

Contents

List of Figures	xi
Preface	xv
Acknowledgements	xvii
Notes Regarding Nomenclature	xix
Abbreviations	xxi
Safety Guidelines for Biochemical Laboratories	xxiii
General Guidelines for Handling Solutions of Protein	xxix
Maintaining a Laboratory Notebook	xxxix

Introduction to Enzymes Catalyzing Oxidation–Reductions with the Coenzyme NAD(P)

Physiological Role of NAD(P)	1
Cosubstrates for NAD(P) in the Reactions Catalyzed by FNR, LuxG, and LDH	2
Does FNR Function as a Monomer or a Dimer?	5
Do FNR, LuxG, and LDH Have Different Michaelis Constants for NAD(P)?	6

Computational Techniques for Biochemistry

Software for Analysis of Data	11
Computational Methods and Bioinformatics for Studying the Structure and Function of Proteins	11
Computational Exercise 1	15
Computational Exercise 2	21

Section 1

FNR

1. Purification and Characterization of Ferredoxin-NADP⁺ Reductase from Chloroplasts of *S. oleracea*

Background: The Role of Ferredoxin-NADP⁺ Reductase in Linear and Cyclic Electron Transport in Photosynthesis 25

FNR Exercise 1	31
FNR Exercise 2	37
FNR Exercise 3	49
FNR Exercise 4	59

FNR Exercise 5	71
FNR Exercise 6	75
FNR Exercise 7	91

Section 2

LuxG

2. Purification and Characterization of a Recombinant FMN Reductase from *P. leiognathi*

Background: Flavin Reductases, Bioluminescence and the <i>Lux</i> Operon	103
LuxG Exercise 1	107
LuxG Exercise 2	117
LuxG Exercise 3	123
LuxG Exercise 4	127
LuxG Exercise 5	133
LuxG Exercise 6	137

Section 3

LDH

3. Purification and Characterization of Bovine L-Lactate Dehydrogenase

Background: Importance of L-Lactate Dehydrogenase in Bacterial and Eukaryotic

Physiology 141

Summary of the Exercises with LDH 147

LDH Exercise 1	149
LDH Exercise 2	153
LDH Exercise 3	157
LDH Exercise 4	167
LDH Exercise 5	175
LDH Exercise 6	185
LDH Exercise 7	189
LDH Exercise 8	197
LDH Exercise 9	201
LDH Exercise 10	205
LDH Exercise 11	207

Section 4

EXPERIMENTAL DESIGN

4. Experimental Design

Background 213

Sources for Biochemical Methods 213

Experimental Design Exercise 1 215

Experimental Design Exercise 2 217

Experimental Design Exercise 3 219

Appendix I: Measurement of Absorbance with Multiwell Plates and an Automated Plate Reader 223

Appendix II: Operation of the Spectronic 20D+ Spectrophotometer 225

Bibliography 227

Index 233