

Contents

Preface to the Third Edition *xv*

List of Abbreviations and Symbols *xvii*

1	General Aspects of Enzyme Analysis	1
1.1	Introduction and Essentials for Enzyme Assays	1
	Standard Books, Series	4
	Databases	4
1.2	Theoretical Basis of Enzyme Assays	4
1.2.1	Order of Enzyme Reactions	4
1.2.2	Importance of the Reaction Order for Enzyme Reactions	6
1.2.3	The Reaction Velocity, Significance, and Practical Aspects	10
1.2.3.1	Determination of the Reaction Velocity, the Progress Curve	10
1.2.3.2	Enzyme Units	15
1.2.3.3	A Short Discussion About Errors in Enzyme Assays	17
1.2.3.4	Practical Rules for the Preparation of Dilution Series	21
1.2.3.5	Statistical Treatment of Enzyme Reactions	23
1.2.4	Treatment of the Michaelis–Menten Equation	24
1.2.4.1	General Considerations	24
1.2.4.2	Linear Representations of the Michaelis–Menten Equation	26
1.2.5	Enzyme Inhibition	28
1.2.6	Multisubstrate Reactions	33
1.3	Essential Conditions for Enzyme Assays	35
1.3.1	Dependence on Solvents and Ionic Strength	35
1.3.2	pH Dependency	36
1.3.2.1	Isoelectric Point	38
1.3.2.2	Buffers: What Must Be Regarded?	38
1.3.2.3	How to Prepare Buffers?	41
1.3.3	Temperature Dependency	42
1.3.4	Stability of Enzymes	47
1.3.4.1	Why Are Enzymes Unstable?	47
1.3.4.2	How Can Enzymes Be Stabilized?	48
1.3.4.3	How to Store Enzymes?	48
1.4	Theory of Coupled Enzyme Reactions	51
1.4.1	Two Coupled Reactions	51
1.4.2	Three Coupled Reactions	54
1.5	Substrate Determination	54

1.5.1	End Point Method	55
1.5.2	Substrate Determination by Coupled Enzyme Reactions	56
1.5.3	Kinetic Method for Substrate Determination	57
1.5.4	Enzymatic Cycling	57
2	Instrumental Aspects	61
2.1	Spectroscopic Methods	61
2.1.1	Absorption (UV/Vis) Photometry	61
2.1.2	Cuvettes	72
2.1.2.1	Shape	72
2.1.2.2	Material	73
2.1.2.3	Cleaning	73
2.1.3	Turbidity Measurement	74
2.1.4	Fluorescence Photometry	75
2.1.5	Luminometry	79
2.1.6	Polarimetry	80
2.2	Electrochemical Methods	81
2.2.1	pH Meter and Glass Electrodes	81
2.2.2	pH Stat	82
2.2.3	Potentiometry	83
2.2.4	Oxygen and Carbon Dioxide Electrodes	83
2.3	Radioactive Labeling	84
2.4	Diverse Methods	84
3	Enzyme Assays	87
3.1	Enzyme Nomenclature	88
3.2	Practical Considerations for Enzyme Assays	90
4	Oxidoreductases, EC 1	95
4.1	General Assay Procedures	95
4.1.1	Optical Assay	95
4.1.2	Fluorimetric Assay	96
4.2	Alcohol Dehydrogenase (ADH), EC 1.1.1.1	96
4.2.1	Reduction Assay	96
4.2.2	Oxidation Assay	97
4.3	Alcohol Dehydrogenase (NADP ⁺), EC 1.1.1.2	98
4.4	Homoserine Dehydrogenase, EC 1.1.1.3	99
4.5	Shikimate Dehydrogenase, EC 1.1.1.25	100
4.6	L-Lactate Dehydrogenase (LDH), EC 1.1.1.27	101
4.6.1	Photometric Reduction Assay	101
4.6.2	Fluorimetric Reduction Assay	102
4.6.3	Oxidation Assay	103
4.7	Malate Dehydrogenase (MDH), EC 1.1.1.37	104
4.8	Malate Dehydrogenase (Oxaloacetate-Decarboxylating) (NAD ⁺), EC 1.1.1.38, and Malate Dehydrogenase (Decarboxylating), EC 1.1.1.39	105

