

Methods in ENZYMOLOGY

Volume 426

Integrins

Edited by

David A. Cheresh





VOLUME FOUR TWENTY SIX

**METHODS IN
ENZYMLOGY**

Integrins

METHODS IN ENZYMOLOGY

Editors-in-Chief

JOHN N. ABELSON AND MELVIN I. SIMON

*Division of Biology
California Institute of Technology
Pasadena, California*

Founding Editors

SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

VOLUME FOUR TWENTY SIX

METHODS IN ENZYMOLGY

Integrins

EDITED BY

DAVID A. CHERESH

Department of Pathology

Moore's Cancer Center

University of California

San Diego, La Jolla



ELSEVIER

AMSTERDAM • BOSTON • HEIDELBERG • LONDON
NEW YORK • OXFORD • PARIS • SAN DIEGO
SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO

Academic Press is an imprint of Elsevier



Academic Press is an imprint of Elsevier
525 B Street, Suite 1900, San Diego, California 92101-4495, USA
84 Theobald's Road, London WC1X 8RR, UK

This book is printed on acid-free paper. 

Copyright © 2007, Elsevier Inc. All Rights Reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage and retrieval system, without permission in writing from the Publisher.

The appearance of the code at the bottom of the first page of a chapter in this book indicates the Publisher's consent that copies of the chapter may be made for personal or internal use of specific clients. This consent is given on the condition, however, that the copier pay the stated per copy fee through the Copyright Clearance Center, Inc. (www.copyright.com), for copying beyond that permitted by Sections 107 or 108 of the U.S. Copyright Law. This consent does not extend to other kinds of copying, such as copying for general distribution, for advertising or promotional purposes, for creating new collective works, or for resale. Copy fees for pre-2007 chapters are as shown on the title pages. If no fee code appears on the title page, the copy fee is the same as for current chapters. 0076-6879/2007 \$35.00

Permissions may be sought directly from Elsevier's Science & Technology Rights Department in Oxford, UK: phone: (+44) 1865 843830, fax: (+44) 1865 853333, E-mail: permissions@elsevier.com. You may also complete your request on-line via the Elsevier homepage (<http://elsevier.com>), by selecting "Support & Contact" then "Copyright and Permission" and then "Obtaining Permissions."

For information on all Elsevier Academic Press publications
visit our Web site at www.books.elsevier.com

ISBN: 978-0-12-373924-7

PRINTED IN THE UNITED STATES OF AMERICA
07 08 09 10 9 8 7 6 5 4 3 2 1

Working together to grow
libraries in developing countries

www.elsevier.com | www.bookaid.org | www.sabre.org

ELSEVIER

BOOK AID
International

Sabre Foundation

CONTENTS

<i>Contributors</i>	<i>xiii</i>
<i>Preface</i>	<i>xix</i>
<i>Volumes in Series</i>	<i>xxi</i>

1. Quantitative Measurements of Integrin-Mediated Adhesion to Extracellular Matrix	1
David Boettiger	
1. Introduction	2
2. Spinning Disc Measurements	3
3. Protocols	4
4. Data Analysis	12
Acknowledgments	19
References	24
2. Investigating Integrin Regulation and Signaling Events in Three-Dimensional Systems	27
Patricia J. Keely, Matthew W. Conklin, Scott Gehler, Suzanne M. Ponik, and Paolo P. Provenzano	
1. Introduction	28
2. Collagen Matrices of Different Densities	31
3. Gel Contraction as a Measure to Quantify Cell Contractility	33
4. Rho Activity Assay from Cells Cultured in 3D Collagen Gels	33
5. Co-Immunoprecipitation of Integrin-Associated Proteins from Cells Cultured in 3D Collagen Gels	35
6. β_1 -Integrin Endocytosis Assays	36
7. Imaging Cell–Matrix Interactions in 3D Collagen Gels	39
References	44
3. Integrins in Cell Migration	47
Keefe T. Chan, Christa L. Cortesio, and Anna Huttenlocher	
1. Introduction	47
2. Methods for Analysis of Integrin-Mediated Cell Migration	49
References	64

4. Integrin Cytoskeletal Interactions	69
Yatish Lad, David S. Harburger, and David A. Calderwood	
1. Introduction	70
2. Models of Integrin Cytoplasmic Tails	72
3. Expression Constructs for Integrin Tail Model Proteins	74
4. Purification of Integrin Cytoplasmic Tails	74
5. Preparation of the Affinity Matrix	76
6. Binding Assays Using Cell Lysates	77
7. Direct Protein–Protein Binding Assays	78
8. Advanced Applications	79
9. Concluding Remarks	81
Acknowledgments	81
References	82
5. Cell Survival in a Three-Dimensional Matrix	85
Alireza Alavi and Dwayne G. Stupack	
1. Introduction	86
2. Protocols	87
3. Conclusions and Perspective	100
References	100
6. Platelet Integrin Adhesive Functions and Signaling	103
Nicolas Prévost, Hisashi Kato, Laurent Bodin, and Sanford J. Shattil	
1. Introduction	104
2. Cell Models	104
3. Microscopy	107
4. Assaying Integrin Adhesive Responses	108
5. Biochemical Analysis of Integrin-Based Signaling	111
References	114
7. Development of Monoclonal Antibodies to Integrin Receptors	117
E. A. Wayner and B. G. Hoffstrom	
1. Introduction	118
2. Methods	121
3. Protocols	139
References	151

