensional Liquid Chromatography	362
F. Affinity Chromatography	363
Immunoaffinity Purification	364
PROTOCOL 10.13 Direct Antibody Coupling to Protein A Beads — 365	
PROTOCOL 10.14 Indirect Antibody Coupling to Protein A Beads — 366	
PROTOCOL 10.15 Preparation of Affinity Columns — 367	
Flow Rate	368
Binding Antigens to Immunoaffinity Matrices	369
Nonspecific Interactions	369
PROTOCOL 10.16 Blocking the Affinity Matrix — 370	
Elution of Antigens from Immunoaffinity Matrices	370
PROTOCOL 10.17 Eluting the Antigen — 372	
Ligand Affinity Chromatography	373

PROTOCOL 10.18 Immobilization of Proteins to *N*-Hydroxysuccinimide Ester Derivatives of Agarose — 373

Toluene Sulfonyl Chloride (Tosyl Chloride) 375

Pseudo-Affinity Adsorbents 375

Lectin Affinity Chromatography 377

PROTOCOL 10.19 Glycoprotein Purification Using Wheat Germ Agglutinin (WGA) — 378

PROTOCOL 10.20 Immobilized Metal Chelate Chromatography (IMAC) — 379

PROTOCOL 10.21 Purifying a Histidine Tagged Recombinant Fusion Protein — 380

PROTOCOL 10.22 Hydroxylapatite Chromatography — 381

Chapter 11 Recombinant Protein Techniques 385

Introduction 385

Recombinant Protein for Antibody Production 386

Protein for Biochemical or Cell Biological Studies 387

A. *In vitro* Transcription and Translation 388

PROTOCOL 11.1 Preparation of the DNA Template — 388

PROTOCOL 11.2 *In vitro* Transcription—Preparation of the mRNA — 389

PROTOCOL 11.3 Guanylyltransferase Catalyzed Addition of a G(5')ppp(5')G Cap to mRNA — 390

☉ PROTOCOL 11.4 *In vitro* Translation: Protein Synthesis — 391

☉ PROTOCOL 11.5 Cotranslational Processing Using Canine Pancreatic Microsomal Membranes — 392

PROTOCOL 11.6 Translocated Products Are Resistant to Protease Digestion — 393

PROTOCOL 11.7 Was the Translational Product Glycosylated? Endoglycosidase H (Endo H) Analysis — 394

Protein Transduction: A Method for Introducing Exogenous Proteins into Cells 395

B. Recombinant Gene Products in *E. coli*: Expression, Identification and Characterization 396

Expression and Purification of *lacZ* and *trpE* Fusion Proteins 397

PROTOCOL 11.8 *lacZ* Induction — 398

PROTOCOL 11.9 Induction of the *trpE* Fusion Protein — 398

PROTOCOL 11.10 Preparation of the Protein Extract — 399

PROTOCOL 11.11 Solubilization of the Fusion Protein — 400

PROTOCOL 11.12 Purification of Eukaryotic Proteins from Inclusion Bodies in *E. coli* — 400

C. Affinity Tags 402

Removing the Tag 403

Glutathione-S-Transferase (GST) Fusion Proteins	404
PROTOCOL 11.13 Production and Analysis of GST Fusion Protein Transformants (Small Scale) — 405	
PROTOCOL 11.14 Purification of GST Fusion Proteins — 406	
PROTOCOL 11.15 Removing the GST from the Fusion Protein — 407	
PROTOCOL 11.16 His-Tag Purification System — 408	
Maltose Binding Protein (MBP) Fusion Proteins	410
Staphylococcal Protein A and ZZ	413
Green Fluorescent Protein (GFP)	415
D. Expression of Foreign Proteins in Eukaryotic Cells	415
Expression and Isolation of Recombinant Proteins from Yeast	415
PROTOCOL 11.17 Preparation of Protein Extracts from Yeast — 416	
Expression of Proteins in Insect Cells Using Baculoviral Vectors	418
Expression of Foreign Proteins in Mammalian Cells	419
Transfection: Expression of Recombinant Proteins in Eukaryotic Systems	420
PROTOCOL 11.18 Transfection of DNA into Eukaryotic Cells with Calcium Phosphate — 421	
PROTOCOL 11.19 Glycerol Shock — 422	
PROTOCOL 11.20 Transfection Using DEAE-Dextran — 423	
PROTOCOL 11.21 Stable Transfections — 424	
PROTOCOL 11.22 Picking Stable Colonies — 425	
Appendices	431
A. Safety Considerations	433
First Aid: Emergency Procedures	433
B. Antibody Preparation	435
Production of Polyclonal Antisera in Rabbits	435
PROTOCOL B.1 Preparation of the Antigen-Adjuvant Emulsion — 436	
PROTOCOL B.2 Intramuscular Immunization (IM) — 436	
PROTOCOL B.3 Intradermal Immunization — 437	
PROTOCOL B.4 Subcutaneous Immunization — 437	
PROTOCOL B.5 Bleeding the Rabbit and Serum Preparation — 437	
PROTOCOL B.6 Precipitation of IgG with Saturated Ammonium Sulfate — 438	
Purification of Antibody Using Protein A Affinity Columns	439
PROTOCOL B.7 Purifying Total Ig — 439	
Numbering Mice	440
C. Solutions	441
Commercial Strengths of Common Laboratory Chemicals	441
Water	441

Molarity	442
Choosing and Preparing Buffers	442
Common Laboratory Solutions	447
Extinction Coefficients	449
D. Nucleic Acids	451
Spectrophotometric Conversions	451
DNA/Protein Conversions	451
Oligonucleotide Concentrations	451
PROTOCOL D.1 Fluorometric Estimation of DNA Concentrations —	451
RNA Precipitation	452
E. Modifications and Motifs	456
Nomenclature	456
Protein Modification Sequences	458
Protein Kinase Recognition Sequence Motifs	460
Subcellular Localization Motifs	461
Protein Databases	462
F. Centrifugation	465
Nomogram	467
General Purpose Centrifuge Rotors	469
Ultracentrifuge Rotors	473
G. Proteases and Proteolytic Enzyme Inhibitors	480
Commonly Used Proteases	480
PROTOCOL G.1 Preparation of Defatted BSA —	482
Protease Inhibitors	483
H. Radioactivity	490
Manual and Machine Film Processing	491
I. Tissue Culture	493
Transwell Permeable Supports	493
J. Miscellaneous	495
PROTOCOL J.1 Siliconizing Glassware —	495
Unit Prefixes	495
The Greek Alphabet	496
Abbreviations	496
HPLC Pump Pressure Conversion	499

xxvi Contents

Dipeptide Masses	500
Mass Differences Considered in Molecular Weight Analysis of Proteins	501
K. List of Suppliers, Vendors, Manufacturers	502
Index	511



<http://www.springer.com/978-0-8176-4340-9>

Protein Analysis and Purification

Benchtop Techniques

Rosenberg, I.M.

2005, XXVI, 520 p., Hardcover

ISBN: 978-0-8176-4340-9

A product of Birkhäuser Basel