4.9	Malate Dehydrogenase (Oxaloacetate-decarboxylating) (NADP ⁺), EC 1.1.1.40	106
4.10	Isocitrate Dehydrogenase (NAD ⁺) (ICDH), EC 1.1.1.41	107
4.11	Isocitrate Dehydrogenase (NADP ⁺) (ICDH), EC 1.1.1.42	108
4.12	Glucose-6-Phosphate Dehydrogenase (NADP ⁺), EC 1.1.1.49 (G6PDH)	109
4.13	Glucose Oxidase (GOD), EC 1.1.3.4	110
4.14	Formate Dehydrogenase (FDH), EC 1.2.1.2	111
4.15	Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH), EC 1.2.1.12	112
4.15.1	Oxidation Assay	112
4.15.2	Reduction Assay Coupled with 3-Phosphoglycerate Kinase (PGK)	113
4.16	Long-Chain-Aldehyde Dehydrogenase, EC 1.2.1.48	114
4.17	Pyruvate Dehydrogenase (Acetyl-transferring) (PDH), EC 1.2.4.1	116
4.17.1	Ferricyanide as Electron Acceptor	116
4.17.2	Dichlorophenolindophenol as Electron Acceptor	117
4.18	Aldehyde Oxidase, EC 1.2.3.1	118
4.19	Oxoglutarate Dehydrogenase (Succinyl-transferring) (OGDH), EC 1.2.4.2	119
4.20	Pyruvate Ferredoxin Oxidoreductase, EC 1.2.7.1	120
4.20.1	Assay with Cytochrome c (cyt c) as Electron Acceptor	121
4.21	Alanine Dehydrogenase, EC 1.4.1.1	121
4.21.1	Oxidation of Alanine	122
4.21.2	Reduction of Pyruvate	122
4.22	Glutamate Dehydrogenase, EC 1.4.1.3	123
4.23	Leucine Dehydrogenase, EC 1.4.1.9	124
4.24	L-Amino-Acid Oxidase, EC 1.4.3.2	125
4.25	D-Amino-Acid Oxidase, EC 1.4.3.3	126
4.26	Monoamine Oxidase, EC 1.4.3.4	126
4.27	Primary Amine Oxidase, EC 1.4.3.21	127
4.27.1	Spectrophotometric Assay	128
4.27.2	Polarographic Assay of O ₂ Uptake with O ₂ Electrode	128
4.27.3	Assays for Benzylamine Oxidase Activity	129
4.28	Diamine Oxidase, EC 1.4.3.22	129
4.29	NADH:Ubiquinone Reductase (H ⁺ -Translocating) EC 1.6.5.3	130
4.29.1	Spectrophotometric Assay	131
4.30	NADH Dehydrogenase, EC 1.6.99.3	131
4.31	Factor-Independent Urate Hydroxylase, EC 1.7.3.3	132
4.32	Dihydrolipoyl Dehydrogenase, EC 1.8.1.4	133
4.32.1	Oxidation of Dihydrolipoamide	134
4.32.2	Reduction of Lipoamide	134
4.33	Glutathione Disulfide Reductase, EC 1.8.1.7	135
4.34	Cytochrome-c Oxidase (COX), EC 1.9.3.1	137
4.34.1	Spectrophotometric Assay	137
4.34.2	Assay with Oxygen Electrode	137
4.35	Catalase, EC 1.11.1.6	138