8. Cell Adhesion, Cellular Tension, and Cell Cycle Control	155
Eric A. Klein, Yuval Yung, Paola Castagnino, Devashish Kothapalli, and Richard K. Assoian	
1. Introduction	156
2. Preparative Methods	156
3. Analytical Methods	165
Acknowledgments	174
References	174
9. Analysis of Integrin Signaling by Fluorescence Resonance Energy Transfer	177
Yingxiao Wang and Shu Chien	
1. Introduction	178
2. Fluorescence Proteins and FRET	179
3. Integrin Signaling	180
4. FRET Analysis of Integrin Signaling	182
5. Future Directions	196
Acknowledgments	197
References	197
10. Studies on Integrins in the Nervous System	203
Sumiko Denda and Louis F. Reichardt	
1. Introduction	204
2. Neuronal Cell Adhesion and Neurite Outgrowth Assays	208
3. Neuronal Culture Procedures	210
4. Biochemical Studies Using Cultured Neurons	214
References	216
11. Methods for Identifying Novel Integrin Ligands	223
Denise K. Marciano, Sumiko Denda, and Louis F. Reichardt	
1. Introduction	224
2. Production of Soluble $\alpha 8^t \beta 1$ -AP	228
3. Solid-Phase Binding Assays with Soluble Integrin Heterodimers	230
4. Histochemistry with Soluble Integrin Heterodimers	231
5. Far Western Blotting Using Integrin Heterodimers	231
6. Ligand Detection Using Intact Integrin Receptors	232
7. $\alpha 3 \beta 1$ Immunolabeling and Purification	233
8. Receptor-Binding Assays	234
References	234

12. Analysis of Integrin Functions in Peri-Implantation Embryos, Hematopoietic System, and Skin	239
Eloi Montanez, Aleksandra Piwko-Czuchra, Martina Bauer, Shao-hua Li, Peter Yurchenco, and Reinhard Fässler	
1. Introduction	240
2. Analysis of Integrin Functions during Peri-Implantation Development	241
3. Analysis of Integrin Functions in Blood	249
4. Analysis of Integrin Functions in Skin	264
5. <i>In Vitro</i> Skin Analysis	277
References	286
 13. Identification and Molecular Characterization of Multiple Phenotypes in Integrin Knockout Mice	 291
Chun Chen and Dean Sheppard	
1. Introduction	292
2. Strategies for Generating Integrin Knockout Mice	292
3. Validation of Observed Phenotypes by Transgenic Rescue <i>In Vivo</i>	293
4. <i>In Vitro</i> Transgenic Rescue	294
5. Generation of Blocking Mouse Monoclonal Antibodies that Recognize Murine Integrins	294
6. Selection of Integrin Knockout Lines to Study	295
7. Selection of Phenotypic Assays	296
8. Identification of Additional Phenotypes in Integrin Knockouts that Survive Embryonic Development	297
9. Identification of Integrin Knockout Phenotypes Based on Educated Guesses from <i>In Vitro</i> Experiments	299
10. Use of Expression Microarrays to Suggest Possible Phenotypic Assays	300
11. Summary	302
References	302
 14. Purification, Analysis, and Crystal Structure of Integrins	 307
Jian-Ping Xiong, Simon L. Goodman, and M. Amin Arnaout	
1. Introduction	308
2. Purification and Analysis of Integrin Heterodimers	308
3. Atomic Structure of Integrins Using Macromolecular Crystallography	320
4. Summary	328
Acknowledgments	329
References	329

15. Electron Microscopy of Integrins	337
Brian D. Adair and Mark Yeager	
1. Introduction	338
2. Purification of Full-Length $\alpha_{IIb}\beta_3$ from Platelets	339
3. Preparation of Specimens for Negative-Stain EM	341
4. Preparation of Specimens for Cryo-EM	346
5. Low-Dose EM	348
6. Image Appraisal, Particle Selection, and Preprocessing	350
7. Initial Particle Analysis and Generation of Reference-Free Aligned Class Averages	355
8. Three-Dimensional Structure Refinement	357
9. Evaluation of the Refinement	362
10. Pseudoatomic Modeling of EM Density Maps	365
11. Future Prospects for Cryo-EM	368
Acknowledgments	369
References	369
16. Intravital Imaging and Cell Invasion	375
Milan Makale	
1. Introduction	376
2. Methods	380
Acknowledgments	398
References	398
17. Using <i>Xenopus</i> Embryos to Investigate Integrin Function	403
Douglas W. DeSimone, Bette Dzamba, and Lance A. Davidson	
1. Introduction	404
2. Visualization of Integrins, Extracellular Matrix, and Cytoskeleton in Embryo Explants	405
3. Live Imaging of Fibronectin Fibrils	410
4. Conclusions	413
Acknowledgments	413
References	414
18. Methods to Study Lymphatic Vessel Integrins	415
Barbara Garmy-Susini, Milan Makale, Mark Fuster, and Judith A. Varner	
1. Introduction	416
2. Isolation of Human Lymphatic Endothelial Cells	418
3. Isolation of Murine Lymphatic Endothelial Cells	418

4. Characterization of LEC Integrin Expression	421
5. <i>In Vitro</i> Cell Adhesion Assays	421
6. Migration Assays	423
7. Matrigel Tube Formation	424
8. Frozen Section Immunofluorescence Microscopy	425
9. Paraffin-Embedded Section Immunohistochemistry	427
10. Growth-Factor-Induced Lymph Node Lymphangiogenesis	428
11. Murine Lymphangioma Model	428
12. Tumor Models	430
13. Endothelial Cell-Specific Integrin α_4 Deletion Mutant	432
14. Intravital Microscopy of Lymph Nodes	433
15. Abdominal Window Implantation	433
16. Intradermal and Footpad Injections	436
References	436
 19. Analysis of Integrin Signaling in Genetically Engineered Mouse Models of Mammary Tumor Progression	 439
Yuliya Pylayeva, Wenjun Guo, and Filippo G. Giancotti	
1. Introduction	440
2. Experimental Approach	442
3. Measurement of Primary Tumor Growth	443
4. Spontaneous and Allograft Lung Metastasis Assays	444
5. Analysis of Tumor Sections	448
6. Detergent Extraction of Tumor Samples	452
7. <i>Ex Vivo</i> Culture of Mammary Tumor Cells	452
8. Genetic Modification of Primary Mammary Tumor Cells	453
9. Disruption of Epithelial Adhesion and Growth Control	456
Acknowledgments	459
References	459
 20. Design and Chemical Synthesis of Integrin Ligands	 463
Dominik Heckmann and Horst Kessler	
1. Introduction	464
2. Design of Integrin Ligands: Overview	465
3. Application of Integrin Ligands for Imaging and Surface Coating	480
4. Experimental Section	488
References	495

