Contents

Preface . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . vii
Contributors . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . xi

PART I  Antibodies as a Tool: From Concept to Design and Application

1 Overview of the Generation, Validation, and Application of Phosphosite-Specific Antibodies .................................... 3
   Kathy Brumbaugh, Wade Johnson, Wen-Chieh Liao, Mong-Shang Lin, J.P. Houchins, Jeff Cooper, Steven Stoess, and Roberto Campos-Gonzalez

2 Selection and Validation of Antibodies for Signal Transduction Immunohistochemistry............................................. 45
   Juraj Bodo and Eric D. Hsi

3 An Overview of Western Blotting for Determining Antibody Specificities for Immunohistochemistry................................. 55
   Biji T. Kurien, Yaser Dorri, Skyler Dillon, Anil Dsouza, and R. Hal Scofield

4 Optimized Protocol to Make Phospho-Specific Antibodies that Work . . . . . . . . . . 69
   Amy J. Archuleta, Crystal A. Stutzke, Kristin M. Nixon, and Michael D. Browning

PART II  Preservation and Unmasking of Tissue Antigens

5 Methodology and Technology for Stabilization of Specific States of Signal Transduction Proteins.................................. 91
   Mats Borén

6 An Enhanced Antigen-Retrieval Protocol for Immunohistochemical Staining of Formalin-Fixed, Paraffin-Embedded Tissues .................... 101
   Sergei I. Syrbu and Michael B. Cohen

PART III  Imaging Techniques and High-Throughput Data Analysis

7 Imaging Techniques in Signal Transduction IHC ............................................. 113
   Jerry Sedgewick

8 Practical Considerations of Image Analysis and Quantification of Signal Transduction IHC Staining .................................. 143
   Michael Grunkin, Jakob Raundahl, and Niels T. Foged

9 Flow Cytometric Analysis of Cell Signaling Proteins ............................................. 155
   Maria A. Suni and Vernon C. Maino
10 CytoSys: A Tool for Extracting Cell-Cycle-Related Expression Dynamics from Static Data .......................................... 171

Jayant Avva, Michael C. Weis, Radina P. Soebiyanto, James W. Jacobberger, and Sree N. Sreenath

PART IV NEUROSCIENCE, CANCER, AND STEM CELL RESEARCH

11 Signaling Events Initiated by Kappa Opioid Receptor Activation: Quantification and Immunocolocalization Using Phospho-Selective KOR, p38 MAPK, and KIR 3.1 Antibodies .............................. 197

Julia C. Lemos, Clarisse A. Roth, and Charles Chavkin

12 Immunohistochemical Assessment of Signal Transduction and Cell-Cycle Networks in Neural Tumors ........................................... 221

Daniel Ciznadija, Afsar Barlas, and Katia Manova

13 Novel Multicolor Immunofluorescence Technique Using Primary Antibodies Raised in the Same Host Species ............................... 233

Jillian Frisch, J.P. Houchins, Michael Grahek, Jordan Schoephoerster, Jodi Hagen, Joseph Sweet, Leopoldo Mendoza, David Schwartz, and Alexander E. Kalyuzhny

14 Activation and Differentiation of Mesenchymal Stem Cells .................. 245

Pravin J. Mishra and Debabrata Banerjee

PART V NOVEL ASSAYS AND TECHNIQUES

15 Double In Situ Detection of Sonic Hedgehog mRNA and pMAPK Protein in Examining the Cell Proliferation Signaling Pathway in Mouse Embryo ................................ 257

Sho Fujisawa, Mesruh Turkekul, Afsar Barlas, Ning Fan, and Katia Manova

16 Identifying Intracellular Sites of Eicosanoid Lipid Mediator Synthesis with EicosaCell Assays .............................................. 277

Christiane Bandeira-Melo, Peter F. Weller, and Patricia T. Bozza

17 Absorption Control in Immunohistochemistry Using Phospho-Peptides Immobilized on Magnetic Beads .................................. 291

Jordan Schoephoerster, Jillian Frisch, Michael Grahek, Chun Wu, Yingwei He, Wei Wang, Jennifer Nguyen, David Schwartz, and Alexander E. Kalyuzhny

Index ................................................................. 301
Signal Transduction Immunohistochemistry
Methods and Protocols
Kalyuzhny, A.E. (Ed.)
2011, XIII, 302 p., Hardcover
A product of Humana Press