4.36	Peroxidase (POD) EC 1.11.1.7	139
4.36.1	Assay with 2,2'-Azino-bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS)	140
4.36.2	Assay with Guaiacol	140
4.36.3	Assay with Dianisidine	141
4.37	Glutathione Peroxidase, EC 1.11.1.9	142
4.37.1	Coupled Assay with Glutathione Reductase	142
4.38	Photinus luciferin 4-Monooxygenase (ATP-Hydrolyzing), EC 1.13.12.7	143
4.39	Alkylglycerol Monooxygenase, EC 1.14.16.5	145
4.39.1	Spectroscopic Assay	145
4.39.2	Coupled Assay with HPLC Detection	145
4.40	Dopamine β -Monooxygenase, EC 1.14.17.1	147
4.41	Tyrosinase, EC 1.14.18.1	148
4.41.1	Dopa Oxidase Assay	148
4.41.2	Dopachrome Assay	149
4.42	Superoxide Dismutase (SOD), EC 1.15.1.1	149
4.42.1	Assay with Pyrogallol	150
4.42.2	Assay with Ferricytochrome c and Xanthine Oxidase	150
4.43	Xanthine Oxidase (XOD), EC 1.17.3.2	151
5	Transferases, EC 2	153
5.1	Ornithine Carbamoyltransferase (OTC), EC 2.1.3.3	153
5.1.1	Method 1 for Color Development	154
5.1.2	Method 2 for Color Development	154
5.2	Choline O-Acetyltransferase, EC 2.3.1.6	155
5.3	Carnitine O-acetyltransferase, EC 2.3.1.7	156
5.3.1	Direct Spectroscopic Assay	156
5.3.2	Assay with DTNB	157
5.4	Dihydrolipoamide Acetyltransferase, EC 2.3.1.12	157
5.4.1	Spectrophotometric Assay	158
5.4.2	Stopped Assay	159
5.5	Fatty Acid Synthase, EC 2.3.1.85	160
5.6	γ -Glutamyltransferase, EC 2.3.2.2	161
5.7	Citrate Synthases, EC 2.3.3.1, EC 2.3.3.3, and EC 2.3.3.16	162
5.8	ATP Citrate Lyase, EC 2.3.3.8	164
5.9	Glycogen Phosphorylase, EC 2.4.1.1	165
5.10	Purine-nucleoside Phosphorylase (PNP), EC 2.4.2.1	166
5.11	Glutathione Transferase, EC 2.5.1.18	167
5.11.1	Spectrophotometric Assay	168
5.11.2	Titrimetric Assay	168
5.12	Aspartate Transaminase (AAT), EC 2.6.1.1	169
5.13	Alanine Transaminase, EC 2.6.1.2	170
5.14	Tyrosine Transaminase (TAT), EC 2.6.1.5; Tryptophan Transaminase (Tam 1), EC 2.6.1.27; Phenylalanine (Histidine) Transaminase, EC 2.6.1.58	171
5.14.1	Tyrosine Transaminase	171

5.14.2	Tryptophan Transaminase	171
5.14.3	Phenylalanine (Histidine) Transaminase:	171
5.15	Hexokinase (HK), EC 2.7.1.1, Glucokinase (GK), EC 2.7.1.2	173
5.16	Pyruvate Kinase (PK), EC 2.7.1.40	174
5.17	Acetate Kinase, EC 2.7.2.1	176
5.18	Phosphoglycerate Kinase (PGK), EC 2.7.2.3	177
5.19	Aspartate Kinase (AK), EC 2.7.2.4	178
5.20	Creatine Kinase (CK), EC 2.7.3.2	180
5.20.1	Coupled Assay	180
5.20.2	pH-Colorimetric Assay	181
6	Hydrolases, EC 3	183
6.1	Triacylglycerol Lipase, EC 3.1.1.3	183
6.1.1	Assay with pH Stat (Auto-titrator)	183
6.1.2	Fluorimetric Assay	184
6.2	Phospholipase A ₂ , EC 3.1.1.4	185
6.3	Acetylcholinesterase (AChE), EC 3.1.1.7	186
6.4	Cholinesterase (ButChE), EC 3.1.1.8	187
6.4.1	pH Stat Assay	187
6.4.2	Colorimetric Assay	188
6.5	Hydroxyacylglutathione Hydrolase, EC 3.1.2.6	189
6.5.1	Direct Assay	189
6.5.2	Assay with DTNB	189
6.6	S-Formylglutathione Hydrolase, EC 3.1.2.12	190
6.7	Alkaline Phosphatase, EC 3.1.3.1	191
6.7.1	Mammalian Alkaline Phosphatase	191
6.7.2	Bacterial Alkaline Phosphatase	192
6.8	Acid Phosphatase, EC 3.1.3.2	192
6.9	5'-Nucleotidase, EC 3.1.3.5	193
6.9.1	Assay by Determination of P _i	194
6.9.2	Assay by Converting Adenosine into Inosine	194
6.10	Glucose-6-Phosphatase, EC 3.1.3.9	195
6.11	3',5'-Cyclic-Nucleotide Phosphodiesterase, EC 3.1.4.17	196
6.12	Steryl-Sulfatase, EC 3.1.6.2	198
6.13	Pancreatic Ribonuclease, EC 3.1.27.5	198
6.14	α-Amylase, EC 3.2.1.1	199
6.15	Glucan 1,4-α-Glucosidase (AMG), EC 3.2.1.3	201
6.15.1	Coupled Assay with HK and G6PDH	201
6.15.2	Photometric Assay with 4-Nitrophenyl- α -D-Glucose	202
6.15.3	Fluorimetric Assay with 4-Methylumbelliferyl- α -D-Glucoside	202
6.16	Cellulases	203
6.16.1	β-1,4-Glucanase, EC 3.2.1.4	203
6.16.2	β-Glucosidase, EC 3.2.1.21	203
6.16.3	Orcinol Assay	204
6.16.4	Activity Staining	204
6.17	Lysozyme, EC 3.2.1.17	206
6.18	Sialidase, EC 3.2.1.18	207