21. Evaluating Integrin Function in Models of Angiogenesis and Vascular Permeability	505
Sara M. Weis	
1. Introduction	506
2. Assessing Role of Integrins During Angiogenesis	511
3. Assessing Role of Integrins in Vascular Permeability	518
4. Concluding Remarks	521
References	523
<i>Author Index</i>	<i>529</i>
<i>Subject Index</i>	<i>559</i>

This page intentionally left blank

CONTRIBUTORS

Brian D. Adair

Department of Cell Biology, The Scripps Research Institute, La Jolla, California

Alireza Alavi

Department of Pathology, School of Medicine, Moores Cancer Center, University of California at San Diego, La Jolla, California

M. Amin Arnaout

Structural Biology Program, Leukocyte Biology and Inflammation Program, Nephrology Division, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts

Richard K. Assoian

Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

Martina Bauer

Max Planck Institute of Biochemistry, Department of Molecular Medicine, Martinsried, Germany

Laurent Bodin

Division of Hematology-Oncology, Department of Medicine, University of California, San Diego, La Jolla, California

David Boettiger

Department of Microbiology, University of Pennsylvania, Philadelphia, Pennsylvania

David A. Calderwood

Department of Pharmacology and Interdepartmental Program in Vascular Biology and Transplantation, Yale University School of Medicine, New Haven, Connecticut

Paola Castagnino

Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

Keefe T. Chan

Department of Molecular and Cellular Pharmacology, University of Wisconsin, Madison, Wisconsin

Chun Chen

Lung Biology Center, Department of Medicine, University of California, San Francisco, San Francisco, California

Shu Chien

Departments of Bioengineering and Medicine, Whitaker Institute of Biomedical Engineering, University of California at San Diego, La Jolla, California

Matthew W. Conklin

Department of Pharmacology, University of Wisconsin Medical School, University of Wisconsin–Madison, Madison, Wisconsin

Christa L. Cortesio

Department of Biomolecular Chemistry, University of Wisconsin, Madison, Wisconsin

Lance A. Davidson

Department of Bioengineering, University of Pittsburgh, Pittsburgh, Pennsylvania

Sumiko Denda

Shiseido Research Center 2, Kanazawa-ku, Yokohama, Japan

Douglas W. DeSimone

Department of Cell Biology, and Morphogenesis and Regenerative Medicine Institute, School of Medicine, University of Virginia, Charlottesville, Virginia

Bette Dzamba

Department of Cell Biology, School of Medicine, University of Virginia, Charlottesville, Virginia

Reinhard Fässler

Max Planck Institute of Biochemistry, Department of Molecular Medicine, Martinsried, Germany

Mark Fuster

Department of Medicine, University of California, San Diego, California

Barbara Garmy-Susini

Moore's UCSD Cancer Center, University of California, San Diego, La Jolla, California

Scott Gehler

Department of Pharmacology, University of Wisconsin Medical School, University of Wisconsin–Madison, Madison, Wisconsin

Filippo G. Giancotti

Sloan-Kettering Division, Weill Graduate School of Medical Sciences, Cornell University, New York, New York

Simon L. Goodman

Preclinical Oncology Research, Merck KGaA, Darmstadt, Germany

Wenjun Guo

Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

David S. Harburger

Department of Pharmacology and Interdepartmental Program in Vascular Biology and Transplantation, Yale University School of Medicine, New Haven, Connecticut

Dominik Heckmann

Department of Chemistry, Technical University München, Garching, Germany

B. G. Hoffstrom

Department of Biological Sciences, Columbia University, New York, New York

Anna Huttenlocher

Departments of Pediatrics and Molecular and Cellular Pharmacology, University of Wisconsin, Madison, Wisconsin

Hisashi Kato

Division of Hematology-Oncology, Department of Medicine, University of California, San Diego, La Jolla, California

Patricia J. Keely

Department of Pharmacology, University of Wisconsin Medical School, University of Wisconsin-Madison, Madison, Wisconsin

Horst Kessler

Department of Chemistry, Technical University München, Garching, Germany

Eric A. Klein

Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

Devashish Kothapalli

Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

Yatish Lad

Department of Pharmacology and Interdepartmental Program in Vascular Biology and Transplantation, Yale University School of Medicine, New Haven, Connecticut

Shaohua Li

Department of Surgery, Robert Wood Johnson Medical School, New Brunswick, New Jersey

Milan Makale

Moore's UCSD Cancer Center, University of California, San Diego, La Jolla, California

Denise K. Marciano

Department of Medicine, Division of Nephrology and Neuroscience Program, Department of Physiology and Howard Hughes Medical Institute, University of California, San Francisco, San Francisco, California

Eloi Montanez

Max Planck Institute of Biochemistry, Department of Molecular Medicine, Martinsried, Germany

Aleksandra Piwko-Czuchra

Max Planck Institute of Biochemistry, Department of Molecular Medicine, Martinsried, Germany

Suzanne M. Ponik

Department of Pharmacology, University of Wisconsin Medical School, University of Wisconsin–Madison, Madison, Wisconsin

Nicolas Prévost

Division of Hematology–Oncology, Department of Medicine, University of California, San Diego, La Jolla, California

Paolo P. Provenzano

Department of Pharmacology, University of Wisconsin Medical School, University of Wisconsin–Madison, Madison, Wisconsin

