Immunochemical Protocols

Methods in Molecular Biology

John M. Walker, SERIES EDITOR

1. **Proteins**, edited by ***John M. Walker***, 1984
2. **Nucleic Acids**, edited by ***John M. Walker***, 1984
3. **New Protein Techniques**, edited by ***John M. Walker***, 1988
4. **New Nucleic Acid Techniques**, edited by ***John M. Walker***, 1988
5. **Animal Cell Culture**, edited by ***Jeffrey W. Pollard and John M. Walker***, 1990
6. **Plant Cell and Tissue Culture**, edited by ***Jeffrey W. Pollard and John M. Walker***, 1990
7. **Gene Transfer and Expression Protocols**, edited by ***E. J. Murray***, 1991
8. **Practical Molecular Virology**, edited by ***Mary K. L. Collins***, 1991
9. **Protocols in Human Molecular Genetics**, edited by ***Christopher G. Mathew***, 1991
10. **Immunochemical Protocols**, edited by ***Margaret M. Manson***, 1992

Methods in Molecular Biology • 10

Immunochemical Protocols

Edited by

Margaret M. Manson

Medical Research Council Laboratories, Carshalton, Surrey, UK

Humana Press **Totowa, New Jersey**

© 1992 Humana Press Inc.
999 Riverview Drive, Suite 208
Totowa, New Jersey 07512

All rights reserved.

No part of this book may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, microfilming, recording, or otherwise without written permission from the Publisher.

Photocopy Authorization Policy:

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by The Humana Press Inc., **provided** that the base fee of US \$3.00 per copy, plus US \$00.20 per page is paid directly to the Copyright Clearance Center at 27 Congress Street, Salem, MA 01970. For those organizations that have been granted a photocopy license from the CCC, a separate system of payment has been arranged and is acceptable to The Humana Press Inc. The fee code for users of the Transactional Reporting Service is: [0-89603-204-3/92 \$3.00 + \$00.20].

Printed in the United States of America. 10 9 8 7 6 5 4 3 2

Library of Congress Cataloging in Publication Data
Main entry under title:

Methods in molecular biology.

Immunochemical Protocols / edited by Margaret M. Manson.

p. cm. – (Methods in molecular biology ; 10)

Includes index.

ISBN 0-89603-204-3 (hard cover)

ISBN 0-89603-270-1 (comb bound)

1. Immunochemistry–Methodology. I. Manson, Margaret M.

II. Series: Methods in molecular biology ; v. 10.

QR183.6.I43 1992

616.07'56–dc20

91-41416

CIP

Preface

Molecular biologists can no longer afford to be experts in only one particular area. They need to be equally competent at handling DNA, RNA, and proteins, moving from one area to another as required by the problem. Advances in the field have been greatly helped by numerous applications of antibody–antigen interactions, so that a basic knowledge of immunology has now become useful.

The emphasis of *Immunochemical Protocols* is not on the production of antibodies, since many people will beg or buy these, but rather on the enormous range of applications to which these powerful reagents can be put. For completeness, however, methods are included for the production of both polyclonal and monoclonal antibodies. Techniques that may be helpful in obtaining antibodies to elusive antigens and to synthetic peptides are also provided. Both qualitative and quantitative methods are described for localizing, characterizing, and quantifying various antigens. These range from the use of antibodies in screening DNA libraries to their application in ELISA assays for quantifying adducts in alkylated DNA.

Each method is described by an author who has regularly used the technique in his or her own laboratory. Not all the techniques necessarily represent the state-of-the-art—they are, however, all dependable methods that regularly achieve the desired result.

Each chapter starts with a brief description of the basic theory behind the technique being described. The main aim of this book, however, is to outline the practical steps necessary for carrying out the method successfully. The Methods section therefore contains a detailed step-by-step description of each protocol. The Notes section compliments the Methods section by indicating any major problems or difficulties that might arise in using the technique, as well as those adaptations, modifications, or alterations that might prove helpful in specific applications.

Immunochemical Protocols thus should be particularly useful to those with no previous experience of a technique, appealing to postgraduates and research workers who wish to try a technique for the first time, as well as to undergraduates (especially project students).