6.18.1	Fluorimetric Assay	207
6.18.2	Activity Staining	208
6.19	α -Glucosidase, EC 3.2.1.20	208
6.19.1	Coupled Assay	208
6.19.1.1	α -Glucosidase Reaction	209
6.19.1.2	Glucose Determination	209
6.19.2	Assay with 4-Nitrophenylglucopyranoside	210
6.20	β -Galactosidase, EC 3.2.1.23	211
6.21	α -Mannosidase, EC 3.2.1.24	212
6.21.1	Photometric Microassay	212
6.21.2	Fluorimetric Assay	213
6.22	β -Fructofuranosidase, EC 3.2.1.26	213
6.23	β -Glucuronidase, EC 3.2.1.31	214
6.23.1	Fluorimetric Assay	215
6.24	β -N-Acetylhexosaminidase, EC 3.2.1.52	215
6.25	Proteases, EC 3.4, General Assays	216
6.25.1	Anson Assay	216
6.25.2	Casein Assay	218
6.25.3	Azocasein Assay	219
6.25.4	Ninhydrin Assay	220
6.26	Leucyl Aminopeptidase (LAP), EC 3.4.11.1, Bacterial Leucyl Aminopeptidase, EC 3.4.11.10	221
6.26.1	Assay with Leucineamide	222
6.26.2	Assay with Leucine- <i>p</i> -nitroanilide	222
6.27	Peptidyl-dipeptidase A, EC 3.4.15.1	223
6.28	α -Chymotrypsin, EC 3.4.21.1	224
6.28.1	Assay with SUPHEPA	224
6.28.2	Assay with GLUPHEPA	225
6.29	Trypsin, EC 3.4.21.4	226
6.30	Pancreatic Elastase, EC 3.4.21.35	227
6.30.1	Assay with Succinyl-Ala-Ala-Ala- <i>p</i> -Nitroanilide	227
6.30.2	Esterase Activity of Elastase	227
6.31	Cathepsin B, EC 3.4.22.1	228
6.32	Pepsin A, EC 3.4.23.1	229
6.33	Asparaginase, EC 3.5.1.1	230
6.34	Glutaminase, EC 3.5.1.2	232
6.34.1	Determination of Ammonia with Nessler's Reagent	232
6.34.2	pH Stat Assay	233
6.35	Urease, EC 3.5.1.5	233
6.35.1	pH Stat Assay	234
6.35.2	Photometric Assay	234
6.36	Guanine Deaminase, EC 3.5.4.3	235
6.36.1	Determination of Ammonia	236
6.37	Adenosinetriphosphatase, EC 3.6.1.3	237
6.38	Mg ²⁺ Importing ATPase, EC 3.6.3.2, Na ⁺ /K ⁺ -Exchanging ATPase, EC 3.6.3.9	238
6.38.1	Assay of Total ATPase Activity	238

