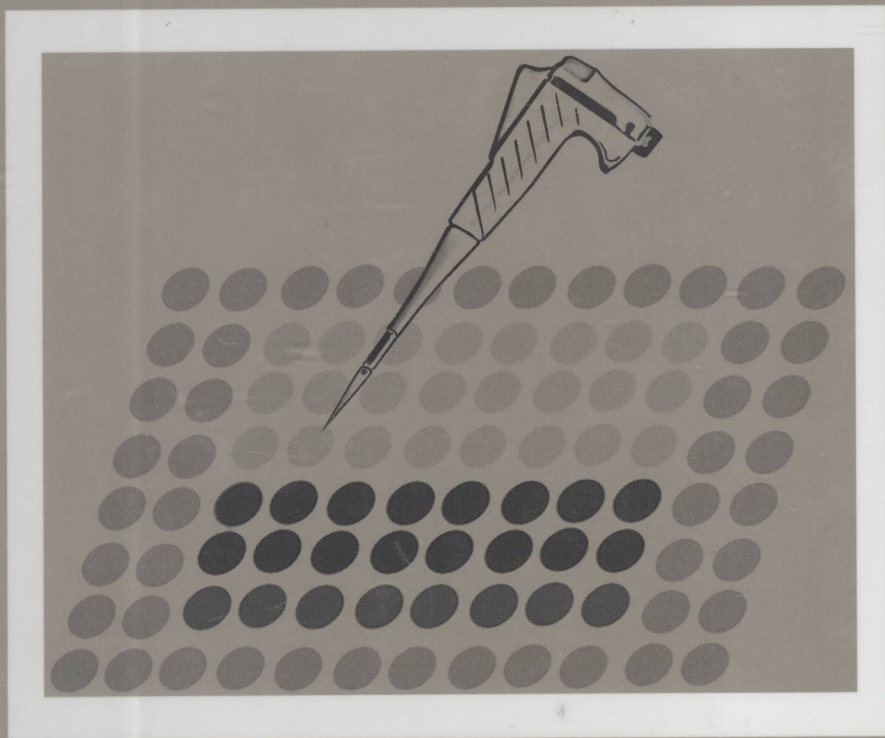


# Pharmaceutical Bioassays

*Methods and Applications*

*Shiqi Peng • Ming Zhao*



WILEY

R917  
P398

---

# PHARMACEUTICAL BIOASSAYS

## Methods and Applications

---

Shiqi Peng

Ming Zhao



E2011001297



WILEY

A JOHN WILEY & SONS, INC., PUBLICATION

Copyright © 2009 by John Wiley & Sons, Inc. All rights reserved.

Published by John Wiley & Sons, Inc., Hoboken, New Jersey.

Published simultaneously in Canada.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, scanning, or otherwise, except as permitted under Section 107 or 108 of the 1976 United States Copyright Act, without either the prior written permission of the Publisher, or authorization through payment of the appropriate per-copy fee to the Copyright Clearance Center, Inc., 222 Rosewood Drive, Danvers, MA 01923, (978) 750-8400, fax (978) 750-4470, or on the web at [www.copyright.com](http://www.copyright.com). Requests to the Publisher for permission should be addressed to the Permissions Department, John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, (201) 748-6011, fax (201) 748-6008, or online at <http://www.wiley.com/go/permission>.

**Limit of Liability/Disclaimer of Warranty:** While the publisher and author have used their best efforts in preparing this book, they make no representations or warranties with respect to the accuracy or completeness of the contents of this book and specifically disclaim any implied warranties of merchantability or fitness for a particular purpose. No warranty may be created or extended by sales representatives or written sales materials. The advice and strategies contained herein may not be suitable for your situation. You should consult with a professional where appropriate. Neither the publisher nor author shall be liable for any loss of profit or any other commercial damages, including but not limited to special, incidental, consequential, or other damages.

For general information on our other products and services or for technical support, please contact our Customer Care Department within the United States at (800) 762-2974, outside the United States at (317) 572-3993 or fax (317) 572-4002.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic formats. For more information about Wiley products, visit our web site at [www.wiley.com](http://www.wiley.com).

***Library of Congress Cataloging-in-Publication Data:***

Pharmaceutical bioassays : methods and applications / Shiqi Peng . . . [et al.].

p. ; cm.

Includes bibliographical references.

ISBN 978-0-470-22760-2 (cloth)

1. Drugs--Testing. 2. Biological assay. I. Peng, Shiqi.

[DNLM: 1. Biological Assay--methods. 2. Pharmaceutical Preparations--analysis.

