

Contents

Foreword *XIX*

Abbreviations, Symbols, Units *XXI*

Preface *XXV*

Part I Fundamentals *1*

Introduction *1*

Principle *1*

Areas of Applications *3*

The Sample *3*

The Buffer *4*

Electroendosmosis *5*

References *6*

1	Electrophoresis	7
1.1	General	7
1.1.1	Electrophoresis in Free Solution	7
1.1.2	Electrophoresis in Supporting Media	12
1.1.3	Gel Electrophoresis	13
1.1.3.1	Gel Types	13
1.1.3.2	Instrumentation for Gel Electrophoresis	17
1.1.3.3	Current and Voltage Conditions	17
1.1.4	Power Supply	19
1.1.5	Separation Chambers	20
1.1.5.1	Vertical Systems	20
1.1.5.2	Horizontal Systems	21
1.2	Electrophoresis in Nonrestrictive Gels	25
1.2.1	Agarose Gel Electrophoresis	25
1.2.1.1	Zone Electrophoresis	25
1.2.1.2	Immuno-electrophoresis	26
1.2.1.3	Affinity Electrophoresis	27
1.2.2	Polyacrylamide Gel Electrophoresis of Low Molecular Weight Substances	28
1.3	Electrophoresis in Restrictive Gels	28

1.3.1	The Ferguson Plot	28
1.3.2	Agarose Gel Electrophoresis	29
1.3.2.1	Proteins	29
1.3.2.2	Nucleic Acids	29
1.3.3	Pulsed-Field Gel Electrophoresis	30
1.3.4	Polyacrylamide Gel Electrophoresis of Nucleic Acids	32
1.3.4.1	DNA Sequencing	32
1.3.4.2	DNA Typing	34
1.3.4.3	Mutation Detection Methods	35
1.3.4.4	Denaturing PAGE of Microsatellites	37
1.3.4.5	Two-dimensional DNA Electrophoresis	37
1.3.5	Polyacrylamide Gel Electrophoresis of Proteins	37
1.3.5.1	Disc Electrophoresis	37
1.3.5.2	Gradient Gel Electrophoresis	39
1.3.5.3	SDS Electrophoresis	40
1.3.5.4	Cationic Detergent Electrophoresis	47
1.3.5.5	Blue Native Electrophoresis	47
1.3.5.6	Rehydrated Polyacrylamide Gels	48
1.3.5.7	Two-Dimensional Electrophoresis Techniques	49
1.3.5.8	GeLC-MS	50
	References	51

2	Isotachopheresis	57
2.1	Migration with the Same Speed	57
2.2	"Ion Train" Separation	59
2.3	Zone Sharpening Effect	59
2.4	Concentration Regulation Effect	59
2.5	Quantitative Analysis	60
	References	61

3	Isoelectric Focusing	63
3.1	Principles	63
3.2	Gels for IEF	65
3.2.1	Polyacrylamide Gels	65
3.2.2	Agarose Gels	67
3.3	Temperature	68
3.4	Controlling the pH Gradient	68
3.5	Kinds of pH Gradients	69
3.5.1	Free Carrier Ampholytes	69
3.5.1.1	Electrode Solutions	70
3.5.1.2	Denaturing IEF: Urea IEF	71
3.5.1.3	Separator IEF	72
3.5.1.4	Plateau Phenomenon	73
3.5.1.5	The Workflow of a Carrier Ampholyte IEF Run	73
3.5.2	Immobilized pH Gradients (IPG)	73