Yuliya Pylyayeva

Cell Biology Program, Memorial Sloan-Kettering Cancer Center, and Sloan-Kettering Division, Weill Graduate School of Medical Sciences, Cornell University, New York, New York

Louis F. Reichardt

Department of Physiology, Neuroscience Program, Howard Hughes Medical Institute, University of California, San Francisco, San Francisco, California

Sanford J. Shattil

Division of Hematology–Oncology, Department of Medicine, University of California, San Diego, La Jolla, California

Dean Sheppard

Lung Biology Center, Department of Medicine, University of California, San Francisco, San Francisco, California

Dwayne G. Stupack

Department of Pathology, School of Medicine, Moore's Cancer Center, University of California at San Diego, La Jolla, California

Judith A. Varner

Moore's UCSD Cancer Center, University of California, San Diego, La Jolla, California

Yingxiao Wang

Department of Bioengineering and Molecular & Integrative Physiology, Neuroscience Program, Center for Biophysics and Computational Biology, Beckman Institute for Advanced Science and Technology, University of Illinois, Urbana-Champaign, Urbana, Illinois

E. A. Wayner

Antibody Development Laboratory, Fred Hutchinson Cancer Research Center, Seattle, Washington

Sara M. Weis

Moore's UCSD Cancer Center, University of California, San Diego, La Jolla, California

Jian-Ping Xiong

Structural Biology Program, Leukocyte Biology and Inflammation Program, Nephrology Division, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts

Mark Yeager

Department of Cell Biology, The Scripps Research Institute, and Division of Cardiovascular Diseases, Scripps Clinic, La Jolla, California

Yuval Yung

Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

Peter Yurchenco

Department of Pathology, Robert Wood Johnson Medical School, Piscataway, New Jersey

This page intentionally left blank

PREFACE

Integrins are a family of cell surface receptors that mediate contact between a cell and its surrounding extracellular matrix and microenvironment. Integrins not only regulate cell attachment (Chapter 1), but also act to transmit signals across the plasma membrane (2, 6, 9). They also serve as mechanotransducers (8) that, in turn, influence the cells' cytoskeleton assembly (4), leading to changes in cell migration/invasion (3, 16) and ultimately influencing cell survival (5) and cell cycle control (8). The techniques associated with understanding how cells respond to integrin ligation in two dimensional setting (1) and in three dimensional microenvironments (2, 5) have greatly advanced the field and our basic understanding of biology and disease. As such, integrins have been shown to play a key role in embryonic development (10, 12), immune recognition, tissue homeostasis, and wound repair (13). In addition, integrins have been shown to regulate various pathological conditions such as cancer, inflammation, and cardiovascular disease (6, 12, 13, 18, 21). In fact, integrin mutations or dysregulation of integrin function are responsible for diseases associated with defective platelet aggregation and clotting, altered immune function, and altered tissue morphogenesis (6, 12, 18, 21). Integrin ligands (11) are typically found in the extracellular matrix and basement membrane and include proteins such as collagens, fibronectins, and laminins. However, during tissue remodeling, cancer, and angiogenesis (21), some specific integrins expressed on invasive cells recognize provisional matrix proteins including fibronectin, vitronectin, fibrin, and osteopontin, among others. For this reason it is important to have techniques available to understand how integrins function to promote cell adhesion to and invasion of the extracellular matrix (1–4). In some cases, integrins can recognize ligands on the surface of other cells. This is particularly true among hemaptoietic cells, those in the blood stream, and in the lymphatic system (6, 18, 21).

Recently, integrins have been recognized as important drug targets. In fact, there has been considerable effort in establishing technology to design integrin antagonists for use in treating various disease conditions (7, 20). For example, inhibitors (antagonists) of the platelet integrin $\alpha\text{IIb}\beta 3$ are used to suppress clot formation in patients with thrombotic disorders (6). Other integrin antagonists suppress immune recognition and thereby regulate inflammatory disease and/or autoimmune diseases such as multiple sclerosis. More recently, clinical trials have established that alpha V integrin antagonists can be used to treat or diagnose human cancer (20, 21). These alpha V

integrin antagonists have been shown to directly suppress tumor growth and invasion and/or suppress the process of tumor angiogenesis. There are three forms of integrin antagonists: antibodies, peptides, and small organic peptidomimetics. Anti-integrin antibodies can directly compete for ligand binding or act as allosteric inhibitors. In general, integrin antibody antagonists tend to be more specific than peptide or peptidomimetic antagonists. The development of specific, function-blocking antibodies to integrins (7) has provided the most important tool for the biologist to understand how integrins function in the context of cells and the intact organism. However, integrin antibodies are generally produced in mice (7) and then subjected to humanization prior to being developed as clinical candidates. The development of integrin inhibitors has been aided by scientific approaches to studying how integrins function on cells (1–6, 8, 18, 21) and how they structurally interact with their ligands (14, 15, 20). In addition, the use of genetic models of mice lacking integrins or expressing mutant integrins have been absolutely critically in understanding the role that integrins play in the intact organism (12, 13, 17, 19). However, in a number of cases integrin knockout mice can have a different phenotypes than what one observes when treating mice with specific integrin antagonists. For example, mice deficient in alpha V integrins can develop with a normal-looking vasculature, yet animals treated with alpha V integrin antagonists show a disrupted angiogenic response (21). This may be due to compensatory changes that occur in response to the genetic knockout or due to molecular redundancy. In either event, it is difficult to compare the phenotypes of wild-type mice treated with integrin antagonists to mice entirely lacking an integrin to begin with.

The integrin field has not only made a significant impact on our understanding of basic cell biology, but it has provided important insight into tissue remodeling in the embryo and the adult. The structural, molecular, and biological techniques have combined to elucidate the role that integrins play in these processes and in the development of a wide array of pathological conditions. The field has now progressed to the point where new therapeutic strategies have been developed or are under development to treat everything from cardiovascular disease to inflammatory disease and cancer. The techniques outlined in this volume provide a complete guide to understanding the structure, function, and biological properties of integrins.