QV 771 P5357 2009]

RM301.27.P475 2009

615'.19--dc22

2009007433

Printed in the United States of America.

10 9 8 7 6 5 4 3 2 1

# PHARMACEUTICAL BIOASSAYS

---

# PREFACE

---

Twenty years ago when I did my research work as a Humboldt Fellow in Prof. Dr. Dr. h.c. Winterfeldt's laboratory at Hannover University, I clearly perceived a trend that chemists were focusing attention on bioassays corresponding with states of disease free, disease onset, and disease progression, which trend pushed our synthetic chemistry to combine with pharmaceutical bioassay. Ten years ago when I was engaged as the dean of the College of Pharmaceutical Sciences in Peking University, pharmaceutical bioassay was considered as one of the platforms in our postgraduate education. Five years ago when I was engaged as the dean of the College of Pharmaceutical Sciences at Capital Medical University, pharmaceutical bioassay became a platform of chemistry, pharmacy, biology, and medicine and was extensively reflected in our undergraduate and postgraduate education.

The trend of chemistry, pharmacy, biology, and medicine to focus attention on bioassays corresponding with states of disease free, disease onset, and disease progression continues today and leads the discovery of efficacious new human and animal therapeutic agents, one of humanity's most vital tasks. The development of pharmaceutical bioassays is an enormous, demanding activity that requires creativity, a vast range of chemical, pharmaceutical, biological, and medical knowledge, as well as great persistence. Nowadays, no education and research would be complete without some exposure to the ways in which bioactive agents and new medicines are defined, discovered, and developed with the support of pharmaceutical bioassays. For those people interested in chemistry, pharmacy, biology, and medical sciences, such knowledge is arguably mandatory.

*Pharmaceutical Bioassay: Methods and Applications* represents a unique attempt to describe assays to evaluate the bioactivities of therapeutic moieties resulting from chemical, biological, and natural processes and to explore the mechanism of action of therapeutic moieties in the cells, tissues, and organs of living beings, as well as in living beings themselves. Such assays are highly important in the pharmaceutical industry and are the prime engine for the advancement of chemistry, pharmacy, biology, and medicine.

It would be impossible to be comprehensive in a book of this size, although hopefully there are no important omissions. I apologize that due to the rapid increase of new

knowledge, I have had to omit some assays, and due to the limit of my appreciation or ability, some topics have not been treated with the depth that they may deserve. The emphasis, in general, has been on the procedures and applications of pharmaceutical bioassays. I hope that the selection and the arrangement of the material may be found satisfactory and useful to all those working in or entering this field.

I am grateful to the Beijing Municipal Commission of Education, which established the Beijing Area Major Laboratory of Peptide and Small Molecular Drugs for us, and also am grateful to the leaders of Capital Medical University, who provided me with excellent equipment, sufficient funds, and had high confidence in my research related to pharmaceutical bioassays; therefore, I hope that this book may be dedicated to the fiftieth anniversary of the founding of Capital Medical University. I am also indebted to my current co-workers and students who help to keep pharmaceutical bioassay alive for me with their enthusiasm for our research. Finally, I am especially grateful to my wife, who has not complained about the extra time I have needed to work during the past decades.

