

Contents

Part I

Fundamentals

0	Introduction	1
1	Electrophoresis	5
1.0	Introduction	5
1.1	Electrophoresis in non-restrictive gels	12
1.1.1	Agarose gel electrophoresis	12
1.1.2	Polyacrylamide gel electrophoresis of low-molecular weight substances	15
1.2	Electrophoresis in restrictive gels	16
1.2.1	The Ferguson plot	16
1.2.2	Agarose gel electrophoresis	17
1.2.3	Polyacrylamide gel electrophoresis (PAGE)	19
2	Isotachophoresis	35
3	Isoelectric focusing	39
3.1	Principles	39
3.2	Free carrier ampholytes	41
3.3	Immobilized pH gradients	44
3.4	Gels for IEF	47
3.5	Temperature	48
3.6	Controlling the pH gradient	49
3.7	Preparative isoelectric focusing	49
3.8	Titration curve analysis	50
4	Blotting	53
4.1	Principle	53
4.2	Transfer methods	53
4.3	Blotting membranes	57
4.4	Buffers for electrophoretic transfers	58
4.5	General staining	60
4.6	Blocking	60
4.7	Special detection	61
4.8	Protein sequencing	62
4.9	Transfer problems	63
5	Instrumentation	65
5.1	Current and voltage conditions	65
5.2	Power supply	67

5.3	Separation chambers	67
5.3.1	Vertical apparatus	67
5.3.2	Horizontal apparatus	68
5.4	Automated electrophoresis	71
5.5	Safety measures	72
5.6	Environmental aspects	73

6 Interpretation of electropherograms 75

6.1	Introduction	75
6.1.1	Purity control	75
6.1.2	Quantitative measurements	75
6.2	Densitometry	77
6.2.1	Applications of densitometry	78
6.2.2	The optics of a densitometer	79
6.2.3	Integration and baseline	82
6.2.4	Evaluation of a densitogram	82

Equipment 86

Methods	86
Instrumentation	86
Laboratory equipment	89
Consumables	90
Chemicals	91

Part II

Methods

Method 1: PAGE of dyes 95

1	Sample preparation	95
2	Stock solutions	95
3	Preparing the casting cassette	95
4	Casting the ultrathin-layer gels	98
5	Electrophoretic separation	98

Method 2: PAGE of DNA fragments 101

1	Sample preparation	102
2	Stock solutions	102
3	Preparing the blank gels	102
4	Electrophoretic separation	105

Method 3: agarose and immunoelectrophoresis 109

1	Sample preparation	109
2	Stock solutions	109
3	Preparing the gels	110
4	Electrophoresis	114
5	Protein detection	117

Method 4: Titration curve analysis 121

- 1 Sample preparation 121
- 2 Stock solutions 121
- 3 Preparing the blank gels 122
- 4 Titration curve analysis 125
- 5 Coomassie and silver staining 128
- 6 Interpreting the curves 130

Method 5: Native PAGE in amphoteric buffers 133

- 1 Sample preparation 134
- 2 Stock solutions 134
- 3 Preparing the empty gels 135
- 4 Electrophoresis 139
- 5 Coomassie and silver staining 142

Method 6: Agarose IEF 145

- 1 Sample preparation 145
- 2 Preparing the agarose gel 146
- 3 Isoelectric focusing 148
- 4 Protein detection 150

Method 7: PAGIEF in rehydrated gels 153

- 1 Sample preparation 153
- 2 Stock solutions 154
- 3 Preparing the blank gels 154
- 4 Isoelectric focusing 157
- 5 Coomassie and silver staining 159
- 6 Densitometric evaluation 161
- 7 Perspectives 164

Method 8: SDS-polyacrylamide electrophoresis 167

- 1 Sample preparation 167
- 2 Stock solutions for the preparation of gels 171
- 3 Preparing the casting cassette 172
- 4 Gradient gel 174
- 5 Electrophoresis 178
- 6 Coomassie and silver staining 180
- 7 Blotting 182
- 8 Densitometry 183
- 9 Perspectives 186

Method 9: Semi-dry blotting of proteins 189

- 1 Transfer buffers 190
- 2 Technical procedure 191
- 3 Staining of blotting membranes 195

Method 10: IEF in immobilized pH gradients 197

- 1 Sample preparation 198
- 2 Stock solutions 198
- 3 Immobiline recipes 199
- 4 Preparing the casting cassette 202
- 5 Preparing the pH gradient gels 203
- 6 Isoelectric focusing 210
- 7 Coomassie and silver staining 211
- 8 Strategies for IPG focusing 214

Method 11: High-resolution 2D electrophoresis 215

- 1 Sample preparation 216
- 2 Stock solutions 217
- 3 Preparing the gel 218
- 4 Separation conditions 222
- 5 Coomassie and silver staining 225

Appendix

A Trouble-shooting guide

- A1 Isoelectric focusing 229
 - A1.1 PAGIEF with carrier ampholytes 229
 - A1.2 Agarose IEF with carrier ampholytes 236
 - A1.3 Immobilized pH gradients 240
- A2 SDS electrophoresis 246
- A3 Semidry blotting 254
- A4 Two-dimensional electrophoresis (IPG-DALT) 260

B References 265

Index 271