Margaret M. Manson

Contents

Preface	v
Contributors	xi
CH. 1. Production of Polyclonal Antisera, <i>Jonathan A. Green and Margaret M. Manson</i>	1
CH. 2. Efficient Elution of Purified Proteins from Polyvinylidene Difluoride Membranes(Immobilon)After Transfer from SDS-PAGE and Their Use as Immunogens, <i>Boguslaw Szewczyk and Donald F. Summers</i>	7
CH. 3. Raising Polyclonal Antibodies Using Nitrocellulose-Bound Antigen, <i>Monique Diano, André Le Bivic, and Michel Hirn</i>	13
CH. 4. Synthesis of Peptides for Use as Immunogens, <i>David C. Hancock and Gerard I. Evan</i>	23
CH. 5. Production and Characterization of Antibodies Against Synthetic Peptides, <i>David C. Hancock and Gerard I. Evan</i>	33
CH. 6. Preparation and Testing of Monoclonal Antibodies to Recombinant Proteins, <i>Christopher J. Dean</i>	43
CH. 7. Screening of Monoclonal Antibodies Using Antigens Labeled with Acetylcholinesterase, <i>Yveline Frobert and Jacques Grassi</i>	65
CH. 8. Purification of Immunoglobulin G (IgG), <i>Michael G. Baines and Robin Thorpe</i>	79
CH. 9. Epitope Mapping, <i>Sara E. Mole</i>	105
CH. 10. Enzyme–Antienzyme Method for Immunohistochemistry, <i>Michael G. Ormerod and Susanne F. Imrie</i>	117
CH. 11. Double Label Immunohistochemistry on Tissue Sections Using Alkaline Phosphatase and Peroxidase Conjugates, <i>Jonathan A. Green and Margaret M. Manson</i>	125
CH. 12. Immunohistochemical Detection of Bromodeoxyuridine-Labeled Nuclei for In Vivo Cell Kinetic Studies, <i>Jonathan A. Green, Richard E. Edwards, and Margaret M. Manson</i>	131

CH. 13. Avidin-Biotin Technology: <i>Preparation of Biotinylated Probes,</i> <i>Edward A. Bayer and Meir Wilchek</i>	137
CH. 14. Avidin-Biotin Technology: <i>Preparation of Avidin Conjugates,</i> <i>Edward A. Bayer and Meir Wilchek</i>	143
CH. 15. Immunochemical Applications of Avidin-Biotin Technology, <i>Edward A. Bayer and Meir Wilchek</i>	149
CH. 16. Preparation of Gold Probes, <i>Julian E. Beesley</i>	163
CH. 17. Immunogold Probes for Light Microscopy, <i>Julian E. Beesley</i>	169
CH. 18. Immunogold Probes in Electron Microscopy, <i>Julian E. Beesley</i>	177
CH. 19. Electron Microscopic Silver Enhancement for Double Labeling with Antibodies Raised in the Same Species, <i>Kurt Bienz and Denise Egger</i>	187
CH. 20. Quantitative and Qualitative Immunoelectrophoresis: <i>General</i> <i>Comments on Principles, Reagents, Equipment, and Procedures,</i> <i>Anne Laine</i>	195
CH. 21. Rocket Immunoelectrophoresis Technique or Electroimmunodiffusion, <i>Anne Laine</i>	201
CH. 22. Crossed Immunoelectrophoresis, <i>Anne Laine</i>	207
CH. 23. Crossed Immunoaffinoelectrophoresis, <i>Anne Laine</i>	215
CH. 24. Immunodetection of Proteins by Western Blotting, <i>Colin J. Henderson and C. Roland Wolf</i>	221
CH. 25. Erasable Western Blots, <i>Scott H. Kaufmann and Joel H. Shaper</i>	235
CH. 26. Colloidal Gold Staining and Immunoprobng on the Same Western Blot, <i>Denise Egger and Kurt Bienz</i>	247
CH. 27. Colloidal Gold Staining and Immunodetection in 2-D Protein Mapping, <i>Anthony H. V. Schapira</i>	255
CH. 28. Fluorescent Protein Staining on Nitrocellulose with Subsequent Immunodetection of Antigen, <i>Boguslaw Szewczyk and Donald F. Summers</i>	261
CH. 29. Competitive ELISA, <i>Kitti Makarananda and Gordon E. Neal</i>	267
CH. 30. Twin-Site ELISAs for <i>fos</i> and <i>myc</i> Oncoproteins Using the AMPAK System, <i>John P. Moore and David L. Bates</i>	273
CH. 31. Preparation of Cytotoxic Antibody-Toxin Conjugates, <i>Alan J. Cumber and Edward J. Wawrzynczak</i>	283

