

Biotechnology Lab Techniques: Culture Media, Microscopy, and Microbial Analysis

Keshawanand Tripathi Yashdeep Srivastava Narendra Kumar Editors



Biotechnology Lab Techniques: Culture Media, Microscopy, and Microbial Analysis

Keshawanand Tripathi

Department of Biotechnology, Invertis University, Bareilly, Uttar Pradesh, India

Yashdeep Srivastava

Department of Biotechnology, Invertis University, Bareilly, Uttar Pradesh. India

Narendra Kumar

School of Biotechnology and Bioengineering, Institute of Advanced Research, Gandhinagar, Gujarat, India



Published, marketed, and distributed by:

Deep Science Publishing USA | UK | India | Turkey Reg. No. MH-33-0523625 www.deepscienceresearch.com editor@deepscienceresearch.com WhatsApp: +91 7977171947

ISBN: 978-93-49307-04-9

E-ISBN: 978-93-49307-52-0

https://doi.org/10.70593/978-93-49307-52-0

Copyright © Keshawanand Tripathi, Yashdeep Srivastava, and Narendra Kumar

Citation: Tripathi, K., Srivastava, Y., & Kumar, N. (Eds.). (2025). *Biotechnology Lab Techniques: Culture Media, Microscopy, and Microbial Analysis*. Deep Science Publishing. https://doi.org/10.70593/978-93-49307-52-0

This book is published online under a fully open access program and is licensed under the Creative Commons "Attribution-Non-commercial" (CC BY-NC) license. This open access license allows third parties to copy and redistribute the material in any medium or format, provided that proper attribution is given to the author(s) and the published source. The publishers, authors, and editors are not responsible for errors or omissions, or for any consequences arising from the application of the information presented in this book, and make no warranty, express or implied, regarding the content of this publication. Although the publisher, authors, and editors have made every effort to ensure that the content is not misleading or false, they do not represent or warrant that the information-particularly regarding verification by third parties-has been verified. The publisher is neutral with regard to jurisdictional claims in published maps and institutional affiliations. The authors and publishers have made every effort to contact all copyright holders of the material reproduced in this publication and apologize to anyone we may have been unable to reach. If any copyright material has not been acknowledged, please write to us so we can correct it in a future reprint.

DEDICATED TO LORD SHIVA



I BOW TO SHIVA ALONG WITH SHAKTI (PARVATI), WHO RESIDES
EVER IN THE LOTUS OF MY HEART, WHO IS THE COLOR OF
CAMPHOR, WHO IS FILLED WITH KINDNESS, WHO IS THE
SUPPORT OF THE WORLD AND WHO WEARS A SNAKE AS A
GARLAND.

Preface

Welcome to the "Biotechnology Lab Techniques: Culture Media, Microscopy, and Microbial Analysis". This comprehensive manual is designed to be an essential companion for students, researchers, and professionals in the field of life sciences. Whether you are just starting your journey into laboratory practices or looking to deepen your understanding of advanced techniques, this handbook provides clear and practical guidance.

The world of life sciences is built upon a foundation of rigorous laboratory work, where precision and technique are paramount. This handbook begins with an introduction to basic laboratory practices, ensuring that readers develop a strong grasp of fundamental skills. From handling laboratory equipment to mastering techniques like smear preparation and staining of microorganisms, each chapter is structured to build upon the last, offering a progressive learning experience.

Central to this handbook are detailed sections on laboratory equipment and tools, essential for conducting experiments effectively. Whether you are operating a compound microscope, utilizing an autoclave for sterilization, or conducting experiments with UV-Vis spectrophotometers, this handbook provides comprehensive insights into their functions and applications.

Preparing media for cultivating microorganisms is a crucial skill covered extensively in this handbook. From nutrient broths to specialized agar types like McConkey and Chocolate agar, each recipe is meticulously detailed to ensure successful growth and isolation of pure microbial colonies. Techniques such as spread plating and streak plating are explained step-by-step, empowering researchers to isolate and study microbes with precision.

Beyond basic techniques, this handbook delves into advanced topics such as the impact of environmental factors like UV radiation and pH on microbial growth. Techniques for assessing cell viability and methods for evaluating antibacterial efficacy of natural products are also explored in detail, reflecting the handbook's commitment to practical relevance in contemporary research.

Additionally, this handbook encompasses techniques in molecular biology and biochemistry, from isolating nucleic acids and proteins to conducting gel electrophoresis and protein estimation assays. These techniques are pivotal for advancing research in genetics, biotechnology, and pharmaceutical sciences.

Furthermore, the handbook extends its scope to include botanical and environmental sciences, featuring methods for estimating chlorophyll content, investigating organogenesis in plants, and assessing biochemical oxygen demand in water samples. Each chapter is authored by experts in their respective fields, ensuring that the content is not only informative but also reliable and up-to-date with current scientific practices.

In conclusion, "Biotechnology Lab Techniques: Culture Media, Microscopy, and Microbial Analysis" is more than just a reference guide; it is a practical companion that equips readers with the knowledge and skills necessary to excel in their scientific endeavors. Whether used in educational settings or research laboratories, this handbook serves as an indispensable tool for navigating the complexities of life sciences.