6.38.2	Assay of Mg ²⁺ -ATPase Activity	239
7	Lyases, EC 4	241
7.1	Pyruvate Decarboxylase (PDC), EC 4.1.1.1	241
7.2	Glutamate Decarboxylase (GAD), EC 4.1.1.15	242
7.3	Fructose-bisphosphate Aldolase, EC 4.1.2.13	244
7.4	Anthranilate Synthase, EC 4.1.3.27	245
7.5	Carbonic Anhydrase (CA), EC 4.2.1.1	246
7.5.1	pH Stat Assay	246
7.5.2	Esterase Assay with 4-Nitrophenylacetate	247
7.6	Fumarate Hydratase, EC 4.2.1.2	248
7.7	Lactoylglutathione Lyase, EC 4.4.1.5	249
7.8	Adenylate Cyclase (AC), EC 4.6.1.1	250
8	Isomerases, EC 5	253
8.1	Xylose Isomerase, EC 5.3.1.5	253
8.1.1	D-Xylose Isomerase Assay	253
8.1.2	D-Xylose Isomerase Microplate Assay	254
8.1.3	D-Glucose Isomerase Assay	255
8.1.4	D-Glucose Isomerase Microplate Assay	256
8.2	Glucose-6-phosphate Isomerase (G6PI), EC 5.3.1.9	256
8.3	Phosphoglucomutase (PGM), EC 5.4.2.2	257
9	Ligases (Synthetases), EC 6	261
9.1	Tyrosine-tRNA Ligase, EC 6.1.1.1	261
9.1.1	Fluorimetric Assay	261
9.1.2	ATP- ³² PP Exchange	262
9.2	Acetate-CoA Ligase (ACL), EC 6.2.1.1	263
9.2.1	Direct Radioactive Assay	264
9.2.2	Coupled Spectroscopic Assay	264
9.3	Glutamine Synthetase, EC 6.3.1.2	266
10	Assays for Multi-enzyme Complexes	269
10.1	Pyruvate Dehydrogenase Complex (PDHC)	269
10.1.1	Overall Activity of PDHC by NAD ⁺ Reduction	270
10.1.2	Overall Activity of PDHC by Dismutation Assay	270
10.2	α-Oxoglutarate Dehydrogenase Complex (OGDHC)	272
10.2.1	Overall Activity by NAD ⁺ Reduction	273
11	Assays for Other Enzyme Relevant Parameters	275
11.1	Substrate Determination	275
11.1.1	Determination of NADP(H) by Enzymatic Cycling	275
11.1.1.1	Cycling Reaction	276
11.1.2	Determination of NAD(H) by Enzymatic Cycling	277
11.2	Protein Determination	279
11.2.1	Biuret Assay	279

11.2.2	BCA Assay	281
11.2.2.1	Assay for Soluble Proteins	281
11.2.2.2	Modification for Immobilized Proteins	282
11.2.3	Lowry Assay	282
11.2.4	Coomassie Binding Assay (Bradford Assay)	283
11.2.4.1	Assay for Soluble Proteins	284
11.2.4.2	Modification for Immobilized Proteins	284
11.2.5	Absorption Method	285
11.2.6	Fluorimetric Assay	287
11.2.7	Ninhydrin Assay	288
11.2.7.1	Ninhydrin Assay with Hydrolysis	288
11.2.7.2	Modified Ninhydrin Assay Without Hydrolysis	289
11.2.8	Protein Assay with 2-Hydroxy-1-naphthaldehyde	290
	General Literature for Protein Assays	291
11.3	Phosphate Determination	291
11.4	Determination of Metal Ions	293
11.4.1	Calcium and Magnesium	293
11.4.2	Iron	294
11.4.2.1	Determination with Ferrozine	295
11.4.2.2	Determination of Fe ^{II} with 1,10-Phenanthroline in the Presence of Fe ^{III}	295
11.4.3	Copper	296
11.4.3.1	Biquinoline Method	296
11.4.3.2	Oxalyldihydrazide Method	297
11.4.4	Manganese	297
11.4.4.1	Colorimetric Assay	298
11.4.4.2	Assay with 1-(2-Pyridylazo)-2-naphthol (PAN)	298
11.4.5	Zinc	299
11.5	Glycoprotein Assays	300
11.5.1	Identification in Electrophoresis Gels	300
11.5.2	Quantitative Analysis of Protein-Bound Hexoses	300
11.6	Cross-linking of Proteins with Dimethylsuberimidate	301
11.7	Concentrating Enzyme Solutions	302
11.7.1	Precipitation	303
11.7.2	Ultrafiltration and Dialysis	306
11.7.3	Ultracentrifugation	307
11.7.4	Lyophilization	307
11.7.5	Other Concentration Methods	307
12	Enzyme Immunoassays	309
12.1	Radioimmunoassays	309
12.2	Principle of Enzyme Immunoassays	309
12.3	Noncompetitive Solid-Phase Enzyme Immunoassay	311
12.4	Competitive Solid-Phase Enzyme Immunoassay	312
12.5	Methods for Enzyme Immunoassays and Immobilization Techniques	312
12.5.1	Protein Coupling to Cyanogen Bromide Activated Agarose	312