I would like to thank the authors of this volume for agreeing to participate in this project as these individuals, having made many of the key contributions to our understanding of the structure, function, and biology of integrins, represent the leaders in this field. I am particularly grateful to Cindy Minor and Jamey Stegmaier and their project management efforts in making this volume possible.

David A. Cheresh

METHODS IN ENZYMOLOGY

VOLUME I. Preparation and Assay of Enzymes

Edited by SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

VOLUME II. Preparation and Assay of Enzymes

Edited by SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

VOLUME III. Preparation and Assay of Substrates

Edited by SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

VOLUME IV. Special Techniques for the Enzymologist

Edited by SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

VOLUME V. Preparation and Assay of Enzymes

Edited by SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

VOLUME VI. Preparation and Assay of Enzymes (*Continued*)

Preparation and Assay of Substrates

Special Techniques

Edited by SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

VOLUME VII. Cumulative Subject Index

Edited by SIDNEY P. COLOWICK AND NATHAN O. KAPLAN

VOLUME VIII. Complex Carbohydrates

Edited by ELIZABETH F. NEUFELD AND VICTOR GINSBURG

VOLUME IX. Carbohydrate Metabolism

Edited by WILLIS A. WOOD

VOLUME X. Oxidation and Phosphorylation

Edited by RONALD W. ESTABROOK AND MAYNARD E. PULLMAN

VOLUME XI. Enzyme Structure

Edited by C. H. W. HIRS

VOLUME XII. Nucleic Acids (Parts A and B)

Edited by LAWRENCE GROSSMAN AND KIVIE MOLDAVE

VOLUME XIII. Citric Acid Cycle

Edited by J. M. LOWENSTEIN

VOLUME XIV. Lipids

Edited by J. M. LOWENSTEIN

VOLUME XV. Steroids and Terpenoids

Edited by RAYMOND B. CLAYTON

VOLUME XVI. Fast Reactions

Edited by KENNETH KUSTIN

VOLUME XVII. Metabolism of Amino Acids and Amines (Parts A and B)

Edited by HERBERT TABOR AND CELIA WHITE TABOR

VOLUME XVIII. Vitamins and Coenzymes (Parts A, B, and C)

Edited by DONALD B. MCCORMICK AND LEMUEL D. WRIGHT

VOLUME XIX. Proteolytic Enzymes

Edited by GERTRUDE E. PERLMANN AND LASZLO LORAND

VOLUME XX. Nucleic Acids and Protein Synthesis (Part C)

Edited by KIVIE MOLDAVE AND LAWRENCE GROSSMAN

VOLUME XXI. Nucleic Acids (Part D)

Edited by LAWRENCE GROSSMAN AND KIVIE MOLDAVE

VOLUME XXII. Enzyme Purification and Related Techniques

Edited by WILLIAM B. JAKOBY

VOLUME XXIII. Photosynthesis (Part A)

Edited by ANTHONY SAN PIETRO

VOLUME XXIV. Photosynthesis and Nitrogen Fixation (Part B)

Edited by ANTHONY SAN PIETRO

VOLUME XXV. Enzyme Structure (Part B)

Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME XXVI. Enzyme Structure (Part C)

Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME XXVII. Enzyme Structure (Part D)

Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME XXVIII. Complex Carbohydrates (Part B)

Edited by VICTOR GINSBURG

VOLUME XXIX. Nucleic Acids and Protein Synthesis (Part E)

Edited by LAWRENCE GROSSMAN AND KIVIE MOLDAVE

VOLUME XXX. Nucleic Acids and Protein Synthesis (Part F)

Edited by KIVIE MOLDAVE AND LAWRENCE GROSSMAN

VOLUME XXXI. Biomembranes (Part A)

Edited by SIDNEY FLEISCHER AND LESTER PACKER

VOLUME XXXII. Biomembranes (Part B)

Edited by SIDNEY FLEISCHER AND LESTER PACKER

VOLUME XXXIII. Cumulative Subject Index Volumes I-XXX

Edited by MARTHA G. DENNIS AND EDWARD A. DENNIS

VOLUME XXXIV. Affinity Techniques (Enzyme Purification: Part B)

Edited by WILLIAM B. JAKOBY AND MEIR WILCHEK

VOLUME XXXV. Lipids (Part B)

Edited by JOHN M. LOWENSTEIN

VOLUME XXXVI. Hormone Action (Part A: Steroid Hormones)

Edited by BERT W. O'MALLEY AND JOEL G. HARDMAN

VOLUME XXXVII. Hormone Action (Part B: Peptide Hormones)

Edited by BERT W. O'MALLEY AND JOEL G. HARDMAN

VOLUME XXXVIII. Hormone Action (Part C: Cyclic Nucleotides)

Edited by JOEL G. HARDMAN AND BERT W. O'MALLEY

VOLUME XXXIX. Hormone Action (Part D: Isolated Cells, Tissues, and Organ Systems)

Edited by JOEL G. HARDMAN AND BERT W. O'MALLEY

VOLUME XL. Hormone Action (Part E: Nuclear Structure and Function)

Edited by BERT W. O'MALLEY AND JOEL G. HARDMAN

VOLUME XLI. Carbohydrate Metabolism (Part B)

Edited by W. A. WOOD

VOLUME XLII. Carbohydrate Metabolism (Part C)

Edited by W. A. WOOD

VOLUME XLIII. Antibiotics

Edited by JOHN H. HASH

VOLUME XLIV. Immobilized Enzymes

Edited by KLAUS MOSBACH

VOLUME XLV. Proteolytic Enzymes (Part B)