3.5.2.1	Preparation of Immobilized pH Gradients	75
3.5.2.2	Applications of Immobilized pH Gradients	76
3.6	Protein Detection in IEF Gels	77
3.7	Preparative Isoelectric Focusing	77
3.7.1	Carrier Ampholyte IEF in Gel	77
3.7.2	Carrier Ampholyte IEF in Free Solution	78
3.7.3	Immobilized pH Gradients	78
3.7.3.1	Isoelectric Membranes	78
3.7.3.2	Off-Gel IEF	79
3.8	Titration Curve Analysis	80
	References	82
4	High-Resolution Two-Dimensional Electrophoresis	85
4.1	IEF in Immobilized pH Gradient Strips	85
4.1.1	Strip Lengths	86
4.1.2	pH Gradient Types	86
4.1.3	The Influence of Salts and Buffer Ions on the Separation	87
4.1.4	Basic IPG Gradients	88
4.1.5	Advantages of Immobilized pH Gradient Strips in 2D Electrophoresis	89
4.1.6	Rehydration of IPG Strips	90
4.1.6.1	Basic pH Gradients	90
4.1.6.2	Reswelling Tray	91
4.1.6.3	Cover Fluid	91
4.1.6.4	Rehydration Time	92
4.1.7	Sample Application on IPG Strips	92
4.1.8	IEF Conditions	95
4.1.8.1	Electrode Pads	95
4.1.8.2	Temperature	95
4.1.8.3	Electric Conditions	95
4.1.8.4	Time	96
4.1.9	Instrumentation	96
4.1.9.1	The Strip Tray Accessory	97
4.1.9.2	Dedicated Instruments for IPG Strips	97
4.1.9.3	Running IEF in IPG Strips	97
4.2	SDS-PAGE	98
4.2.1	Equilibration of the IPG Strips	98
4.2.2	Technical Concepts for the Second Dimension (SDS-PAGE)	99
4.2.2.1	Vertical Set-ups	99
4.2.2.2	Horizontal Set-ups	99
4.2.3	Gel Types	101
4.2.3.1	Gel Sizes	101
4.2.3.2	Vertical Gels	101
4.2.3.3	Horizontal Gels	102

4.2.4	Gel Casting	102
4.2.4.1	Gels for Multiple Vertical Systems	102
4.2.4.2	Gels for Horizontal Systems	104
4.2.5	Running the SDS Gels	105
4.2.5.1	Vertical Systems	105
4.2.5.2	Horizontal Systems	106
4.3	Proteomics	106
	References	108
5	Protein Sample Preparation	111
5.1	Protein Quantification Methods	111
5.2	Preparation of Native Samples	112
5.3	Samples for SDS Electrophoresis	113
5.3.1	SDS Treatment	113
5.3.1.1	Nonreducing SDS Treatment	114
5.3.1.2	Reducing SDS Treatment	115
5.3.1.3	Reducing SDS Treatment with Subsequent Alkylation	116
5.3.2	Clean-up and Protein Enrichment	117
5.3.2.1	Precipitation	117
5.3.2.2	Protein Enrichment by Affinity Beads	118
5.4	Samples for High-Resolution 2D PAGE	118
5.4.1	Cell Washing	119
5.4.2	Cell Disruption	119
5.4.3	Sample Acquisition and Storage	119
5.4.4	Protease Inactivation	122
5.4.5	Phosphatase Inactivation	122
5.4.6	Alkaline Conditions	123
5.4.7	Removal of Contaminants	123
5.4.7.1	Precipitation Methods	123
5.4.7.2	Affinity Beads	125
5.4.8	Prefractionation	125
5.4.8.1	Depletion of Highly Abundant Proteins	125
5.4.8.2	Equalizer Technology	125
5.4.8.3	Preseparation of Cell Organelles	126
5.4.8.4	Prefractionation according to Isoelectric Points	126
5.4.9	Special Case: Plant Proteins	127
	References	127
6	Protein Detection	131
6.1	Fixation	131
6.1.1	IEF Gels	132
6.1.2	Agarose Gels	132
6.1.3	SDS Polyacrylamide Gels	132
6.2	Poststaining Methods	133
6.2.1	Organic Dyes	133

