Methods in Enzymology

Volume 74

Immunochemical Techniques

Part C

EDITED BY

John J. Langone

Helen Van Vunakis

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Preface

The availability of immunochemical techniques to quantify antigens has revolutionized analytical technology. Immunoassays not only offer accurate estimates of individual substances in complex mixtures, but also can be uniquely useful in studies of antigen and antibody structure.

This volume continues the coverage of general immunochemical procedures begun in Volumes 70 and 73, Parts A and B, respectively, and also includes chapters that illustrate how antibodies have been used to gain important new information about the structure and activity of enzymes and receptors. Since immune complexes are increasingly important in clinical biochemistry and medicine, several chapters deal with their detection, estimation, and isolation. The opportunities to use antibodies as analytical reagents are growing at a rapid rate as techniques are simplified and the scientific community of nonimmunologists becomes acquainted with the basic principles required to produce and use antibodies to solve diverse research problems. We hope that Volume 74, Part C, along with Parts A and B, will display the basic array of immunochemical techniques and will inspire wider application of these methods.

Again we thank the authors for their excellent contributions, Carla Langone for dealing with the correspondence, and Dr. Adrian Gee for his expertise in indexing the volumes.

JOHN J. LANGONE

HELEN VAN VUNAKIS

METHODS IN ENZYMOLOGY

EDITED BY

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I. Preparation and Assay of Enzymes
II. Preparation and Assay of Enzymes
III. Preparation and Assay of Substrates
IV. Special Techniques for the Enzymologist
V. Preparation and Assay of Enzymes
VI. Preparation and Assay of Enzymes (*Continued*)
Preparation and Assay of Substrates
Special Techniques
VII. Cumulative Subject Index

Table of Contents

CONTRIBUTORS TO VOLUME 74 . . . . . .

PREFACE . . . . . .

VOLUMES IN SERIES . . . . . .

Section I. Immunoassay Methods for the Detection of Antigens and Antibodies

A. Fluorescence Immunoassay Methods

1. Equilibrium and Kinetic Inhibition Assays Based upon Fluorescence Polarization — WALTER B. DANDLIKER, MAO-LIN HSU, JACQUES LEVIN, AND B. RAMANATH RAO 3

2. Fluorescence Excitation Transfer Immunoassay (FETI) — EDWIN F. ULLMAN AND PYARE L. KHANNA 28

3. Indirect Quenching Fluoroimmunoassay — R. D. NARGESSI AND J. LANDON 60

4. The Homogeneous Substrate-Labeled Fluorescent Immunoassay — JOHN F. BURD 79

5. The Fluorescence Immunoassay Using Plane Surface Solid Phases (FIAPS) — H. H. SEDLACEK, H. GRIGAT, T. RENK, AND F. R. SEILER 87

B. Immunoassays Dependent on Other Detection Methods

6. Particle Counting Immunoassay (PACIA) — P. L. MASSON, C. L. CAMBIASO, D. COLLET-CASSART, C. G. M. MAGNUSSON, C. B. RICHARDS, AND C. J. M. SINDIC 106