Edited by LASZLO LORAND

VOLUME XLVI. Affinity Labeling

Edited by WILLIAM B. JAKOBY AND MEIR WILCHEK

VOLUME XLVII. Enzyme Structure (Part E)

Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME XLVIII. Enzyme Structure (Part F)

Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME XLIX. Enzyme Structure (Part G)

Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME L. Complex Carbohydrates (Part C)

Edited by VICTOR GINSBURG

VOLUME LI. Purine and Pyrimidine Nucleotide Metabolism

Edited by PATRICIA A. HOFFEE AND MARY ELLEN JONES

VOLUME LII. Biomembranes (Part C: Biological Oxidations)

Edited by SIDNEY FLEISCHER AND LESTER PACKER

VOLUME LIII. Biomembranes (Part D: Biological Oxidations)

Edited by SIDNEY FLEISCHER AND LESTER PACKER

VOLUME LIV. Biomembranes (Part E: Biological Oxidations)

Edited by SIDNEY FLEISCHER AND LESTER PACKER

VOLUME LV. Biomembranes (Part F: Bioenergetics)

Edited by SIDNEY FLEISCHER AND LESTER PACKER

VOLUME LVI. Biomembranes (Part G: Bioenergetics)

Edited by SIDNEY FLEISCHER AND LESTER PACKER

VOLUME LVII. Bioluminescence and Chemiluminescence

Edited by MARLENE A. DeLUCA

VOLUME LVIII. Cell Culture

Edited by WILLIAM B. JAKOBY AND IRA PASTAN

VOLUME LIX. Nucleic Acids and Protein Synthesis (Part G)

Edited by KIVIE MOLDAVE AND LAWRENCE GROSSMAN

VOLUME LX. Nucleic Acids and Protein Synthesis (Part H)

Edited by KIVIE MOLDAVE AND LAWRENCE GROSSMAN

VOLUME 61. Enzyme Structure (Part H)

Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME 62. Vitamins and Coenzymes (Part D)

Edited by DONALD B. McCORMICK AND LEMUEL D. WRIGHT

VOLUME 63. Enzyme Kinetics and Mechanism (Part A: Initial Rate and Inhibitor Methods)

Edited by DANIEL L. PURICH

VOLUME 64. Enzyme Kinetics and Mechanism

(Part B: Isotopic Probes and Complex Enzyme Systems)

Edited by DANIEL L. PURICH

VOLUME 65. Nucleic Acids (Part I)

Edited by LAWRENCE GROSSMAN AND KIVIE MOLDAVE

VOLUME 66. Vitamins and Coenzymes (Part E)

Edited by DONALD B. McCORMICK AND LEMUEL D. WRIGHT

VOLUME 67. Vitamins and Coenzymes (Part F)

Edited by DONALD B. McCORMICK AND LEMUEL D. WRIGHT

VOLUME 68. Recombinant DNA

Edited by RAY WU

VOLUME 69. Photosynthesis and Nitrogen Fixation (Part C)

Edited by ANTHONY SAN PIETRO

VOLUME 70. Immunochemical Techniques (Part A)

Edited by HELEN VAN VUNAKIS AND JOHN J. LANGONE

VOLUME 71. Lipids (Part C)

Edited by JOHN M. LOWENSTEIN

VOLUME 72. Lipids (Part D)

Edited by JOHN M. LOWENSTEIN

VOLUME 73. Immunochemical Techniques (Part B)

Edited by JOHN J. LANGONE AND HELEN VAN VUNAKIS

VOLUME 74. Immunochemical Techniques (Part C)

Edited by JOHN J. LANGONE AND HELEN VAN VUNAKIS

VOLUME 75. Cumulative Subject Index Volumes XXXI, XXXII, XXXIV–LX

Edited by EDWARD A. DENNIS AND MARTHA G. DENNIS

VOLUME 76. Hemoglobins

Edited by ERALDO ANTONINI, LUIGI ROSSI-BERNARDI, AND EMILIA CHIANCONE

VOLUME 77. Detoxication and Drug Metabolism

Edited by WILLIAM B. JAKOBY

VOLUME 78. Interferons (Part A)

Edited by SIDNEY PESTKA

VOLUME 79. Interferons (Part B)

Edited by SIDNEY PESTKA

VOLUME 80. Proteolytic Enzymes (Part C)

Edited by LASZLO LORAND

VOLUME 81. Biomembranes (Part H: Visual Pigments and Purple Membranes, I)

Edited by LESTER PACKER

VOLUME 82. Structural and Contractile Proteins (Part A: Extracellular Matrix)

Edited by LEON W. CUNNINGHAM AND DIXIE W. FREDERIKSEN

VOLUME 83. Complex Carbohydrates (Part D)

Edited by VICTOR GINSBURG

VOLUME 84. Immunochemical Techniques (Part D: Selected Immunoassays)

Edited by JOHN J. LANGONE AND HELEN VAN VUNAKIS

VOLUME 85. Structural and Contractile Proteins (Part B: The Contractile Apparatus and the Cytoskeleton)

Edited by DIXIE W. FREDERIKSEN AND LEON W. CUNNINGHAM

VOLUME 86. Prostaglandins and Arachidonate Metabolites

Edited by WILLIAM E. M. LANDS AND WILLIAM L. SMITH

VOLUME 87. Enzyme Kinetics and Mechanism (Part C: Intermediates, Stereo-chemistry, and Rate Studies)

Edited by DANIEL L. PURICH

VOLUME 88. Biomembranes (Part I: Visual Pigments and Purple Membranes, II)

Edited by LESTER PACKER

VOLUME 89. Carbohydrate Metabolism (Part D)

Edited by WILLIS A. WOOD

VOLUME 90. Carbohydrate Metabolism (Part E)

Edited by WILLIS A. WOOD

VOLUME 91. Enzyme Structure (Part I)

Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME 92. Immunochemical Techniques (Part E: Monoclonal Antibodies and General Immunoassay Methods)

Edited by JOHN J. LANGONE AND HELEN VAN VUNAKIS

VOLUME 93. Immunochemical Techniques (Part F: Conventional Antibodies, Fc Receptors, and Cytotoxicity)

Edited by JOHN J. LANGONE AND HELEN VAN VUNAKIS

VOLUME 94. Polyamines

Edited by HERBERT TABOR AND CELIA WHITE TABOR

VOLUME 95. Cumulative Subject Index Volumes 61–74, 76–80

Edited by EDWARD A. DENNIS AND MARTHA G. DENNIS

VOLUME 96. Biomembranes [Part J: Membrane Biogenesis: Assembly and Targeting (General Methods; Eukaryotes)]

Edited by SIDNEY FLEISCHER AND BECCA FLEISCHER

VOLUME 97. Biomembranes [Part K: Membrane Biogenesis: Assembly and Targeting (Prokaryotes, Mitochondria, and Chloroplasts)]

Edited by SIDNEY FLEISCHER AND BECCA FLEISCHER

VOLUME 98. Biomembranes (Part L: Membrane Biogenesis: Processing and Recycling)

Edited by SIDNEY FLEISCHER AND BECCA FLEISCHER

VOLUME 99. Hormone Action (Part F: Protein Kinases)

Edited by JACKIE D. CORBIN AND JOEL G. HARDMAN

VOLUME 100. Recombinant DNA (Part B)

Edited by RAY WU, LAWRENCE GROSSMAN, AND KIVIE MOLDAVE

VOLUME 101. Recombinant DNA (Part C)

Edited by RAY WU, LAWRENCE GROSSMAN, AND KIVIE MOLDAVE

VOLUME 102. Hormone Action (Part G: Calmodulin and Calcium-Binding Proteins)

Edited by ANTHONY R. MEANS AND BERT W. O'MALLEY

VOLUME 103. Hormone Action (Part H: Neuroendocrine Peptides)

Edited by P. MICHAEL CONN

VOLUME 104. Enzyme Purification and Related Techniques (Part C)

Edited by WILLIAM B. JAKOBY

VOLUME 105. Oxygen Radicals in Biological Systems

Edited by LESTER PACKER

VOLUME 106. Posttranslational Modifications (Part A)

Edited by FINN WOLD AND KIVIE MOLDAVE

VOLUME 107. Posttranslational Modifications (Part B)

Edited by FINN WOLD AND KIVIE MOLDAVE

VOLUME 108. Immunochemical Techniques (Part G: Separation and Characterization of Lymphoid Cells)

Edited by GIOVANNI DI SABATO, JOHN J. LANGONE, AND HELEN VAN VUNAKIS

VOLUME 109. Hormone Action (Part I: Peptide Hormones)

Edited by LUTZ BIRNBAUMER AND BERT W. O'MALLEY

VOLUME 110. Steroids and Isoprenoids (Part A)

Edited by JOHN H. LAW AND HANS C. RILLING

VOLUME 111. Steroids and Isoprenoids (Part B)

Edited by JOHN H. LAW AND HANS C. RILLING

VOLUME 112. Drug and Enzyme Targeting (Part A)

Edited by KENNETH J. WIDDER AND RALPH GREEN

VOLUME 113. Glutamate, Glutamine, Glutathione, and Related Compounds

Edited by ALTON MEISTER

VOLUME 114. Diffraction Methods for Biological Macromolecules (Part A)

Edited by HAROLD W. WYCKOFF, C. H. W. HIRS, AND SERGE N. TIMASHEFF

VOLUME 115. Diffraction Methods for Biological Macromolecules (Part B)

Edited by HAROLD W. WYCKOFF, C. H. W. HIRS, AND SERGE N. TIMASHEFF

VOLUME 116. Immunochemical Techniques

(Part H: Effectors and Mediators of Lymphoid Cell Functions)

Edited by GIOVANNI DI SABATO, JOHN J. LANGONE, AND HELEN VAN VUNAKIS

VOLUME 117. Enzyme Structure (Part J)

Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME 118. Plant Molecular Biology

Edited by ARTHUR WEISSBACH AND HERBERT WEISSBACH

VOLUME 119. Interferons (Part C)

Edited by SIDNEY PESTKA

VOLUME 120. Cumulative Subject Index Volumes 81–94, 96–101

VOLUME 121. Immunochemical Techniques (Part I: Hybridoma Technology and Monoclonal Antibodies)

Edited by JOHN J. LANGONE AND HELEN VAN VUNAKIS

VOLUME 122. Vitamins and Coenzymes (Part G)

Edited by FRANK CHYTIL AND DONALD B. MCCORMICK

VOLUME 123. Vitamins and Coenzymes (Part H)

Edited by FRANK CHYTIL AND DONALD B. MCCORMICK

VOLUME 124. Hormone Action (Part J: Neuroendocrine Peptides)

Edited by P. MICHAEL CONN

VOLUME 125. Biomembranes (Part M: Transport in Bacteria, Mitochondria, and Chloroplasts: General Approaches and Transport Systems)

Edited by SIDNEY FLEISCHER AND BECCA FLEISCHER

VOLUME 126. Biomembranes (Part N: Transport in Bacteria, Mitochondria, and Chloroplasts: Protonmotive Force)

Edited by SIDNEY FLEISCHER AND BECCA FLEISCHER

VOLUME 127. Biomembranes (Part O: Protons and Water: Structure and Translocation)

Edited by LESTER PACKER

VOLUME 128. Plasma Lipoproteins (Part A: Preparation, Structure, and Molecular Biology)

Edited by JERE P. SEGREST AND JOHN J. ALBERS

VOLUME 129. Plasma Lipoproteins (Part B: Characterization, Cell Biology, and Metabolism)