SHIQI PENG

*Capital Medical University*

---

# CONTRIBUTORS

---

**Shiqi Peng**, Capital Medical University, Beijing, China

**Ming Zhao**, Capital Medical University, Beijing, China

**Chunying Cui**, Capital Medical University, Beijing, China

**Guohui Cui**, Capital Medical University, Beijing, China

---

# CONTENTS

---

**Preface**

**xxi**

**Contributors**

**xxiii**

## **1 Methods and Applications of Anticancer Bioassays / 1**

*Shiqi Peng*

- 1.1 MTT Assay for Six Carcinoma Cells / 2
- 1.2 Flow Cytometric Assay for Cell Apoptosis / 3
- 1.3 DNA Fragmentation Assay / 3
- 1.4 Bcl-XL/BH3 Interaction Assay / 3
- 1.5 Dissociation-Enhanced Lanthanide Fluoro-Immunoassay (DELFIA) / 4
- 1.6 Ishikawa Cell and Rat Assay for Detecting Antiestrogens / 5
- 1.7 ATP Assay for Eight Cells / 7
- 1.8 AP Activity Assay / 7
- 1.9 Tumor Endothelial Cell Tube Formation Assay / 8
- 1.10 Antiangiogenic Assay / 9
- 1.11 *In Vivo* Hollow Fiber Assay / 10
- 1.12 VX2 Rabbit Lung Assay / 11
- 1.13 Insulin-Like Growth Factor-I-Induced Kinase Receptor Activation Assay / 11
- 1.14 Insulin-Like gD.trkA-Induced Kinase Receptor Activation Assay / 12
- 1.15 UV Spectra-Based Calf Thymus DNA Intercalation Assay / 12
- 1.16 Fluorescence Spectra-Based Calf Thymus DNA Intercalation Assay / 13
- 1.17 P-Glycoprotein Pump in MCF-7R Cells Assay / 13
- 1.18 P-Glycoprotein Pump-Related Efflux Carriers Assay / 14
- 1.19 [<sup>3</sup>H]Substrate Transport Inhibition Assay / 15
- 1.20 Lactate Dehydrogenase Release Assay / 15
- 1.21 Functional Assay of Mitochondrial P-gp / 16
- 1.22 Resistance Index Value Assay / 16
- References and Notes / 17



## 2 Methods and Applications of Antiviral Assays / 23

*Shiqi Peng, Ming Zhao, and Chunying Cui*

- 2.1 Nonradioactive HIV-1 RT Activity Assay / 24
- 2.2 Respiratory Syncytial Virus Assay / 24
- 2.3 Influenza Virus Types A and B Assay / 24
- 2.4 Nasal Exhaled NO Concentration Assay / 25
- 2.5 Nasal NOS2 mRNA Quantity Assay / 25
- 2.6 RT-PCR and Swine Assay for Anti-HEV Antibody / 26
- 2.7 HIV-1 Protease and Reverse Transcriptase Kinetic Assay / 26
- 2.8 Anti-HIV Assay / 27
- 2.9 Robust Antiviral Assays / 28
- 2.10 HIV/SIV Fusion Assay / 28
- 2.11 Rapid DNA Hybridization Assay / 29
- 2.12 Antiviral Screening Assay for HepAD38 Cell Cultures / 29
- 2.13 Trak-C HCV Core Assay / 31
- References and Notes / 33

## 3 Methods and Applications of Antitubercular Assays / 39

*Shiqi Peng, Ming Zhao, and Chunying Cui*

- 3.1 *Mycobacterium tuberculosis* Assay / 39
- 3.2 DNA Polymerase  $\beta$  Lyase Assay / 40
- 3.3 Agar Dilution Assay for *In Vitro* Antitubercular Activity / 41
- 3.4 Microplate Alamar Blue Assay for *In Vitro* Antitubercular Activity / 41
- 3.5 Radiometric Respiratory Assay for *In Vitro* Antitubercular Activity / 42
- 3.6 *Mycobacterium bovis* BCG Inhibition Assay / 42
- References and Notes / 43

## 4 Methods and Applications of Thrombus-Related Assays / 45

*Ming Zhao*

- 4.1 *In Vitro* Anti-Platelet Aggregation Assay / 46
- 4.2 Fibrinolytic Area Assay / 47
- 4.3 TXB<sub>2</sub> and PGD<sub>2</sub> TLC Assay / 47
- 4.4 TXA<sub>2</sub> Synthase Activity Assay / 48
- 4.5 [Ca<sup>2+</sup>]<sub>i</sub> Measuring Assay / 48
- 4.6 Arachidonic Acid Liberation Assay / 48
- 4.7 Serotonin Secretion Assay / 48
- 4.8 cAMP Release Assay / 49
- 4.9 *Ex Vivo* Anti-Platelet Aggregation Assay for Patients / 49
- 4.10 ATP Release Assay / 50
- 4.11 PAF-Induced Mice Mortality Assay / 50