7. Spin Immunoassay — GEORGE C. YANG AND EDMUND S. COPELAND 140

8. Membrane Immunoassay: A Spin Membrane Immunoassay for Thyroxine — C. T. TAN, SAMUEL W. CHAN, AND J. CARLETON HSIA 152

9. Detection of Antigens and Haptens by Inhibition of Passive Immune Hemolysis — TIBOR BORSOS AND JOHN J. LANGONE 161

10. Quantification of Complement Fixation by the C1 Fixation and C1 Fixation and Transfer Tests — TIBOR BORSOS 165

11. Probing Immune Reactions by Laser Light Scattering Spectroscopy — EGIDIJUS E. UZGIRIS 177

C. Use of Antibodies to Study Enzymes

12. Radioimmunoassay of Creatine Kinase Isoenzymes	Robert Roberts and Charles W. Parker	198
13. Specific Radioimmunoassays for Rabbit Liver Fructose Bisphosphatase, Pyruvate Kinase, and Glycerol-3-phosphate Dehydrogenase	Carlo M. Veneziale, James C. Donofrio, Joel B. Hansen, Mark L. Johnson, and Mary Y. Mazzotta	210
14. Specific Radioimmunoassays for Rabbit Skeletal and Cardiac Muscle 6-Phosphofructokinase and Pyruvate Kinase	Carlo M. Veneziale, Joel B. Hansen, and Mark L. Johnson	232
15. Preparation of Site-Specific Anti-Cytochrome *c* Antibodies and Their Application	Ronald Jemmerson and Emanuel Margoliash	244
16. Production and Application of Antibodies to Rat Liver Cytochrome *P*-450	Laurence S. Kaminsky, Michael J. Fasco, and F. Peter Guengerich	262
17. Radioimmunoassay Determination of Circulating Pancreatic Endopeptidases	Corey Largman, James W. Brodrick, and Michael C. Geokas	272
18. Radioimmunoassay of Human Pancreatic Amylase	Michio Ogawa, Yuichi Takatsuka, Takeshi Kitahara, Kishio Matsuura, and Goro Kosaki	290
19. Radioimmunoassay of the Regulatory Subunit of Type I cAMP-Dependent Protein Kinase	C. L. Kapoor, J. A. Beavo, and A. L. Steiner	299
20. Radioimmunoassay of Bovine Type II cAMP-Dependent Protein Kinase	Norman Fleischer, Dwijen Sarkar, Charles Rubin, and Jack Erlichman	310
21. Immunotitration of 3-Hydroxy-3-Methylglutaryl-CoA Reductase	Terence J. Scallen, Joel E. Hardgrave, and Renu A. Heller	320
22. Production of Antibodies to Catalase and Their Effect on Enzyme Activity	Robert N. Feinstein and Bernard N. Jaroslow	343
23. Radioimmunoassay of Human Adenosine Deaminase	Peter E. Daddona, Michael A. Frohman, and William N. Kelley	351
24. Quantitation of Human Cuprozinc Superoxide Dismutase (SOD-1) by Radioimmunoassay and Its Possible Significance in Disease	Bert C. Del Villano and J. A. Tischfield	359
25. Radioimmunoassay and Immunotitration of Human Serum Dopamine β-Hydroxylase	Joel Dunnette and Richard Weinshilboum	370

D. Use of Antibodies to Study Receptors

26. Antibodies to Prolactin Receptors and Growth Hormone Receptors — RICHARD G. DRAKE AND HENRY G. FRIESEN — 380

27. Measurement of Thyrotropin Receptor Antibodies — BERNARD REES SMITH AND REGINALD HALL — 405

28. Quantitation of Estradiol Receptors by Radioimmunoassay — ARISTIDE FLORIDI — 420

29. Production and Assay of Antibodies to Acetylcholine Receptors — JON LINDSTROM, BRETT EINARSON, AND SOCRATES TZARTOS — 432

30. Production and Application of an Antibody Specific for the Cardiac β-Adrenergic Receptor — SIMEON M. WRENN, JR. — 460

31. Insulin Receptor Antibodies — STEVEN JACOBS AND PEDRO CUATRECASAS — 471

Section II. Data Analysis

32. Concentrations of Binding Protein and Labeled Analyte That Are Appropriate for Measuring at Any Analyte Concentration Range in Radioimmunoassays — CLARKE J. HALFMAN — 481

33. Microprocessor-Based Radioimmunoassay Data Analysis — KRESO UKRAINCIK AND WILLIAM PIKNOSH — 497

Section III. Immune Complexes

34. The Raji, Conglutinin, and Anti-C3 Assays for the Detection of Complement-Fixing Immune Complexes — ARGYRIOS N. THEOFILOPOULOS — 511

35. The [^{125}I]C1q Binding Assay for the Detection of Soluble Immune Complexes — RUDOLF H. ZUBLER, NICOLE CARPENTIER, AND PAUL-H. LAMBERT — 530

