



Comprehensive Laboratory Manual of Life Science

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PREFACE

After the enormous success of first edition of Laboratory Manual of Microbiology, Biochemistry and Molecular Biology, we are happy to bring the second book with some additional sections. However, our goal remains the same. Our focus in writing this manual has been to bring together the protocols in simple and understandable manner to provide the students the laboratory practices as an enjoyable learning experience. We have tried to clear the basic concept behind the particular section or experiment.

This is based on the feedback of students and other users of the laboratory manual and has been written from the point of view of making this book a comprehensive laboratory manual having the experiments from all important sections prescribed in syllabi of most of the universities for practical training of UG and PG students. First book from authors had protocols from Microbiology, Biochemistry and Molecular Biology which covered a major percentage of practicals, however it was felt that Immunology and Environmental Biotechnology also have important share in many courses. Therefore, these two sections were added. Immunology is the basis of all disease diagnosis in today's scenario. Seven immunology related experiments have been included to form the basis of understanding the immune response. Although ready to use kits are available these days for the diagnosis of diseases and pathogens causing disease the experiments given in the book will definitely help in developing in understanding about the practical aspects of the subject.

Environmental science is an interdisciplinary field. Because environmental disharmonies occur as a result of the interaction between humans and the natural world, we must include both when seeking solutions to environmental problems. Therefore, we found it a necessity to include a separate section on Environment Biology. This Environmental science section

includes the analysis of samples of air, water, hazardous waste and sediment/ soil. The laboratory experiments emphasize sampling, extraction and instrumental analysis. Environmental Biotechnology section also covers all the practicals commonly followed by different universities.

The first part is the Introduction; the second part is about Instruments: Principle and Precautions. The third part is about the Experiments which includes many protocols related to Microbiology, Biochemistry, Molecular Biology, Immunology, and Environmental Science. In the end, we have included the Appendix and Index also.

This book will definitely be more useful to the graduate and postgraduate science students.

Mamta Baunthiyal
Indu Ravi
Jyoti Saxena

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ABBREVIATIONS

A	- Absorbance
AB	- Antibody
AIDS	- Acquired immunodeficiency Syndrome
Ag	- Antigen
APS	- Ammonium persulphate
BOD	- Biological Oxygen demand
BPB	- Bromophenol blue
BSA	- Bovine Serum Albumin
BTH	- Benzo (1,2,3) thiadiazole -7-carbothionic acid S-methyl ester
bp	- Base pairs
CAT	- Chloramphenicol acetyl transferase
COD	- Chemical Oxygen demand
CTAB	- Cetyltrimethylammonium bromide
Da	- Daltons
DAB	- 3,3'-Diamino benzidine
DEPC	- Diethyl pyrocarbonate
DO	- Dissolved oxygen
DMAB	- <i>p</i> -Dimethyl amino benzaldehyde
DMF	- N, N-dimethyl formamide
DMSO	- Dimethyl sulfoxide
DTT	- Dithiothreitol
DW	- Distilled water
EDTA	- Ethylenediamine tetra acetic acid
EIA	- Enzyme immunoassay
ELISA	- Enzyme linked immunosorbent assay
EMB	- Eosine- methylene blue
FW	- Formula weight
g	- gram
GAA	- Glacial acetic acid
GFP	- Green fluorescent protein
GUS	- β - Glucuronidase
H	- Hours
HEPA	- High Efficiency Particulate Air
HEPES	- N-2- hydroxyethylpiperazine-N'-2-ethanesulphonic acid

HIV	- Human immunodeficiency virus
H ₂ O ₂	- Hydrogen peroxide
HCl	- Hydrochloric acid
HPLC	- High performance liquid chromatography
IgG	- Immunoglobulin G
IR	- Infra red
IPTG	- Isopropyl-thiogalactoside
Kbp	- kilobase pairs
KDa	- kilodaltons
Km	- Michaelis-Menton constant
L	- litre
LB media	- Luria Bertani media
LD ₅₀	- Lethal Dose 50
LMW	- Low molecular weight marker
M	- Molar
mg	- milligram
min	- minutes
ml	- milliliter
Mm	- Mili molar
Mr	- Relative molecular weight
MW	- Molecular weight
µg	- microgram
µkat	- microkatal
µl	- microlitre
µm	- micromoles
N	- Normal
Nm	- nano meters
OD	- Optical Density
PBS	- phosphate buffer saline
PCR	- Polymerase chain reaction
PDA	- Potato Dextrose Agar
Pfu	- Plaque forming units
Pi	- inorganic phosphate
Ppm	- parts per million
Psi	- Pounds per square inch
PVDF	- Polyvinylidene difluoride
RBC	- Red blood cell
RCF	- Relative centrifugal force

Rf	-	Relative front
RPM	-	Revolutions per minute
S	-	Svedberg unit
SDS	-	PAGE-Sodium dodecyl sulphate polyacrylamide gel electrophoresis
Sec	-	seconds
TBS	-	Tris buffered saline
TE	-	Tris EDTA
TEMEND	-	N,N,N', N'-Tetra methyl ethylene diamine (hydroxyl methyl) amino methane
Tm	-	melting temperature
TLC	-	Thin layer chromatography
TOC	-	Total Organic Carbon
Tris	-	Tris (hydroxymethyl) aminomethane
TS	-	Total solids
UV	-	Ultra violet
V	-	velocity
Vmax	-	maximum velocity
v/v	-	volume by volume
WBC	-	White blood cell
w/v	-	weight by volume
X-gal	-	5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside

