Monoclonal Antibody Protocols

Methods in Molecular Biology™

John M. Walker, SERIES EDITOR

45. **Monoclonal Antibody Protocols**, edited by **William C. Davis**, 1995
44. **Agrobacterium Protocols**, edited by **Kevan M. A. Gartland and Michael R. Davey**, 1995
43. **In Vitro Toxicity Testing Protocols**, edited by **Sheila O'Hare and Chris K. Atterwill**, 1995
42. **ELISA: Theory and Practice**, by **John R. Crowther**, 1995
41. **Signal Transduction Protocols**, edited by **David A. Kendall and Stephen J. Hill**, 1995
40. **Protein Stability and Folding**, edited by **Bret A. Shirley**, 1995
39. **Baculovirus Expression Protocols**, edited by **Christopher D. Richardson**, 1995
38. **Cryopreservation and Freeze-Drying Protocols**, edited by **John G. Day and Mark R. McLellan**, 1995
37. **In Vitro Transcription and Translation Protocols**, edited by **Martin J. Tymms**, 1995
36. **Peptide Analysis Protocols**, edited by **Michael W. Pennington and Ben M. Dunn**, 1994
35. **Peptide Synthesis Protocols**, edited by **Ben M. Dunn and Michael W. Pennington**, 1994
34. **Immunocytochemical Methods and Protocols**, edited by **Lorette C. Javois**, 1994
33. **In Situ Hybridization Protocols**, edited by **K. H. Andy Choo**, 1994
32. **Basic Protein and Peptide Protocols**, edited by **John M. Walker**, 1994
31. **Protocols for Gene Analysis**, edited by **Adrian J. Harwood**, 1994
30. **DNA-Protein Interactions**, edited by **G. Geoff Kneale**, 1994
29. **Chromosome Analysis Protocols**, edited by **John R. Gosden**, 1994
28. **Protocols for Nucleic Acid Analysis by Nonradioactive Probes**, edited by **Peter G. Isaac**, 1994
27. **Biomembrane Protocols: II. Architecture and Function**, edited by **John M. Graham and Joan A. Higgins**, 1994
26. **Protocols for Oligonucleotide Conjugates**, edited by **Sudhir Agrawal**, 1994
25. **Computer Analysis of Sequence Data: Part II**, edited by **Annette M. Griffin and Hugh G. Griffin**, 1994
24. **Computer Analysis of Sequence Data: Part I**, edited by **Annette M. Griffin and Hugh G. Griffin**, 1994
23. **DNA Sequencing Protocols**, edited by **Hugh G. Griffin and Annette M. Griffin**, 1993
22. **Optical Spectroscopy, Microscopy, and Macroscopic Techniques**, edited by **Christopher Jones, Barbara Mulloy, and Adrian H. Thomas**, 1994
21. **Protocols in Molecular Parasitology**, edited by **John E. Hyde**, 1993
20. **Protocols for Oligonucleotides and Analogs**, edited by **Sudhir Agrawal**, 1993

Earlier volumes are still available. Contact Humana for details.

Monoclonal Antibody Protocols

Edited by

William C. Davis

*Department of Veterinary Microbiology and Pathology,
College of Veterinary Medicine,
Washington State University, Pullman, WA*

Humana Press

Totowa, New Jersey

© 1995 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. *Methods in Molecular Biology™* is a trademark of The Humana Press Inc.

All authored papers, comments, opinions, conclusions, or recommendations are those of the author(s), and do not necessarily reflect the views of the publisher.

This publication is printed on acid-free paper. ©
ANSI Z39.48-1984 (American Standards Institute)
Permanence of Paper for Printed Library Materials.

Photocopy Authorization Policy:

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by Humana Press Inc., provided that the base fee of US \$4.00 per copy, plus US \$00.20 per page, is paid directly to the Copyright Clearance Center at 222 Rosewood Drive, Danvers, MA 01923. 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 Humana Press Inc. The fee code for users of the Transactional Reporting Service is: [0-89603-308-2/95 \$4.00 + \$00.20].

Printed in the United States of America. 10 9 8 7 6 5 4 3 2 1

Library of Congress Cataloging in Publication Data

Main entry under title:

Methods in molecular biology™.

Monoclonal antibody protocols / edited by William C. Davis.

p. cm. — (Methods in molecular biology™ ; 45)

Includes index.

ISBN 0-89603-308-2 (alk. paper)

1. Monoclonal antibodies—Laboratory manuals. I. Davis, W. C. (William C.) II. Series:
Methods in molecular biology™ (Totowa, NJ) ; 45.

QR186.85.M66285 1995

616.07'93—dc20

95-13504

CIP

Preface

Since the initial description of techniques to immortalize antibody-producing B-lymphocytes by fusion with tissue culture-adapted myeloma cells, methods have been developed to produce monoclonal antibodies of defined specificity in multiple animal species. Stable hybrids can be readily produced in mice using a number of myeloma and hybridoma cell lines. To obviate the problem of identifying fusion partners in other animal species, xenohybrids have been produced using B-lymphocytes from the relevant species and mouse myeloma cells. The use of xenohybrids has minimized the problem of obtaining stable antibody-producing hybrids in all species examined thus far. Although alternative techniques are being developed to produce monoclonal antibodies by molecular methods, hybridoma technology will remain the technology of choice for producing monoclonal antibodies for a variety of applications in research and industry.

The objective of *Monoclonal Antibody Protocols* is to provide investigators with a set of methods for producing and using monoclonal antibodies in biomedical, agricultural, and biological sciences. The book is not intended to provide methodology for all possible applications, but rather a series of methods presented in an easy-to-follow format that can be used by new and established investigators, graduate and postgraduate fellows, and technical staff. The methods include descriptions of techniques for immunization in vivo and in vitro to obtain the antibodies of interest; production of monoclonal antibodies in mice, humans, and domestic species; isotyping and characterizing antibodies; producing antibodies in a bioreactor to eliminate the need of using mice to produce immune ascites; purification of IgG and IgM antibodies; preparation of immunoaffinity columns; conjugation of antibodies with fluorophores, biotin, and enzymes; and also descriptions of popular applications such as the use of monoclonal antibodies in immunoaffinity purification of mol-

ecules, Western blotting, and chemiluminescence, immunohistochemistry, electron microscopy, flow cytometry, and magnetic particle separation of leukocyte subpopulations.

Each chapter has been written by an author or authors with considerable experience in the use of the methodology. Where possible, an attempt has been made to introduce recent technological advances that facilitate the development and use of monoclonal antibodies. Each chapter starts with a brief description of the technique and application, followed by a detailed step-by-step outline of the methods. A notes section is included to provide additional information on problems that are occasionally encountered in the use of the technology and recommendations on how to identify the source of the problem and the steps needed to modify the procedures to obtain the expected results. Chapters are cross-referenced where related techniques are described to reduce redundancy and facilitate use of the methodology.

Monoclonal Antibody Protocols should prove effective as an easy-to-use laboratory manual for developing and using monoclonal antibodies.

William C. Davis

Contents

Preface	v
Contributors	ix
CH. 1. Methods of Immunization to Enhance the Immune Response to Specific Antigens In Vivo in Preparation for Fusions Yielding Monoclonal Antibodies <i>Jon A. Rudbach, John L. Cantrell, and J. T. Ulrich</i>	1
CH. 2. Methods of Immunization to Enhance the Immune Response to Specific Antigens In Vitro <i>Margaret E. Schelling</i>	9
CH. 3. Culture Conditions that Optimize Outgrowth of Hybridomas <i>Mary J. Hamilton and William C. Davis</i>	17
CH. 4. Production of Stable Heterohybridomas Producing Human Monoclonal Antibodies <i>Morris D. Cooper and Risa Kirkpatrick</i>	29
CH. 5. Production of Stable Bovine–Murine Interspecies Hybrids <i>Brenda V. Jones and Christopher J. Howard</i>	41
CH. 6. Production of Monoclonal Antibodies in Swine <i>Araceli L. Lumanglas and Bosco Shang Wang</i>	49
CH. 7. Production of Monoclonal Antibodies in Horses <i>Lance E. Perryman and Patricia H. Mason</i>	55
CH. 8. Use of Electric-Field-Mediated Cell Fusion to Produce Hybridomas Secreting Monoclonal Antibodies <i>Xi Zhao</i>	61
CH. 9. Rapid Isotyping of Mouse Monoclonal Antibodies <i>Philippe Pouletty</i>	69
CH. 10. Methods of Measuring Antibodies in Sera/Plasma, Ascites, and Tissue-Culture Media <i>Jon A. Rudbach, John L. Cantrell, and J. T. Ulrich</i>	79
CH. 11. Measurement of Immunoglobulin Synthesis Using the ELISPOT Assay <i>Morris D. Cooper and Risa Kirkpatrick</i>	83
CH. 12. Use of Monoclonal Antibodies in Immunohistochemistry <i>Shan-Rong Shi, Jeffrey B. Prince, Christopher M. Jones, Krishan L. Kalra, and Atul K. Tandon</i>	89