6.2.1.1	Monodisperse Coomassie Brilliant Blue Staining	133
6.2.1.2	Colloidal Coomassie Brilliant Blue Staining	133
6.2.1.3	Acid Violet 17 Staining for IEF Gels	134
6.2.2	Silver Staining	134
6.2.2.1	Colloidal Silver Staining	134
6.2.2.2	Silver Nitrate Staining	134
6.2.2.3	Ammoniacal Silver Staining	135
6.2.3	Negative Staining	136
6.2.3.1	Copper Staining	136
6.2.3.2	Imidazole Zinc Staining	136
6.2.4	Fluorescent Staining	136
6.2.5	Specific Detection	138
6.2.5.1	Proteins with Posttranslational Modifications	138
6.2.5.2	Isoenzymes	139
6.2.6	Stain-Free Technology	140
6.3	Prelabeling	140
6.3.1	Prelabeling with Fluorescent Tags	140
6.3.2	Radioactive Labeling of Living Cells	141
6.3.3	Labeling with Stable Isotopes	141
6.4	Difference Gel Electrophoresis (DIGE)	143
6.4.1	Minimum Lysine Labeling	143
6.4.2	Saturation Cysteine Labeling	144
6.4.3	The Internal Standard	146
6.4.4	Experimental Design	147
6.4.5	Major Benefits of 2D DIGE	147
6.4.6	Specific Labeling of Cell-Surface Proteins	148
6.4.7	Comparative Fluorescence Gel Electrophoresis	148
6.5	Imaging, Image Analysis, Spot Picking	149
6.5.1	Quantitative Evaluation	149
6.5.1.1	Quantification Prerequisites	149
6.5.1.2	Critical Issues in Quantification	150
6.5.2	Imaging Systems	151
6.5.2.1	Optical Density	152
6.5.2.2	Densitometry	152
6.5.2.3	CCD Cameras	153
6.5.3	Image Analysis	154
6.5.3.1	One-Dimensional Gel Software	155
6.5.3.2	Two-Dimensional Gel Software	156
6.5.4	Protein Identification and Characterization	158
6.5.4.1	Spot-Picking	159
	References	160
7	Blotting	165
7.1	Transfer Methods	165
7.1.1	Diffusion Blotting	165

7.1.2	Capillary Blotting	165
7.1.3	Pressure Blotting	166
7.1.4	Vacuum Blotting	167
7.1.5	Electrophoretic Blotting	168
7.1.5.1	Tank Blotting	168
7.1.5.2	Semidry Blotting	169
7.1.5.3	Electrophoretic Blotting of Film-Backed Gels	171
7.2	Blotting Membranes	171
7.3	Buffers for Electrophoretic Transfers	172
7.3.1	Proteins	172
7.3.1.1	Tank Blotting	172
7.3.1.2	Semidry Blotting	173
7.3.2	Nucleic Acids	174
7.3.2.1	Tank Blotting	174
7.3.2.2	Semidry Blotting	174
7.4	General Staining	174
7.5	Blocking	175
7.6	Specific Detection	175
7.6.1	Hybridization	175
7.6.2	Enzyme Blotting	176
7.6.3	Immunoblotting	176
7.6.4	Lectin Blotting	179
7.6.5	Stripping, Reprobing	179
7.6.6	Double Blotting	180
7.7	Protein Sequencing	180
7.8	Transfer Issues	180
7.9	Electro-Elution of Proteins from Gels	181
	References	183

Part II Equipment and Methods 187

Equipment	187
Methods	187
Small Molecules	187
Proteins	187
DNA	188
Instrumentation	188
Accessories	189
Consumables	190

8 Special Laboratory Equipment 191

9 Consumables 193

10 Chemicals 195

10.1 Reagents 195

Method 1	PAGE of Dyes	197
M1.1	Sample Preparation	197
M1.2	Stock Solutions	197
M1.3	Preparing the Casting Cassette	198
M1.3.1	Gasket	198
M1.3.2	Slot-Former	198
M1.3.3	Assembling the Gel Cassette	199
M1.4	Casting Ultra-Thin-Layer Gels	200
M1.5	Electrophoretic Separation	201
M1.5.1	Removing the Gel from the Cassette	201
Method 2	Agarose and Immunoelectrophoresis	205
M2.1	Sample Preparation	205
M2.2	Stock Solutions	206
M2.3	Preparing the Gels	206
M2.3.1	Agarose Gel Electrophoresis	206
M2.3.1.1	Preparing the Slot-Former	207
M2.3.1.2	Assembling the Gel Cassette	207
M2.3.2	Immunoelectrophoresis Gels	209
M2.3.2.1	Punching Out the Sample Wells and Troughs	210
M2.4	Electrophoresis	211
M2.4.1	Grabar-Williams Technique	212
M2.4.2	Laurell Technique	212
M2.5	Protein Detection	214
M2.5.1	Coomassie Staining (Agarose Electrophoresis)	214
M2.5.2	Immunofixing of Agarose Electrophoresis	214
M2.5.3	Coomassie Staining (Immunoelectrophoresis)	215
M2.5.4	Silver Staining	215
	References	216
Method 3	Titration Curve Analysis	217
M3.1	Sample Preparation	217
M3.2	Stock Solutions	217
M3.3	Preparing the Blank Gels	218
M3.3.1	Preparing the Casting Cassette	218
M3.3.2	Assembling the Gel Cassette	219
M3.3.3	Filling the Gel Cassette	220
M3.3.4	Removing the Gel from the Cassette	221
M3.3.5	Washing the Gel	221
M3.4	Titration Curve Analysis	222
M3.4.1	Reswelling the Rehydratable Gel	222
M3.4.2	Formation of the pH Gradient	222
M3.4.3	Native Electrophoresis in the pH Spectrum	223
M3.5	Coomassie and Silver Staining	224
M3.5.1	Colloidal Coomassie Staining	224