|      |  |      |
|------|--|------|
| 4.12 | PGE <sub>2</sub> and TXB <sub>2</sub> ELISA  | / 50 |
| 4.13 | Thrombelastograph Assay  | / 51 |
| 4.14 | Image-Monitored FeCl <sub>3</sub> -Induced Thrombosis Assay for Rat                | / 51 |
| 4.15 | Weight-Monitored FeCl <sub>3</sub> -Induced Thrombosis Assay for Rats              | / 52 |
| 4.16 | Occlusion Time-Monitored FeCl <sub>3</sub> -Induced Thrombosis Assay for Pig       | / 52 |
| 4.17 | Doppler Blood Flow-Monitored FeCl <sub>3</sub> -Induced Thrombosis Assay for Mouse | / 53 |
| 4.18 | Rat Groin Flap Assay   | / 54 |
| 4.19 | Ferret Acute Thrombosis Assay  | / 55 |
| 4.20 | Rat Acute Thrombosis Assay   | / 56 |
| 4.21 | Arteriovenous Shunt Assay  | / 56 |
| 4.22 | Plasma Clotting Time Assay   | / 56 |
| 4.23 | Thromboembolic Photochemical Assay for Repeated Stroke in Mice                     | / 57 |
| 4.24 | Euglobulin Clot Lysis Assay  | / 58 |
| 4.25 | Clot Formation and Lysis (CloFAL) Assay  | / 59 |
| 4.26 | Fibrin Microplate Assay  | / 59 |
| 4.27 | Fibrinolytic Activity Assay  | / 60 |
| 4.28 | Fibrinolysis Assay   | / 60 |
| 4.29 | Thrombolytic Assay   | / 61 |
|      | References and Notes   | / 62 |

## 5 Methods and Applications of Anticoagulation Assays / 65

Ming Zhao

|      |  |      |
|------|--|------|
| 5.1  | Ecarin Chromogenic Assay                                     | / 66 |
| 5.2  | Anticoagulation Activity Assay in an <i>In Vitro</i> System  | / 67 |
| 5.3  | Anticoagulation Activity Assays in an <i>In Vivo</i> System  | / 67 |
| 5.4  | Rat Thrombosis Assay   | / 68 |
| 5.5  | <i>In Vivo</i> Microvascular IVC Blood Flow Assay            | / 68 |
| 5.6  | Owren PT Assay   | / 68 |
| 5.7  | Rabbit Double-Balloon Injury Assay                           | / 69 |
| 5.8  | A Rapid Point-of-Care Assay for Enoxaparin                   | / 70 |
| 5.9  | Thromboplastin Clotting Assay                                | / 71 |
| 5.10 | Rat Assay for Reproducible Stasis-Induced Venous Thrombosis  | / 71 |
| 5.11 | <i>In Vivo</i> ACT II/Ecarin Clotting Time Assay             | / 72 |
| 5.12 | <i>Ex Vivo</i> and <i>In Vivo</i> Anticoagulation Assay      | / 73 |
| 5.13 | Plasma-Based Ecarin Clotting Time Assay for r-Hirudin        | / 74 |
| 5.14 | Protamine Titration for Heparin in Whole Blood               | / 75 |
| 5.15 | Automated Assay Evaluating Response of Kaolin ACT to Heparin | / 76 |
| 5.16 | Platelet/Monocyte Interaction-Based PM Exposure Mouse Assay  | / 77 |

- 5.17 Mouse Tail-Bleeding Time Assay / 78
- 5.18 MRI Assay for Rabbit Atherosclerotic Lesions / 78
- 5.19 Spiral Computed Tomography Assay / 79
- 5.20 Duplex Ultrasound Assay / 80
- 5.21 Standard Hemochron Assay / 81
- 5.22 Platelet Serotonin Release Assay / 81
- 5.23 Activated Partial Thromboplastin Time Assay / 82
- 5.24 Rat Stroke Outcome Assay / 82
- References and Notes / 83

## **6 Methods and Applications of Blood Pressure-Related Assays / 87**

*Ming Zhao*

- 6.1 Human Plasma New Pressor Protein Assay / 88
- 6.2 Pulmonary Hypertension Assay / 89
- 6.3 Coronary Arteries Constriction Assay / 90
- 6.4 MRI and Brain Natriuretic Peptide Assays / 90
- 6.5 Competition Enzyme-Linked Immunosorbent Assay / 91
- 6.6 Right Ventricular Pressure Assay / 92
- 6.7 Plasma Nitrite/Nitrate Concentration Assay / 92
- 6.8 Adeno-Associated Virus Vector-Caused Rat Pulmonary Artery Pressure Assay / 92
- 6.9 Adeno-Associated Virus Vector-Caused Rat Protein and mRNA Assay / 93
- 6.10 N-Terminal pro-Brain Natriuretic Peptide (N-T proBNP) Assay / 93
- 6.11 Middle Cerebral Artery Occlusion Assay / 94
- 6.12 Vascular Endothelial Growth Factor Level Assay / 95
- 6.13 Antispasmodic Agent *In Vivo* Action Assay / 95
- 6.14 Temperature Assay in Awake Subjects / 96
- References and Notes / 97

## **7 Methods and Applications of Assays Related to Parkinson's Disease and Graves' Disease / 101**

*Shiqi Peng*

- 7.1 Flow Cytometric Assay for Cellular DNA Content and Caspase-3 / 102
- 7.2 Swine Resuscitation Assay / 103
- 7.3 HTLV-tax1 or pMuLV-SV-nlslacZ Vectors Transfected Cell Assay / 104
- 7.4 Parkinsonian Rat Assay / 104
- 7.5 ELISA for Nerve Growth Factor Antigen / 105
- 7.6 Assay for  $\beta$ -Nerve Growth Factor Levels in Cerebrospinal Fluid / 106

|      |  |     |
|------|--|-----|
| 7.7  | MDCK Scatter Assay /   | 107 |
| 7.8  | Facial Nerve and Spinal Root Avulsions /   | 108 |
| 7.9  | TRAb Assays /  | 108 |
| 7.10 | Human Thyrotropin Receptor (hTSHR) Assay /   | 109 |
| 7.11 | Soluble ICAM-1, TSAb, and TBIAb Activity Assays /                                      | 111 |
| 7.12 | Microarray Immunoassay for hTSHr Production /  | 112 |
| 7.13 | Affinity Assay for [ <sup>35</sup> S]GTPγS Binding to Gas/olf /                        | 113 |
| 7.14 | Tissue Segment Binding Assay for α <sub>1B</sub> -Adrenoceptor /                       | 114 |
| 7.15 | Membrane Binding Assay with Rat Cerebral Cortex for<br>α <sub>1B</sub> -Adrenoceptor / | 115 |
| 7.16 | Whole Cell Binding Assay for α <sub>1B</sub> -Adrenoceptor /                           | 115 |
| 7.17 | Functional Assay for α <sub>1A,B</sub> -Adrenoceptor /                                 | 116 |
| 7.18 | Rat Neuroprotective Assay /  | 116 |
| 7.19 | GABA-Benzodiazepine Receptor Assay /   | 117 |
| 7.20 | Forced Swimming and Tail Suspension Assays /   | 117 |
| 7.21 | IGF-I Kinase Receptor Activation (KIRA) Assay /  | 118 |
| 7.22 | gD.trkA Kinase Receptor Activation (KIRA) Assay /                                      | 118 |
| 7.23 | gD.trk KIRA-ELISA /  | 118 |
| 7.24 | Assays for Cannabinoid Receptors in Rat Cerebella or Mouse<br>Brains /                 | 120 |
|      | References and Notes /   | 121 |

## 8 Methods and Applications of Alzheimer's Disease Assays / 125

*Shiqi Peng*

|      |   |     |
|------|---|-----|
| 8.1  | Assay for Oxidative Stress in Cerebral Cortex of AD Mice /                | 126 |
| 8.2  | Reporter Assay for Primary Neuronal Cultures /                            | 127 |
| 8.3  | Electrophoretic Mobility Shift Assay (EMSA) /                             | 127 |
| 8.4  | Binding Assays Using Aggregated Aβ Peptide in Solution /                  | 127 |
| 8.5  | Assay for Muscarinic Receptor 1 in Alzheimer's<br>Dementia Model /        | 128 |
| 8.6  | β-Secretase Activity Assay /  | 128 |
| 8.7  | Aβ Fibril Binding Assay /   | 129 |
| 8.8  | TLC and Microplate Assays for Acetylcholinesterase<br>Inhibitors /        | 129 |
| 8.9  | Immunocapture Assay Measuring Specific Enzyme Activity<br>of Neprilysin / | 130 |
| 8.10 | <i>In Vivo</i> AChE Inhibition Assay /                                    | 131 |
| 8.11 | Single Particle Assay for Aβ Aggregates /                                 | 132 |
| 8.12 | Indirect Immunofluorescence Assay /                                       | 133 |
| 8.13 | Mouse Behavioral Assays /   | 133 |
| 8.14 | Center of Pressure (CoP) Assay in Mice /                                  | 135 |
| 8.15 | Assay for Plasma Levels of DJ-1 /   | 136 |
| 8.16 | Membrane Filter Assay for Tau Aggregation /                               | 137 |

- 8.17 Assays for Motor Neuron Degeneration / 138
- 8.18 HPLC Assay for Neuroprotective Agent in Mice Plasma / 140
- 8.19 Tissue Culture Assays for SOD1 Mutations / 141
- 8.20 Luciferase-Based Reporter Assay / 142
- References and Notes / 142

## **9 Methods and Applications of Antiosteoporosis Assays / 145**

*Ming Zhao*

- 9.1 Rat Bone Mineral Density Assay / 146
- 9.2 Osteoblastic Cell Proliferation and Alkaline Phosphatase (ALP) Activity Assays / 146
- 9.3 Murine Osteoblastic MC3T3-E1 Cell Calcification and Van Kossa Assays / 147
- 9.4 Osteoclast Generation Assay for Male Senile Rat / 148
- 9.5 Bone Resorption and Recovery Related Assays / 148
- 9.6 Mouse Bone Mineral Density Assay / 149
- 9.7 PPAR- $\gamma$  Competitor Assay / 150
- 9.8 Human Serum Estrogen Level Assay / 150
- 9.9 Luciferase Activity Assay / 150
- 9.10 IL-1 $\beta$  and TNF- $\alpha$  Level Assay / 150
- 9.11 ER Binding and Receptor Activity Assays / 151
- 9.12 Fluorescent Estrogen Receptor Assay / 152
- 9.13 ELISA for Urinary Helical Peptide / 153
- 9.14 Urine Midmolecule Osteocalcin Assay / 153
- 9.15 BMD and Osteocalcin Assay / 154
- 9.16 *In Vivo* Antiosteoporosis Assay on Mice / 154
- References and Notes / 155

## **10 Methods and Applications of Immunomodulating Assays / 157**

*Shiqi Peng*

- 10.1 Rat Mast Cell Histamine-Release Assay / 158
- 10.2 Rabbit Aortic Force Assay / 159
- 10.3 Dopaminergic Cell Death-Based Neural Transplantation Assay / 159
- 10.4 Mast Cell Degranulation Assay / 160
- 10.5 Basophils Assay as Allergen / 161
- 10.6 RBL-2H3 Cell Desensitization Assay / 162
- 10.7 Migration Assay of Dendritic Cell from PBMCs / 162
- 10.8 Mouse EAE Induction Assay / 163
- 10.9 COSTIM Assay for DC/T-Cells / 163
- 10.10 Cytokine Assay for IL-6, IL-10, IL-12, and TNF- $\alpha$  of DCs / 164
- 10.11 ELISPOT Assay for DC IFN- $\gamma$  / 165

|       |  |     |
|-------|--|-----|
| 10.12 | DC Function Assay for Evaluating Toll-like Receptor Function /   | 166 |
| 10.13 | Migration Assay of Dendritic Cell from Bone Marrow of A/J Mice / | 167 |
| 10.14 | Lymphoid Organ Assay /   | 168 |
| 10.15 | ELISA of IFN- $\gamma$ from Human Myelomonocytic KG-1 Cells /    | 170 |
| 10.16 | Human Whole Blood IFN- $\gamma$ Assays /                         | 171 |
| 10.17 | Sheep Whole Blood IFN- $\gamma$ Assays /                         | 171 |
| 10.18 | ELISPOT Assay for IFN- $\gamma$ /                                | 172 |
| 10.19 | IFN- $\beta$ RG Assay /  | 173 |
| 10.20 | Anti-rHuEPO NAb Assay /  | 173 |
| 10.21 | Chloramphenicol Acetyltransferase Assay /                        | 174 |
| 10.22 | Chemotaxis Assay /   | 175 |
| 10.23 | Fibroblast-Populated Microsphere Assay /                         | 175 |
| 10.24 | Fibroblast-Populated Concentric Microsphere Assay /              | 176 |
| 10.25 | Radial Assay of Chemotaxis /                                     | 178 |
| 10.26 | Antibody Forming Cell Assay /                                    | 179 |
| 10.27 | Immunosuppressive Assay /  | 179 |
| 10.28 | Cell-Based ELISA /   | 180 |
| 10.29 | Large Animal Lung Transplantation Assay /                        | 181 |
|       | References and Notes /   | 182 |

## **11 Methods and Applications of Anti-Inflammatory Assays / 187**

*Ming Zhao*

|       |   |     |
|-------|---|-----|
| 11.1  | Adhesion Formation Assay /  | 188 |
| 11.2  | Ligand Complex-Based Adhesion Assay /                               | 189 |
| 11.3  | Human Umbilical Vein Endothelial Cell Assay /                       | 190 |
| 11.4  | Pleurisy Mouse Assay /  | 191 |
| 11.5  | Proliferation of PBMC Assay /                                       | 192 |
| 11.6  | COX-1, COX-2, and 5-LOX Assay with [1- $^{14}$ C]Arachidonic Acid / | 193 |
| 11.7  | COX-1 and COX-2 Assay with Human Whole Blood /                      | 193 |
| 11.8  | <i>o</i> -Hydroxyleukotriene B <sub>4</sub> Assay /                 | 194 |
| 11.9  | Leukocyte Rolling and Adherence Assay /                             | 194 |
| 11.10 | CCR5 Receptor Binding Assay /                                       | 195 |
| 11.11 | Tissue Binding Affinity Assay /                                     | 195 |
| 11.12 | G93A-SOD1 Transgenic Mouse Assay /                                  | 196 |
| 11.13 | MCP-1-Induced ERK1 and ERK2 Phosphorylation Assay /                 | 197 |
| 11.14 | LPS- and IL-6-Induced ERK1 and ERK2 Phosphorylation Assay /         | 198 |
| 11.15 | ELA4.NOB-1/CTLL Cell Assay /  | 199 |
| 11.16 | FK506 Binding Protein 51 (FKBP51) mRNA Assay /                      | 200 |
| 11.17 | Xylene-Induced Ear Edema Assay /                                    | 201 |
|       | References and Notes /  | 201 |

## **12 Methods and Applications of Antioxidant Activity Assays / 205**

*Shiqi Peng*

- 12.1 Blood and Plasma Total Antioxidant Capacity (TAC) Assay / 206
- 12.2 Ferric Reducing-Antioxidant Power (FRAP) Assay / 207
- 12.3 Human LDL Oxidation Assay / 207
- 12.4 DPPH Radical Cation Scavenging Assay / 207
- 12.5 ABTS<sup>+</sup> Radical Cation Scavenging Assay / 208
- 12.6 Lipid Peroxidation Assay Using Rat Brain Tissue / 208
- 12.7 Flow-Through Chemiluminescence (FTCL) Assay / 209
- 12.8 Superoxide Radical Scavenging Assay / 209
- 12.9 Deoxyribose Assay for Hydroxyl Radical Scavenging Activity / 209
- 12.10 DNA Nicking Assay for Hydroxyl Radical Scavenging Activity / 210
- 12.11 Oxidative Lag-Time Assay / 210
- 12.12 TBARS and Electrophoresis Assay / 210
- 12.13 Reporter and Electrophoretic Mobility Shift Assay (EMSA) / 211
- 12.14 [Ca<sup>2+</sup>]<sub>cyt</sub> Assay / 211
- 12.15 Quantitative Real-Time PCR Assay / 212
- 12.16 FT-IR-Based Assay for Antioxidation Activity of Ionol and Piperidone / 212
- 12.17 HPLC Assay for Antioxidation Potential of Polyphenol / 213
- 12.18 ROS Production Assay / 213
- 12.19 Rabbit LDL Oxidation Assay / 213
- 12.20 ROS Scavenging Assays / 214
- 12.21 DNA Damage Assay / 215
- 12.22 Egg Yolk TBARS Assay / 216
- 12.23 Mouse Catalase (CAT) Assay / 216
- 12.24 Rat Tissue TBARS Assay / 216
- 12.25 Antioxidant Activity Assay for  $\beta$ -Carotene/Linoleic Acid System / 217
- 12.26 Rat Brain Tissue NO Assay / 217
- 12.27 Rat Brain Antioxidative Enzyme Assay / 218
- 12.28 Rat Brain Hippocampi Protein Oxidation Assay / 218
- References and Notes / 219

## **13 Methods and Applications of Analgesic Assays / 223**

*Ming Zhao*

- 13.1 Algogenic Activity Assay for Rat Ureteral Stone / 224
- 13.2 Cold Pressor-Based Assay for Acute Pain of Healthy Volunteers / 224
- 13.3 Heat-Based Assay for Acute Pain of Healthy Volunteers / 225
- 13.4 Electrical Stimulation-Based Assay for Acute Pain of Healthy Volunteers / 225

- 13.5 Radiant Heat Tail-Flick Assay for Mice / 226
- 13.6 Pain Behaviors/Responses Assays in Rats / 226
- 13.7 Hot Plate Assay in Rats / 227
- 13.8 Plantar Assay in Rats / 227
- 13.9 Hot Plate Assay in Mice / 227
- 13.10 Paw and Tail Formalin Assays in Mice / 228
- 13.11 Rat Assays for Bone Cancer Pain / 229
- 13.12 Mouse Assay for Hindpaw Cancer Pain / 229
- 13.13 Visceral Pain Assay / 230
- 13.14 Canine Nociceptive Thermal Escape Assay in Dog / 230
- 13.15 Carrageenan Assay in Rats / 232
- 13.16 Electrophysiologic Assay for Mice with Tumor-Evoked Hyperalgesia / 232
- 13.17 Mouse Assay for Bone Cancer Pain / 233
- References and Notes / 234

#### **14 Methods and Applications of Epilepsy Assays / 237**

*Ming Zhao, Shiqi Peng, and Guohui Cui*

- 14.1 HISS Assay / 238
- 14.2 Mouse Locomotor Activity Assay / 239
- 14.3 Mouse Diathesis-Stress Assay / 240
- 14.4 Social Interaction Assay / 240
- 14.5 Spatial Learning Ability Assay for Recurrent Seizure Rats / 241
- 14.6 Timed PTZ Infusion Assay for Mice / 241
- 14.7 Timed PTZ Seizure Assay for Mice / 242
- 14.8 Maximal Electroconvulsions Threshold Assay for Mice / 243
- 14.9 MES Assay / 243
- 14.10 6-Hz Psychomotor Seizure Assay for Mice / 244
- 14.11 Subcutaneous Bicuculline and Picrotoxin Assay for Mice / 244
- 14.12 NMDA-Induced Convulsions Assay for Mice / 244
- 14.13 AGS Assay / 245
- 14.14 Kindled Rat Assay for Focal Seizures / 245
- 14.15 Cobalt/Homocysteine Assay for Status Epilepticus of Rats / 246
- 14.16 PTZ-Induced Kindling Assay / 247
- 14.17 ICES Assay for Mice / 247
- References and Notes / 248

#### **15 Methods and Applications of Diabetes Assays / 251**

*Ming Zhao, Shiqi Peng, and Guohui Cui*

- 15.1 Islet Xenograft Assay for Diabetic Mice / 252
- 15.2 Assays for Spontaneous Diabetes and Adoptive Transfer of Diabetes / 252



- 15.3 Oral Glucose Tolerance Assays for Patients / 253
- 15.4 Injection Glucose Tolerance Assays for Rats / 253
- 15.5 Renal Cortical TGF- $\beta$ 1 Protein Assays for Rats / 254
- 15.6 Low-Dose Streptozotocin-Treated Heminephrectomized Rat Assay / 254
- 15.7 Rat Early Diabetic Nephropathy Assay / 255
- 15.8 Urinary Endothelin-1 Excretion Assay for Type 2 Diabetes Rats / 255
- 15.9 Subtotally Nephrectomized Rat Assay / 256
- 15.10 Type 2 Diabetes Mice Assay / 256
- References / 257

## 16 Methods and Applications of Assays for Toxins from Microorganisms / 259

*Shiqi Peng*

- 16.1 Colorimetric Yeast Assay for Trichothecene Mycotoxins / 260
- 16.2 Colorimetric Cell Proliferation Assay / 261
- 16.3 Yeast DEL Assay / 262
- 16.4 NCCLS and EUCAST Assays / 263
- 16.5 Alcohol Dehydrogenase-Based Colorimetric Assays / 264
- 16.6 Colorimetric Assay for Iron in Yeast / 265
- 16.7 Microplate Redox Assays of *E. coli* / 265
- 16.8 Ciliate *Tetrahymena thermophila* Assay for Trichothecene Mycotoxins / 266
- 16.9 Fluorescent Dyes-Based Cell Viability Assay for Triton X-100 Toxicity / 267
- 16.10 MTT Assay for Fusarium Mycotoxins / 268
- 16.11 Mortality and Frass Production Assay for Toxicity of Bacterial Strains / 269
- 16.12 Cell Toxicity Assay / 270
- 16.13 Dhase Inhibition Assay / 271
- 16.14 Sediment Toxicity Assay / 271
- 16.15 Toxicity Assay of Particle-Associated Arsenite and Mercury / 272
- 16.16 Genetic Toxicity Assay / 273
- 16.17 Microtox Assays / 275
- 16.18 DNA Piezoelectric Biosensor Assay / 275
- 16.19 Protein Phosphatase Inhibition Assay and ELISA of Microcystins / 276
- 16.20 *Lepidium sativum* Assay for Microcystin Toxicity / 277
- 16.21 Antiproliferative Assay for Interleukin-4 / 278
- References and Notes / 278