12.5.2	Coupling of Diaminohexyl Spacer	313
12.5.3	Periodate Activation of Cellulose	314
12.5.4	Introduction of Thiol Groups into Proteins (Antibodies)	315
12.5.5	Conjugation of a Protein (Antibody) with an Enzyme (Peroxidase)	316
12.5.6	Conjugation of β -Galactosidase to Proteins (Antibodies) by MBS	316
12.5.7	Conjugation of Alkaline Phosphatase to Antibodies by Glutaraldehyde	317
13	Binding Measurements	319
13.1	Different Types of Binding	319
13.1.1	General Considerations	319
13.1.2	How Can Specific Reversible Binding be Identified?	320
13.1.3	Experimental Aspects	322
13.2	Binding Measurements by Size Discrimination	325
13.2.1	Equilibrium Dialysis	325
13.2.1.1	Binding of Indole to Bovine Serum Albumin	327
13.2.2	Evaluation of Binding Experiments	329
13.2.3	Ultrafiltration	330
13.2.4	Gel Filtration	331
13.2.5	Ultracentrifugation	332
13.3	Spectroscopic Methods	333
13.3.1	Difference Spectroscopy	334
13.3.1.1	Difference Spectroscopic Titration of Ligands Binding to Catalase	336
13.3.1.2	Evaluation of Spectroscopic Binding Curves	339
13.3.2	Fluorescence Spectroscopy	341
13.3.2.1	Binding of ANS to Bovine Serum Albumin	341
13.4	Other Binding Methods	344
13.4.1	Radioactive Labeling	344
13.4.2	Surface Plasmon Resonance (SPR)	345
14	Enzymes in Technical Applications	347
14.1	Modes of Enzyme Immobilization	347
14.1.1	Adsorption	348
14.1.2	Entrapment	350
14.1.3	Encapsulation	350
14.1.4	Cross-linking	351
14.1.5	Covalent Immobilization to Solid Supports	351
14.1.5.1	Supports	351
14.1.5.2	Spacer	353
14.2	Methods for Enzyme Immobilization	354
14.2.1	Microencapsulation in Nylon Beads	355
14.2.2	Entrapment in Polyacrylamide	355
14.2.3	Covalent Immobilization on Glass Surfaces	356
14.2.4	Covalent Immobilization on Controlled-Pore Glass (CPG)	358
14.2.5	Covalent Immobilization to Polyamide	360

14.2.5.1	O-Alkylation with Triethyloxonium Tetrafluoroborate	361
14.2.5.2	Immobilization to Amino Groups after Partial Hydrolysis of Polyamide	363
14.2.5.3	Immobilization to Carboxyl Groups After Partial Hydrolysis of Polyamide	364
14.2.6	Immobilization to Polyester	365
14.2.7	Immobilization by Alkaline Hydrolysis and Activation with Tosylchloride	367
14.2.8	Alkaline Hydrolysis and Activation by Carbonyldiimidazol	368
14.3	Analysis of Immobilized Enzymes	368
14.3.1	General Principles	368
14.3.2	Continuous Photometric Assays for Immobilized Enzymes	369
14.3.3	Cofactors in Reactions with Immobilized Enzymes	371
14.4	Enzyme Reactors	372
14.4.1	Batch Reactor (Stirred-Tank Reactor)	373
14.4.2	Membrane Reactor	373
14.4.3	Solid-Bed Reactor	374
14.4.4	Immobilized Cells	375
14.5	Biosensors	375
14.5.1	Enzyme Electrodes	375
14.5.2	Immunoelectrodes	379
14.5.3	Other Biosensors	379
14.6	Immobilized Enzymes in Therapy	381

Index 383