Edited by JOHN J. ALBERS AND JERE P. SEGREST

VOLUME 130. Enzyme Structure (Part K)

Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME 131. Enzyme Structure (Part L)

Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

VOLUME 132. Immunochemical Techniques (Part J: Phagocytosis and Cell-Mediated Cytotoxicity)

Edited by GIOVANNI DI SABATO AND JOHANNES EVERSE

VOLUME 133. Bioluminescence and Chemiluminescence (Part B)

Edited by MARLENE DELUCA AND WILLIAM D. MCELROY

VOLUME 134. Structural and Contractile Proteins (Part C: The Contractile Apparatus and the Cytoskeleton)

Edited by RICHARD B. VALLEE

VOLUME 135. Immobilized Enzymes and Cells (Part B)

Edited by KLAUS MOSBACH

VOLUME 136. Immobilized Enzymes and Cells (Part C)

Edited by KLAUS MOSBACH

VOLUME 137. Immobilized Enzymes and Cells (Part D)

Edited by KLAUS MOSBACH

VOLUME 138. Complex Carbohydrates (Part E)

Edited by VICTOR GINSBURG

VOLUME 139. Cellular Regulators (Part A: Calcium- and Calmodulin-Binding Proteins)

Edited by ANTHONY R. MEANS AND P. MICHAEL CONN

VOLUME 140. Cumulative Subject Index Volumes 102–119, 121–134

VOLUME 141. Cellular Regulators (Part B: Calcium and Lipids)

Edited by P. MICHAEL CONN AND ANTHONY R. MEANS

VOLUME 142. Metabolism of Aromatic Amino Acids and Amines

Edited by SEYMOUR KAUFMAN

VOLUME 143. Sulfur and Sulfur Amino Acids

Edited by WILLIAM B. JAKOBY AND OWEN GRIFFITH

VOLUME 144. Structural and Contractile Proteins (Part D: Extracellular Matrix)

Edited by LEON W. CUNNINGHAM

VOLUME 145. Structural and Contractile Proteins (Part E: Extracellular Matrix)

Edited by LEON W. CUNNINGHAM

VOLUME 146. Peptide Growth Factors (Part A)

Edited by DAVID BARNES AND DAVID A. SIRBASKU

VOLUME 147. Peptide Growth Factors (Part B)

Edited by DAVID BARNES AND DAVID A. SIRBASKU

VOLUME 148. Plant Cell Membranes

Edited by LESTER PACKER AND ROLAND DOUCE

VOLUME 149. Drug and Enzyme Targeting (Part B)

Edited by RALPH GREEN AND KENNETH J. WIDDER

VOLUME 150. Immunochemical Techniques (Part K: *In Vitro* Models of B and T Cell Functions and Lymphoid Cell Receptors)

Edited by GIOVANNI DI SABATO

VOLUME 151. Molecular Genetics of Mammalian Cells

Edited by MICHAEL M. GOTTESMAN

VOLUME 152. Guide to Molecular Cloning Techniques

Edited by SHELBY L. BERGER AND ALAN R. KIMMEL

VOLUME 153. Recombinant DNA (Part D)

Edited by RAY WU AND LAWRENCE GROSSMAN

VOLUME 154. Recombinant DNA (Part E)

Edited by RAY WU AND LAWRENCE GROSSMAN

VOLUME 155. Recombinant DNA (Part F)

Edited by RAY WU

VOLUME 156. Biomembranes (Part P: ATP-Driven Pumps and Related Transport: The Na, K-Pump)

Edited by SIDNEY FLEISCHER AND BECCA FLEISCHER

VOLUME 157. Biomembranes (Part Q: ATP-Driven Pumps and Related Transport: Calcium, Proton, and Potassium Pumps)

Edited by SIDNEY FLEISCHER AND BECCA FLEISCHER

VOLUME 158. Metalloproteins (Part A)

Edited by JAMES F. RIORDAN AND BERT L. VALLEE

VOLUME 159. Initiation and Termination of Cyclic Nucleotide Action

Edited by JACKIE D. CORBIN AND ROGER A. JOHNSON

VOLUME 160. Biomass (Part A: Cellulose and Hemicellulose)

Edited by WILLIS A. WOOD AND SCOTT T. KELLOGG

VOLUME 161. Biomass (Part B: Lignin, Pectin, and Chitin)

Edited by WILLIS A. WOOD AND SCOTT T. KELLOGG

VOLUME 162. Immunochemical Techniques (Part L: Chemotaxis and Inflammation)

Edited by GIOVANNI DI SABATO

VOLUME 163. Immunochemical Techniques (Part M: Chemotaxis and Inflammation)

Edited by GIOVANNI DI SABATO

VOLUME 164. Ribosomes

Edited by HARRY F. NOLLER, JR., AND KIVIE MOLDAVE

VOLUME 165. Microbial Toxins: Tools for Enzymology

Edited by SIDNEY HARSHMAN

VOLUME 166. Branched-Chain Amino Acids

Edited by ROBERT HARRIS AND JOHN R. SOKATCH

VOLUME 167. Cyanobacteria

Edited by LESTER PACKER AND ALEXANDER N. GLAZER

VOLUME 168. Hormone Action (Part K: Neuroendocrine Peptides)

Edited by P. MICHAEL CONN

VOLUME 169. Platelets: Receptors, Adhesion, Secretion (Part A)

Edited by JACEK HAWIGER

VOLUME 170. Nucleosomes

Edited by PAUL M. WASSARMAN AND ROGER D. KORNBERG

VOLUME 171. Biomembranes (Part R: Transport Theory: Cells and Model Membranes)

Edited by SIDNEY FLEISCHER AND BECCA FLEISCHER

VOLUME 172. Biomembranes (Part S: Transport: Membrane Isolation and Characterization)

Edited by SIDNEY FLEISCHER AND BECCA FLEISCHER