Ch. 13.	Use of Monoclonal Antibodies in Immunoelectron Microscopy <i>Carlos E. Suarez and Ruth Brown</i>	109
Ch. 14.	Use of Monoclonal Antibodies for Western Blotting with Enhanced Chemiluminescent Detection <i>Susan J. Fowler</i>	115
Ch. 15.	The Use of Directly and Indirectly Labeled Monoclonal Antibodies in Flow Cytometry <i>Carleton C. Stewart and Sigrid J. Stewart</i>	129
Ch. 16.	Use of Monoclonal Antibodies and Flow Cytometry to Cluster and Analyze Leukocyte Differentiation Molecules <i>William C. Davis, Jennifer E. Davis, and Mary J. Hamilton</i> ...	149
Ch. 17.	In Vivo and In Vitro Production of Monoclonal Antibodies: <i>Bioreactors vs Immune Ascites</i> <i>Uwe Marx and Wolfram Merz</i>	169
Ch. 18.	Separation of Monoclonal Antibodies from Cell-Culture Supernatants and Ascites Fluid Using Thiophilic Agarose <i>Thorkild C. Bøg-Hansen</i>	177
Ch. 19.	Detection, Purification, and Utilization of Murine Monoclonal IgM Antibodies <i>Ian D. Walker</i>	183
Ch. 20.	Preparation and Use of Immunoaffinity Columns with Monoclonal Antibodies Without Purification from Ascites and Tissue- Culture Medium <i>Thorkild C. Bøg-Hansen and William C. Davis</i>	189
Ch. 21.	Antigen Purification by Monoclonal Antibody Immunoaffinity Chromatography <i>Alberta L. Brassfield</i>	195
Ch. 22.	Coupling of Monoclonal Antibodies with Fluorophores <i>Rosaria P. Haugland</i>	205
Ch. 23.	Coupling of Monoclonal Antibodies with Biotin <i>Rosaria P. Haugland and Wendy W. You</i>	223
Ch. 24.	Coupling of Monoclonal Antibodies with Enzymes <i>Rosaria P. Haugland</i>	235
Ch. 25.	Use of Monoclonal Antibodies with Magnetic Particles to Separate Cell Subpopulations by Negative Selection <i>Dennis E. Vaccaro and Joan E. Markinac</i>	245
Ch. 26.	Use of Monoclonal Antibodies with Magnetic Particles to Separate Cell Subpopulations by Positive Selection <i>Dennis E. Vaccaro and Joan E. Markinac</i>	253
Index		261

Contributors

THORKILD C. BØG-HANSEN • *The Protein Laboratory, The Panum Institute, University of Copenhagen, Denmark*

ALBERTA L. BRASSFIELD • *Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, WA*

RUTH BROWN • *Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, CA*

JOHN L. CANTRELL • *RIBI ImmunoChem Research, Inc., Hamilton, MT*

MORRIS D. COOPER • *Department of Medical Microbiology and Immunology, Southern Illinois University School of Medicine, Springfield, IL*

JENNIFER E. DAVIS • *Fred Hutchinson Cancer Research Center, Seattle, WA*

WILLIAM C. DAVIS • *Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, WA*

SUSAN J. FOWLER • *Amersham International, Amersham, Bucks, UK*

MARY J. HAMILTON • *Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, WA*

ROSARIA P. HAUGLAND • *Molecular Probes Inc., Eugene, OR*

CHRISTOPHER J. HOWARD • *Institute for Animal Health, Compton Laboratory, Berkshire, UK*

BRENDA V. JONES • *Institute for Animal Health, Compton Laboratory, Berkshire, UK*

CHRISTOPHER M. JONES • *BioGenex, San Ramon, CA*

KRISHAN L. KALRA • *BioGenex, San Ramon, CA*

RISA KIRKPATRICK • *Department of Medical Microbiology and Immunology, Southern Illinois University School of Medicine, Springfield, IL*

- ARACELI L. LUMANGLAS • *Laboratory of Immunoendocrinology, Agricultural Research Division, American Cyanamid Company, Princeton, NJ*
- JOAN E. MARKINAC • *PerSeptive Diagnostics, Inc., Cambridge, MA*
- UWE MARX • *Institute for Medical Immunology, Charité University Clinic, Medical Faculty of Humboldt University, Berlin, Germany*
- PATRICIA H. MASON • *Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, WA*
- WOLFRAM MERZ • *Integra Biosciences, Inc., Woburn, MA*
- LANCE E. PERRYMAN • *College of Veterinary Medicine, North Carolina State University, Raleigh, NC*
- PHILIPPE POULETTY • *SangStat Medical Corp., Menlo Park, CA*
- JEFFREY B. PRINCE • *BioGenex, San Ramon, CA*
- JON A. RUDBACH • *RIBI ImmunoChem Research, Inc., Hamilton, MT*
- MARGARET E. SCHELLING • *Program in Genetics and Cell Biology, Washington State University, Pullman, WA*
- SHAN-RONG SHI • *BioGenex, San Ramon, CA*
- CARLETON C. STEWART • *Laboratory of Flow Cytometry, Roswell Park Cancer Institute, Buffalo, NY*
- SIGRID J. STEWART • *Laboratory of Flow Cytometry, Roswell Park Cancer Institute, Buffalo, NY*
- CARLOS E. SUAREZ • *Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, CA*
- ATUL K. TANDON • *BioGenex, San Ramon, CA*
- J. T. ULRICH • *RIBI ImmunoChem Research, Inc., Hamilton, MT*
- DENNIS E. VACCARO • *PerSeptive Diagnostics, Inc., Cambridge, MA*
- IAN D. WALKER • *School of Veterinary Medicine, Veterinary Preclinical Centre, University of Melbourne, Parkville, Victoria, Australia*
- BOSCO SHANG WANG • *Laboratory of Immunoendocrinology, Agricultural Research Division, American Cyanamid Company, Princeton, NJ*
- ANA WEIMIN YOU • *Molecular Probes Inc., Eugene, OR*
- XI ZHAO • *InCell Inc., Santa Clara, CA*