M3.5.2	Acid Violet 17 Staining	224
M3.5.3	Five-Minute Silver Staining of Dried Gels	225
M3.6	Interpreting the Curves	225
	References	227

Method 4 Native PAGE in Amphoteric-Buffers 229

M4.1	Sample Preparation	230
M4.2	Stock Solutions	230
M4.3	Preparing the Empty Gels	231
M4.3.1	Slot-Former	231
M4.3.2	Assembling the Casting Cassette	232
M4.3.3	Polymerization Solutions	233
M4.3.4	Filling the Cooled Gel Cassette	234
M4.3.5	Removing the Gel from the Casting Cassette	234
M4.3.6	Washing the Gel	234
M4.4	Electrophoresis	235
M4.4.1	Rehydration in Amphoteric Buffers	235
M4.5	Coomassie and Silver Staining	240
M4.5.1	Colloidal Coomassie Staining	240
M4.5.2	Acid Violet 17 Staining	240
M4.5.3	Five-Minute Silver Staining of Dried Gels	241
	References	242

Method 5 Agarose IEF 243

M5.1	Sample Preparation	243
M5.2	Preparing the Agarose Gel	244
M5.2.1	Making the Spacer Plate Hydrophobic	244
M5.2.2	Assembling the Casting Cassette	244
M5.2.3	Preparation of Electrode Solutions	246
M5.3	Isoelectric Focusing	247
M5.4	Protein Detection	249
M5.4.1	Coomassie Blue Staining	249
M5.4.2	Immunofixation	249
M5.4.3	Silver Staining	250
	References	251

Method 6 PAGIEF in Rehydrated Gels 253

M6.1	Sample Preparation	253
M6.2	Stock Solutions	254
M6.3	Preparing the Blank Gels	254
M6.3.1	Making the Spacer Plate Hydrophobic	254
M6.3.2	Assembling the Casting Cassette	255
M6.3.3	Filling the Gel Cassette	256
M6.3.4	Removing the Gel from the Casting Cassette	257
M6.3.5	Washing the Gel	257

M6.4	Isoelectric Focusing	257
M6.4.1	Rehydration Solution with Carrier Ampholytes (SERVALYT™, Pharmalyte™)	257
M6.4.2	Reswelling the Gel	257
M6.4.3	Separation of Proteins	259
M6.4.4	Sample Application	259
M6.5	Coomassie and Silver Staining	260
M6.5.1	Colloidal Coomassie Staining	260
M6.5.2	Acid Violet 17 Staining	261
M6.5.3	Five-Minute Silver Staining of Dried Gels	261
M6.5.4	The Most Sensitive Silver Staining Procedure for IEF	262
M6.6	Perspectives	264
	References	266
Method 7	Horizontal SDS-PAGE	267
M7.1	Sample Preparation	267
M7.1.1	Nonreducing SDS Treatment	267
M7.1.2	Reducing SDS Treatment	268
M7.1.3	Reducing SDS Treatment with Alkylation	268
M7.2	Prelabeling with Fluorescent Dye	269
M7.2.1	Labeling	269
M7.2.2	Detection	269
M7.3	Stock Solutions for Gel Preparation	270
M7.4	Preparing the Casting Cassette	271
M7.4.1	Preparing the Slot-Former	271
M7.4.2	Assembling the Casting Cassette	272
M7.5	Gradient Gel	273
M7.5.1	Pouring the Gradient	273
M7.6	Electrophoresis	277
M7.6.1	Preparing the Separation Chamber	277
M7.6.2	Placing the Gel on the Cooling Plate	277
M7.6.3	Electrophoresis	278
M7.7	Protein Detection	279
M7.7.1	Hot Coomassie Staining	279
M7.7.2	Colloidal Staining	280
M7.7.2.1	Stock Solutions	280
M7.7.2.2	Fixation Solution	280
M7.7.2.3	Staining Solution	280
M7.7.2.4	Staining Procedure	281
M7.7.3	Reversible Imidazole–Zinc Negative Staining	281
M7.7.4	Silver Staining	281
M7.7.4.1	Blue Toning	282
M7.7.5	Fluorescent Staining with SERVA Purple	283
M7.7.5.1	Stock Solutions	283