Dr. Keshawanand Tripathi Dr. Yashdeep Srivastava Dr. Narendra Kumar

Contents

1.	Fundamentals of Laboratory Practices: Principles, Techniques, and Safety Protocols
	Narendra Kumar, Keshawanand Tripathi, Yashdeep Srivastava
2.	Essential Laboratory Equipment and Tools: Functions, Handling, and Applications
	Narendra Kumar, Keshawanand Tripathi, Yashdeep Srivastava, Sarvesh Kumar Pandey
3.	Composition, Optimization, and Preparation of Culture Media for Microbiological and Biotechnological Applications
	Narendra Kumar, Keshawanand Tripathi, Yashdeep Srivastava
4.	Techniques for Isolation of Pure Microbial Colonies: Principles, Methods, and Applications
	Narendra Kumar, Keshawanand Tripathi, Yashdeep Srivastava
5.	Standardized Techniques for Microbial Smear Preparation: Principles,
	Staining Protocols, and Microscopic Evaluation
	Narendra Kumar, Keshawanand Tripathi, Yashdeep Srivastava
6.	Staining Techniques for Microorganisms: Principles, Methods, and Diagnostic
	Applications46
	Narendra Kumar, Keshawanand Tripathi, Yashdeep Srivastava
7.	Effects of Ultraviolet (UV) Radiation on Microbial Growth: Mechanisms,
	Responses, and Applications57
	Narendra Kumar, Keshawanand Tripathi, Yashdeep Srivastava,

8.	Influence of pH on Microbial Growth: Mechanisms, Adaptations, and
	Industrial Implications60
	Narendra Kumar, Keshawanand Tripathi, Shashank Upadhyay, Sarvesh Kumar Pandey, Yashdeep Srivastava
9.	Trypan Blue Dye Exclusion Assay: Principles, Protocols, and Applications in Cell Viability Assessment
10.	Evans Blue Dye Exclusion Assay: Mechanisms, Protocols, and Applications in Cell Viability Analysis
11.	Paraffin Block Preparation and Histological Staining: Techniques for Tissue Structure Analysis
12.	Paraffin Preservation of Microbial Cultures: Techniques, Mechanisms, and Long-Term Storage Applications
13.	Lyophilization of Microbial Cultures: Principles, Techniques, and Applications in Long-Term Preservation
14.	Assessment of Antibacterial Efficacy of Selected Natural Products: Methods, Mechanisms, and Applications

15.	Quantitative Analysis of Bacterial Growth: Methods for Measuring Growth Curves and Kinetic Parameters
16.	Enumeration of Soil Microbiota: Calculation of Colony Forming Units (CFU) for Microbial Quantification
17.	Replica Plating Technique for Identifying <i>E. coli</i> Auxotrophic Mutants: Principles, Methods, and Application
18.	Measurement of Water Potential: Comparative Analysis of Three Distinct Methods in Biological Systems
19.	Experimental Demonstration of Transpiration Pull: Mercury Method for Measuring Xylem Water Transport
20.	Estimation of Biochemical Oxygen Demand (BOD): Principles, Methodology, and Water Quality Assessment
21.	Quantitative Estimation of Dissolved Oxygen (DO) in Water Samples: Principles, Methods, and Environmental Significance

22.	Isolation of Bacterial Chromosomal DNA: Principles, Techniques, and Analytical Applications
23.	Extraction and Purification of Plasmid DNA from Bacteria: Techniques and Applications in Molecular Biology
24.	CTAB-Based Extraction of Total Plant Genomic DNA: Principles, Protocols, and Applications
25.	Trizol-Based RNA Extraction: Principles, Protocol, and Applications in Molecular Biology
26.	Synthesis of Complementary DNA (cDNA): Principles, Methodology, and Applications in Gene Expression Studies
27.	Agarose Gel Electrophoresis of DNA: Principles, Protocols, and Applications in Molecular Analysis
28.	Isolation and Purification of Recombinant Proteins: Principles, Techniques, and Biotechnological Applications
29.	SDS-PAGE Analysis of Proteins: Principles, Methodology, and Applications in Proteomics

30.	Experimental Validation of Lambert-Beer's Law: Principles,
	Spectrophotometric Analysis, and Applications151
	Yashdeep Srivastava, Keshawanand Tripathi, Narendra Kumar, Sarvesh Kumar Pandey,
	Santosh Kumar Mishra
31.	Quantitative Protein Estimation Using the Bradford Assay: Principles,
	Protocols, and Applications
	Yashdeep Srivastava, Keshawanand Tripathi, Narendra Kumar
32	Biuret Assay for Protein Estimation: Principles, Methodology, and
02.	Analytical Applications
	Keshawanand Tripathi, Yashdeep Srivastava, Santosh Kumar Mishra, Narendra Kumar
33.	Lowry Method for Protein Quantification: Principles, Protocols, and
	Applications in Biochemical Analysis
	Keshawanand Tripathi, Yashdeep Srivastava, Santosh Kumar Mishra, Narendra Kumar
	,,,
34.	Spectrophotometric Estimation of Phytosterols: Principles, Methodology,
	and Analytical Applications165
	Yashdeep Srivastava, Keshawanand Tripathi, Narendra Kumar
35	Quantification of Leaf Chlorophyll Content: Spectrophotometric
33.	
	Estimation and Physiological Implications
	Yashdeep Srivastava, Keshawanand Tripathi, Narendra Kumar
36.	Investigation of Organogenesis in Bacopa monnieri: Mechanisms, Tissue
	Culture Techniques, and Developmental Insights171
	Yashdeep Srivastava, Keshawanand Tripathi, Narendra Kumar