CH. 32.	Immunoaffinity Purification and Quantification of Antibody–Toxin Conjugates, <i>Edward J. Wawrzynczak and Alan J. Cumber</i>	295
CH. 33.	An Immuno-Slot-Blot Assay for Detection and Quantitation of Alkyldeoxyguanosines in DNA, <i>Barbara I. Ludeke</i>	307
CH. 34.	Production of Monoclonal Antibodies for the Detection of Chemically Modified DNA, <i>Michael J. Tilby</i>	321
CH. 35.	Sensitive Competitive Enzyme-Linked Immunoassay for Quantitation of Modified Bases in DNA, <i>Michael J. Tilby</i>	329
CH. 36.	An Immunochemical Assay for Detecting Transition of B-DNA to Z-DNA, <i>T. J. Thomas</i>	337
CH. 37.	Cell Sorting Using Immunomagnetic Beads, <i>Eddie C. Y. Wang, Leszek K. Borysiewicz, and Anthony P. Weetman</i>	347
CH. 38.	Cell Preparation for Flow Cytometry, <i>Michael G. Ormerod</i>	359
CH. 39.	Preparation of Rat Lung Cells for Flow Cytometry, <i>Janet Martin and Ian N. H. White</i>	363
CH. 40.	The Isolation of Rat Hepatocytes for Flow Cytometry, <i>Reginald Davies</i>	369
CH. 41.	Flow Cytometric Analysis of Cells Using an Antibody to a Surface Antigen, <i>Michael G. Ormerod</i>	373
CH. 42.	Multiple Immunofluorescence Analysis of Cells Using Flow Cytometry, <i>Michael G. Ormerod</i>	381
CH. 43.	Cell Kinetic Studies Using a Monoclonal Antibody to Bromodeoxyuridine, <i>George D. Wilson</i>	387
CH. 44.	Production and Use of Nonradioactive Hybridization Probes, <i>Victor T.-W. Chan and James O'D. McGee</i>	399
CH. 45.	Cellular Human and Viral DNA Detection by Nonisotopic <i>In Situ</i> Hybridization, <i>C. Simon Herrington and James O'D. McGee</i>	409
CH. 46.	Chromosomal Mapping of Genes by Nonisotopic <i>In Situ</i> Hybridization, <i>Bhupendra Bhatt and James O'D. McGee</i>	421
CH. 47.	Nonisotopic <i>In Situ</i> Hybridization: <i>Immunocytochemical Detection of Specific Repetitive Sequences on Chromosomes and Interphase Nuclei</i> <i>John A. Crolla</i>	431

CH. 48. Biotinylated Probes in Colony Hybridization, <i>Michael J. Haas</i>	441
CH. 49. Screening of λ gt11 cDNA Libraries Using Monoclonal Antibodies, <i>Duncan F. Webster, William T. Melvin, M. Danny Burke,</i> <i>and Francis J. Carr</i>	451
CH. 50. Expression of Foreign Genes in Mammalian Cells Using an Antibody Fusion System, <i>Simon J. Forster, Francis J. Carr, William J. Harris,</i> <i>and Anita A. Hamilton</i>	461
Index	475

Contributors

MICHAEL G. BAINES • *National Institute for Biological Standards and Control, Potters Bar, UK*

DAVID L. BATES • *Novo Nordisk Diagnostics Ltd., Cambridge, UK*

EDWARD A. BAYER • *Department of Biophysics, The Weizmann Institute of Science, Rehovot, Israel*

JULIAN E. BEESLEY • *Wellcome Research Laboratories, Beckenham, UK*

BHUPENDRA BHATT • *Department of Haematology, King's College School of Medicine and Dentistry, London, UK*

KURT BIENZ • *Institute for Medical Microbiology, University of Basel, Switzerland*

LESZEK K. BORYSIEWICZ • *Department of Medicine, Addenbrooke's Hospital, Cambridge, UK*

M. DANNY BURKE • *Scotgen Limited, University of Aberdeen, Scotland*

FRANCIS J. CARR • *Scotgen Limited, University of Aberdeen, Scotland*

VICTOR T.-W. CHAN • *Oncology Division, Beth Israel Hospital, Boston, MA*

JOHN A. CROLLA • *Wessex Regional Genetics Laboratory, General Hospital, Salisbury, UK*

ALAN J. CUMBER • *Drug Targeting Laboratory, Institute of Cancer Research, Sutton, Surrey, UK*

REGINALD DAVIES • *Medical Research Council Toxicology Unit, Carshalton, Surrey, UK*

CHRISTOPHER J. DEAN • *Institute of Cancer Research, The Haddow Laboratories, Sutton, Surrey, UK*

MONIQUE DIANO • *Biologie de la Differentiation Cellulaire, Faculte des Sciences de Luminy, Marseilles, France*

RICHARD E. EDWARDS • *Medical Research Council Toxicology Unit, Carshalton, Surrey, UK*

DENISE EGGER • *Institute for Medical Microbiology, University of Basel, Switzerland*

GERARD I. EVAN • *Imperial Cancer Research Fund Laboratories, London, UK*

SIMON J. FORSTER • *Scotgen Limited, University of Aberdeen, Scotland*

YVELINE FROBERT • *Section de Pharmacologie et d'Immunologie, Department de Biologie, Gif-sur-Yvette, France*