M7.7.5.2	Staining Protocol	283
M7.7.5.3	Detection	284
M7.8	Blotting	284
M7.9	Perspectives	285
M7.9.1	Gel Characteristics	285
M7.9.2	SDS Electrophoresis in Washed and Rehydrated Gels	285
M7.9.3	SDS Disc Electrophoresis in a Rehydrated and Selectively Equilibrated Gel	285
M7.9.4	Peptide Separation	286
	References	287

Method 8 Vertical PAGE 289

M8.1	Sample Preparation and Prelabeling	290
M8.2	Stock Solutions for SDS- PAGE	290
M8.3	Single Gel Casting	291
M8.3.1	Discontinuous SDS-Polyacrylamide Gels	292
M8.3.2	Porosity Gradient Gels	293
M8.4	Multiple Gel Casting	295
M8.4.1	Multiple Discontinuous SDS Polyacrylamide Gels	296
M8.4.2	Multiple SDS Polyacrylamide Gradient Gels	298
M8.5	Electrophoresis	299
M8.5.1	Running Conditions	300
M8.6	SDS Electrophoresis of Small Peptides	301
M8.7	Blue Native PAGE	303
M8.8	Two-Dimensional Electrophoresis	306
M8.9	DNA Electrophoresis	307
M8.10	Long-Shelf-Life Gels	308
M8.11	Protein Detection	308
M8.12	Preparing Glass Plates with Bind-Silane	308
M8.12.1	Coating a Glass Plate with Bind-Silane	309
M8.12.2	Removal of Gel and Bind-Silane from a Glass Plate	309
	References	310

Method 9 Semidry Blotting of Proteins 311

M9.1	Transfer Buffers	313
M9.2	Technical Procedure	314
M9.2.1	Gels Without Support Film	315
M9.2.2	Gels on Film Backing	315
M9.2.2.1	Using a Nitrocellulose (NC) Blotting Membrane	316
M9.2.2.2	Using a PVDF Blotting Membrane	316
M9.2.2.3	Transfer from Cut-Off Gels	317
M9.3	Staining of Blotting Membranes	318
	References	320

Method 10 IEF in Immobilized pH Gradients 321

- M10.1 Sample Preparation 322
- M10.2 Stock Solutions 322
- M10.3 Immobiline Recipes 323
 - M10.3.1 Custom-Made pH Gradients 323
- M10.4 Preparing the Casting Cassette 327
 - M10.4.1 Making the Spacer Plate Hydrophobic 327
 - M10.4.2 Assembling the Casting Cassette 327
- M10.5 Preparing the pH Gradient Gels 328
 - M10.5.1 Pouring the Gradient 328
 - M10.5.1.1 Setting Up the Casting Apparatus 328
 - M10.5.1.2 Filling the Cassette 329
 - M10.5.1.3 Washing the Gel 331
 - M10.5.1.4 Storage 332
 - M10.5.1.5 Rehydration 332
- M10.6 Isoelectric Focusing 332
 - M10.6.1 Placing the Gel on the Cooling Plate 332
 - M10.6.2 Sample Application 335
 - M10.6.3 Electrode Solutions 335
 - M10.6.4 Focusing Conditions 335
 - M10.6.5 Measuring the pH Gradient 336
- M10.7 Staining 336
 - M10.7.1 Colloidal Coomassie Staining 336
 - M10.7.2 Acid Violet 17 Staining 337
 - M10.7.3 Staining Procedure 337
 - M10.7.4 Silver Staining 337
 - M10.7.5 Practical Tip 337
- M10.8 Strategies for IPG Focusing 337
 - References 339

