

Contents

HEALTH WARNING

ABBREVIATIONS

1. AN INTRODUCTION TO POLYACRYLAMIDE GEL ELECTROPHORESIS	1
B. David Hames	
Introduction	1
Properties of Polyacrylamide Gel	3
Chemical structure	3
Polymerisation catalysts	4
Effective pore size	4
Experimental Approach	5
Rod or slab gels	5
Dissociating or non-dissociating buffer system	6
Continuous or discontinuous (multiphasic) buffer system	7
Choice of pH	11
Choice of polymerisation catalyst	12
Choice of gel concentration	12
Molecular weight estimation	14
Apparatus	18
Gel holders and electrophoresis tanks	18
Additional items of equipment required for electrophoresis	21
Preparation and Electrophoresis of Polyacrylamide Gels	23
Reagents	23
Stock solutions	25
Gel mixture preparation	26
Preparation of rod gels	28
Preparation of slab gels	33
Sample preparation	36
Sample loading and electrophoresis	40
Analysis of Gels Following Electrophoresis	42
Recovery of gels	42
Protein staining and quantitation	44
Detection of radioactive proteins	50
Detection of glycoproteins and phosphoproteins	59
Detection of proteins using immunological methods	60
Detection of enzymes	60
Recovery of Separated Proteins	61
Localisation of protein bands	62
Elution of proteins	63
Modifications to the Basic Techniques	64
Molecular weight analysis of oligopeptides	64

Separation of special classes of proteins	65
Concentration gradient gels	71
Large numbers of gels	76
Micro-rod and micro-slab gels	77
Agarose-acrylamide composite gels	79
Homogeneity and Identity	80
Artifacts and Troubleshooting	83
Acknowledgements	86
References	86
References of general interest	86
References in the text,	86
 2. "QUANTITATIVE" AND PREPARATIVE POLYACRYLAMIDE GEL ELECTROPHORESIS	 93
Andreas Chrambach and David Rodbard	
Introduction	93
A Survey of Apparatus for Quantitative PAGE	93
Choice of Gel	96
Choice of Buffer System	98
Multiphasic versus continuous zone electrophoresis	98
Multiphasic buffer systems available: the Jovin output	99
Quantitative PAGE Procedure	102
Strategy	102
Optimisation of pH	103
Choice of stacking limits	111
Choice of optimal conditions for protein stability and resolution	112
Optimisation of pore size: the Ferguson plot	114
Identity testing	122
Size and charge isomerism	124
Physical characterisation	126
Optimally resolving pore size	132
Preparative PAGE	134
Gel slice extraction	135
Successive zone elution	136
Acknowledgements	141
References	141
 3. GEL ISOTACHOPHORESIS	 145
Nga Y. Nguyen and Andreas Chrambach	
Introduction	145
Procedures	146
Optimisation of conditions for steady-state stacking	146
Test of separation within the stack	147
Preparative isotachophoresis by gel slice extraction	148

Preparative isotachopheresis by successive zone elution	152
Preparative steady-state stacking by successive zone elution	154
References	154
4. ANALYTICAL AND PREPARATIVE GEL ELECTROFOCUSING	157
Birgit An der Lan and Andreas Chrambach	
When to use Gel Electrofocusing	157
Apparatus	158
Gel tube versus horizontal slab apparatus	158
Voltage control devices	161
Gradient monitoring devices	162
Gel	163
Polyacrylamide gel	164
Sephadex gel	164
Agarose gel	164
Formation of pH Gradients	165
Synthetic ampholytes versus buffers	165
Amphoteric versus non-amphoteric carrier constituents	166
Flat versus steep pH gradients	167
pH gradient linearity	167
Electrofocusing at high ionic strength	168
Electrofocusing in the presence of detergents	169
Choice of Anolyte and Catholyte	169
Electrofocusing Dynamics	170
pH gradients	170
Proteins	173
Conductance	174
Procedure for Analytical Gel Electrofocusing	174
Preparative Gel Electrofocusing	183
Apparatus	183
Procedure	184
References	185
5. TWO-DIMENSIONAL GEL ELECTROPHORESIS	189
John Sinclair and David Rickwood	
Introduction	189
Apparatus	190
First-dimensional gels	190
Second-dimensional gels	190
General Techniques for Two-dimensional Gels	193
Solutions and apparatus	193
Preparation of sample	194
Preparation of the first-dimensional gel	195
Equilibration of the first-dimensional gel for the second dimension	195
Preparation of the second-dimensional gel	196