36. Quantitation of Circulating Immune Complexes by Combined PEG Precipitation and Immunoglobulin-Specific Radioimmunoassay (PICRIA) — HANS C. SIERSTED, IVAN BRANDSLUND, SVEN-ERIK SVEHAG, AND JENS C. JENSENIUS — 538

37. Detection and Quantitation of Immune Complexes with a Rapid Polyethylene Glycol Precipitation Complement Consumption Method (PEG-CC) — IVAN BRANDSLUND, HANS C. SIERSTED, JENS C. JENSENIUS, AND SVEN-ERIK SVEHAG — 551

38. Detection and Quantitation of Circulation Immune Complexes by the C1q–Protein A Binding Assay (C1q–PABA) — GRACIELA GLIKMANN AND SVEN-ERIK SVEHAG — 571

39. C3c-Binding ELISA for the Detection of Immunoconglutinins and Immunoglobulin Aggregates — Aarno Hautanen and Ewert Linder — 588

40. Enzyme Immunoassay for the Demonstration and Quantification of Immune Complexes — René Masseyeff, Richard Maiolini, Laurent Roda, and Bernard Ferrua — 608

41. Assay of Soluble Immune Complexes Using Radiolabeled Rheumatoid Factor — Patrick Naish, Joan Barratt, and Malcolm Sindrey — 617

42. Quantification of Immune Complexes by Nephelometry — Klaus Höffken and Carl G. Schmidt — 628

43. Isolation of Soluble Immune Complexes from Human Serum: Combined Use of Polyethylene Glycol Precipitation, Gel Filtration, and Affinity Chromatography on Protein A–Sepharose — Gabriel Virella, J. Michael Kilpatrick, Francoise Chenais, and H. Hugh Fudenberg — 644

44. Purification and Analysis of Immune Complexes with the Aid of Rheumatoid Factor-Coated Tubes — Z. Gilead, Y. Gazitt, G. Klein, and D. Sulitzeanu — 664

Author Index 677

Subject Index 699

Section I

Immunoassay Methods for the Detection of Antigens and Antibodies

A. Fluorescence Immunoassay Methods
Articles 1 through 5

B. Immunoassays Dependent on Other Detection Methods
Articles 6 through 11

C. Use of Antibodies to Study Enzymes
Articles 12 through 25

D. Use of Antibodies to Study Receptors
Articles 26 through 31

[1] Equilibrium and Kinetic Inhibition Assays Based upon Fluorescence Polarization

By WALTER B. DANDLIKER, MAO-LIN HSU, JACQUES LEVIN, and B. RAMANATH RAO

Introduction

Assays utilizing the high specificities of antibodies or other receptors make it possible to detect and quantify many substances present in only minute traces in complex biological materials. In the past two decades these assays have become one of the main analytical tools of biomedical science[1,2] and efforts continue toward increasing the sensitivity, specificity, and convenience of such assays even further.[3] All of the methods in use depend upon labeling either the ligand being quantified or the receptor molecule (antibody, hormone receptor, etc.) being used to provide recognition. (In a few special cases where natural fluorescence of ligand or receptor can be utilized, labeling may be unnecessary.) The general types of label that afford the requisite sensitivity include radiolabels, enzyme labels, and fluorescence or phosphorescence labels. The readout of an assay then finally depends upon a determination of the amount of label present: by counting (radioimmunoassay or combined enzymatic radioimmunoassay), by measurement of an enzymatically produced product, by fluorescence or phosphorescence intensity (with or without time resolution) and by fluorescence polarization.

The polarization of fluorescence operates differently than the other methods of detection in that it gives a direct measure of the bound/free ratio instead of simply being a measure of amount of label present, i.e., the polarization is independent of concentration and for a given bound/free ratio remains constant over many orders of magnitude of change in the absolute concentrations. This characteristic provides a definite advantage for some situations in that the essential measurement can be made without any physical separation of bound and free. For example, where the measurement must be rapid or carried out by automated methods, the simplification provided by polarization measurements may be of crucial importance. However, in order to gain these advantages, the impact of several

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