Method 11 High-Resolution 2D Electrophoresis 341

- M11.1 Sample Preparation 342
 - M11.1.1 Sample Clean-Up 343
- M11.2 Prelabeling of Proteins with Fluorescent Dyes 346
 - M11.2.1 Labeling of One Sample 346
 - M11.2.2 DIGE Labeling 347
 - M11.2.2.1 Experimental Design 347
 - M11.2.2.2 Sample Preparation 347
 - M11.2.2.3 Reconstitution of the CyDyes 348
 - M11.2.2.4 Minimal Labeling of the Lysines 349
 - M11.2.2.5 Saturation Labeling of the Cysteines 350
 - M11.2.2.6 Preparation for Loading the Samples onto the IPG Strips 351
 - M11.2.2.7 Detection of DIGE Spots 352
- M11.3 Stock Solutions for Gel Preparation 352
- M11.4 Preparing the Gels 354

M11.4.1	IPG Strips	354
M11.4.2	SDS Polyacrylamide Gels	358
M11.5	Separation Conditions	359
M11.5.1	First Dimension (IPG-IEF)	359
M11.5.1.1	IPG-IEF with Conventional Equipment	360
M11.5.1.2	IPG-IEF with IPG Strip Kit (Figure)	360
M11.5.1.3	IPG-IEF in Individual Ceramic Trays	362
M11.5.1.4	Equipment and Trays for Cup Loading	363
M11.5.2	Equilibration	366
M11.5.3	Second Dimension (SDS Electrophoresis)	366
M11.5.3.1	Vertical Gels	366
M11.5.3.2	Horizontal Gels	367
M11.6	Staining Procedures	370
M11.6.1	Staining of Multiple Gels	371
M11.6.2	Colloidal Coomassie Staining	371
M11.6.2.1	Stock Solutions	371
M11.6.2.2	Fixation Solution	372
M11.6.2.3	Staining Solution	372
M11.6.2.4	Staining Procedure:	372
M11.6.3	Reversible Imidazole–Zinc Negative Staining	372
M11.6.4	Silver Staining	373
M11.6.4.1	Mass Spectrometry Analysis of Silver-Stained Spots	374
M11.6.4.2	Blue Toning	374
M11.6.5	Fluorescent Staining with SERVA Purple	374
M11.6.5.1	Stock Solutions	374
M11.6.5.2	Staining Protocol	375
M11.6.5.3	Detection	376
	References	377

Method 12 PAGE of DNA Fragments 379

M12.1	Stock Solutions	380
M12.2	Preparing the Gels	381
M12.3	Sample Preparation	385
M12.4	Electrophoresis	386
M12.5	Silver Staining	391

Appendix Troubleshooting 393

A1.1	Frequent Mistakes	393
A1.1.1	Miscalculation of the Cross-Linking Factor of a Polyacrylamide Gel	393
A1.1.2	Polymerization Temperature and Time for a Polyacrylamide Gel	393
A1.1.3	Creating Aggregates in SDS Samples	394
A1.1.4	Titration of the Running Buffer in SDS Electrophoresis	394
A1.1.5	Incomplete Removal of PBS from Cells	395

A1.1.6	Over-focusing of IPG Strips in 2D PAGE	395
A1.1.6.1	Protein Degradation in Basic pH Gradients	395
A1.1.6.2	The "Thiourea Effect"	395
A1.2	Isoelectric Focusing	396
A1.2.1	PAGIEF with Carrier Ampholytes	396
A1.2.2	Agarose IEF with Carrier Ampholytes	402
A1.2.3	Immobilized pH Gradients	405
A1.3	SDS Electrophoresis	410
A1.3.1	Horizontal SDS-PAGE	410
A1.3.2	Vertical PAGE	418
A1.4	Two-Dimensional Electrophoresis	419
A1.5	Semi-Dry Blotting	426
A1.6	DNA Electrophoresis	431

Index	435
-------	-----