Analysis of the distribution of polypeptides	197
Comparative analysis of two-dimensional gels	199
Two-dimensional Separations of Proteins on the Basis of Isoelectric Points and Molecular Weights	200
First-dimensional separation	201
Second-dimensional separation	203
Modifications to the basic technique	205
Separation of Special Classes of Proteins	209
Ribosomal proteins	209
Histones	211
Nuclear proteins	216
References	217
 6. PEPTIDE MAPPING BY LIMITED PROTEOLYSIS USING SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS	 219
B. David Hames	
Introduction	219
Apparatus	220
Methodology	220
Experimental procedure	220
Modifications to the basic technique	225
Interpretation of Data	227
References	228
 7. IMMUNOELECTROPHORESIS	 229
Richard D. Jurd	
Introduction	229
Preparation of Antigens and Antibodies	229
Antigens	229
Antibody production	230
Commercially available antibody	233
Assay of antibody activity	233
Purification of IgG antibody	233
Simple Immunoelectrophoresis	236
Apparatus	236
Reagents	236
Method	237
Radioimmunoelectrophoresis	239
Enzymatic Detection Methods	241
Other Modifications of Immunoelectrophoresis	241
Cross-over electrophoresis	241
“Rocket” immunoelectrophoresis	241
Two-dimensional immunoelectrophoresis (crossed immunoelectrophoresis)	243
Troubleshooting	246

References	247
General references	247
References in the text	247

APPENDIX I : BIBLIOGRAPHY OF POLYPEPTIDE DETECTION METHODS

	249
General Polypeptide Detection Methods	249
Staining methods	249
Fluorescent dye methods	249
Direct detection methods	250
Detection Methods Based on the Use of Radioisotopes	251
Methods to Detect Specific Classes of Polypeptide	251
Glycoproteins	251
Phosphoproteins	252
Nucleoproteins	252
Proteins with available thiol groups	253
Cadmium-containing proteins	253
Collagen and procollagen	253
Immunological Methods	253
Enzyme Detection Methods	254

APPENDIX II : REAGENTS FOR THE ISOTOPIC LABELLING OF PROTEINS

	265
Summary	265
Reagents	266
Acetic anhydride	266
Bolton and Hunter reagent	266
Bromoacetic acid	267
Chloroacetic acid	267
<i>p</i> -Chloromercuribenzenesulphonic acid	268
<i>p</i> -Chloromercuribenzoic acid	268
Dansyl chloride	268
DFP (di-isopropyl phosphorofluoridate)	269
Ethyl acetimidate	269
<i>N</i> -ethylmaleimide	270
1-Fluoro-2,4-dinitrobenzene	270
Formaldehyde	270
Iodine	271
Iodoacetamide	272
Iodoacetic acid	272
Isethionyl acetimidate	273
Maleic anhydride	273
Methyl 3,5-diiodohydroxybenzimidate	273
Phenyl isothiocyanate	274
Potassium borohydride	275

Sodium borohydride	275
Succinic anhydride	276
<i>N</i> -Succinimidyl propionate	276
Acknowledgement	277
APPENDIX III : MOLECULAR WEIGHTS AND ISOELECTRIC POINTS OF SELECTED MARKER PROTEINS	279
APPENDIX IV : SUPPLIERS OF SPECIALIST ITEMS FOR ELECTROPHORESIS	281
INDEX	283