JACQUES GRASSI • *Section de Pharmacologie et d'Immunologie, Department de Biologie, Gif-sur-Yvette, France*

JONATHAN A. GREEN • *Medical Research Council Toxicology Unit, Carshalton, Surrey, UK*

MICHAEL J. HAAS • *Eastern Regional Research Center, U. S. Department of Agriculture, Philadelphia, PA*

ANITA A. HAMILTON • *Scotgen Limited, University of Aberdeen, Scotland*

DAVID C. HANCOCK • *Imperial Cancer Research Fund Laboratories, London, UK*

WILLIAM J. HARRIS • *Scotgen Limited, University of Aberdeen, Scotland*

COLIN J. HENDERSON • *Imperial Cancer Research Fund, Molecular Pharmacology and Drug Metabolism Group, Edinburgh, Scotland*

C. SIMON HERRINGTON • *University of Oxford, Nuffield Department of Pathology and Bacteriology, John Radcliffe Hospital, Oxford, UK*

MICHEL HIRN • *Faculte des Sciences de Luminy, Marseilles, France*

SUSANNE F. IMRIE • *Institute of Cancer Research, The Haddow Laboratories, Sutton, Surrey, UK*

SCOTT H. KAUFMANN • *Oncology Center, Johns Hopkins Hospital and Department of Pharmacology and Molecular Science, Johns Hopkins University School of Medicine, Baltimore, MD*

ANNE LAINE • *INSERM, Lille Cedex, France*

ANDRÉ LE BIVIC • *Biologie de la Differentiation Cellulaire, Faculte des Sciences de Luminy, Marseilles, France*

BARBARA I. LUDEKE • *Department of Neuropathology, University Hospital, Zurich, Switzerland*

KITTI MAKARANANDA • *Department of Pharmacology, Mahidol University, Bangkok, Thailand*

MARGARET M. MANSON • *Medical Research Council Toxicology Unit, Carshalton, Surrey, UK*

JANET MARTIN • *Medical Research Council Toxicology Unit, Carshalton, Surrey, UK*

JAMES O'D. MCGEE • *University of Oxford, Nuffield Department of Pathology and Bacteriology, John Radcliffe Hospital, Oxford, UK*

WILLIAM T. MELVIN • *Department of Molecular and Cell Biology, University of Aberdeen, Scotland*

SARA E. MOLE • *CRC Human Cancer Genetics Research Group, Department of Pathology, University of Cambridge, UK*

JOHN P. MOORE • *Aaron Diamond AIDS Research Center, New York*

GORDON E. NEAL • *Medical Research Council Laboratories, Toxicology Unit, Carshalton, Surrey, UK*

MICHAEL G. ORMEROD • *Institute of Cancer Research, The Haddow Laboratories, Sutton, Surrey, UK*

ANTHONY H. V. SCHAPIRA • *Department of Neurological Science, Royal Free Medical School, London, UK and Institute of Neurology, London, UK*

JOEL H. SHAPER • *Oncology Center, Johns Hopkins Hospital and Department of Pharmacology and Molecular Science, Johns Hopkins University School of Medicine, Baltimore, MD*

DONALD F. SUMMERS • *Department of Cellular, Viral, and Molecular Biology, University of Utah School of Medicine, Salt Lake City, UT*

BOGUSLAW SZEWCZYK • *Department of Biochemistry, University of Gdansk, Poland*

T. J. THOMAS • *Department of Medicine, Division of Rheumatology, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, New Brunswick, NJ*

ROBIN THORPE • *National Institute for Biological Standards and Control, Potters Bar, England*

MICHAEL J. TILBY • *Leukaemia Research Fund Unit, Newcastle University, Newcastle-upon-Tyne, UK*

EDDIE C. Y. WANG • *Department of Medicine, Addenbrooke's Hospital, Cambridge, UK*

EDWARD J. WAWRZYNCZAK • *Drug Targeting Laboratory, Institute of Cancer Research, Sutton, Surrey, UK*

DUNCAN F. WEBSTER • *Department of Molecular and Cell Biology, University of Aberdeen, Scotland*

ANTHONY P. WEETMAN • *Department of Medicine, Addenbrooke's Hospital, Cambridge, UK*

IAN N. H. WHITE • *Medical Research Council Toxicology Unit, Carshalton, Surrey, UK*

MEIR WILCHEK • *Department of Biophysics, The Weizmann Institute of Science, Rehovot, Israel*

GEORGE D. WILSON • *Cancer Research Campaign, Gray Laboratory, Mount Vernon Hospital, Northwood, Middlesex, UK*

C. ROLAND WOLF • *Imperial Cancer Research Fund, Molecular Pharmacology and Drug Metabolism Group, Edinburgh, Scotland*