

Contents

1	Introduction	1
	What Is Immunocytochemistry?	1
	What Can Immunocytochemistry Tell Us?	2
	An Outline of the Immunocytochemistry Procedure	4
	What Is Included in This Book?	5
2	Antibodies	7
	Introduction	7
	Antibody Molecules	8
	Making Antibodies	10
	Talking About Antibodies	13
	Finding and Getting Antibodies	14
	Choice of Primary (1°) Antibodies	15
	Antibodies Handling and Storing	16
	Recommended Storage Freezer, -20°C	16
	Recommended Storage Refrigerator, 4°C	16
3	Sample Preparation/Fixation	17
	Introduction	17
	Fixation Theory	18
	Chemical Fixatives	19
	Vehicle	22
	Applying Fixatives	24
	Dissecting the Area of Interest	25
	Protocol – Fixation	26
	Components for Paraformaldehyde Fixative	26
	Procedure	27
	Perfusion Procedure	27
	Perfusion Equipment	28
	Drop-in-Fixation	28
4	Tissue Sectioning	29
	Introduction	29
	Embedding Tissue by Freezing	30

Theory of Freezing Tissue	30
Freezing Tissue	32
Cryostat Sectioning	33
Tissue Processing	37
Vibratome, Freezing Microtome, and Microwave	39
Fresh Frozen Tissue	41
Embedding Tissue with Paraffin	41
Cryostat Protocol	42
5 Blocking and Permeability	45
Introduction	45
Nonspecific Antibody Binding to Tissue and Cells	45
Blocking for Nonspecific Antibody Binding	47
Permeabilize Tissue and Cells to Allow Antibody Penetration	49
Effects of Blocking Agents on Antibody Penetration	51
Combined Incubation Step	53
6 Labels for Antibodies	55
Introduction	55
Fluorescence Theory	56
Four Generations of Fluorescent Labels	58
Immunocytochemistry Fluorophores and Flow Cytometry	59
Choosing Fluorochromes	61
Enzyme Theory	61
Enzyme Substrates	61
Particulate Label	63
Choice of Fluorescent or Enzymes for Immunocytochemistry	64
7 Application Methods	65
Introduction	66
Direct Immunocytochemistry	66
Direct Immunocytochemistry Advantages	67
Direct Immunocytochemistry Disadvantages	67
Indirect Immunocytochemistry	67
Indirect Immunocytochemistry Advantages	68
Indirect Immunocytochemistry Disadvantages	68
Avidin–Biotin Molecules	68
Direct Avidin–Biotin Immunocytochemistry	69
Direct Avidin–Biotin Method Advantages	70
Direct Avidin–Biotin Method Disadvantages	70
Indirect Avidin–Biotin Immunocytochemistry	70
Indirect Avidin–Biotin Advantages	71
Indirect Avidin–Biotin Disadvantages	71
Avidin–Biotin Complex (ABC) Immunocytochemistry	71
Avidin–Biotin Complex (ABC) Advantages	73
Avidin–Biotin Complex (ABC) Disadvantages	73

Tyramide Signal Amplification (TSA) Immunocytochemistry	73
Tyramide Signal Amplification Advantages	74
Tyramide Signal Amplification Disadvantages	75
ABC with TSA	75
ABC with TSA Advantages	77
ABC with TSA Disadvantages	77
8 Controls	79
Introduction	79
Three Immunocytochemistry Controls	79
1. 1° Antibody Controls	80
2. 2° Antibody Controls	84
3. Labeling Controls	85
9 Method and Label Decision	89
Introduction	89
Choose Application Label and Method	89
Experimental Design Chart	93
10 Single Antibody Procedure	97
Introduction	97
Experimental Design Chart	98
Incubation Conditions	98
Antibody Dilutions	100
Antibody Dilution Matrix	102
2° Antibody Controls	102
Rinses	104
Mounting Media	105
Final Procedure	106
Steps in a Single 1° Antibody Indirect Immunocytochemistry Experiment	106
Steps in a Single 1° Antibody Immunocytochemistry Experiment for Ag A	107
11 Multiple Antibodies Different Species	111
Introduction	111
Combining Two 1° Antibody Incubations	112
Experimental Design Chart	112
Designing 2° Antibody Controls	113
Rules for Multiple Label Experiments	113
Complete Final Procedure	115
(D) Block and Permeabilize	116
(E) Rinse after Block and Permeabilize	116
(F) 1° Antibodies	117
(G) Rinse After 1° Antibody	117
(H) 2° Antibody	117
(I) Rinse After 2° Antibody	117

12 Multiple Antibodies from the Same Species	119
Introduction	120
Combine Two 1° Antibodies from the Same Species	
with Block-Between Method	120
Experimental Design Chart for Block-Between Method	122
Design the 2° Antibody Control for the Same Species	
with Block-Between	124
Final Procedure for Two 1° Antibody Same Species	
with Block-Between	127
(A) Prepare Cell Culture	127
(B) Fix Culture	127
(C) Block and Permeabilize	127
(D) Rinse After Block and Permeabilize	128
(E) Incubate First 1° Antibody	128
(F) Rinse After First 1° Antibody	128
(G) Incubate First 2° Antibody	128
(H) Rinse After First 2° Antibody	128
(I) Block Antibodies in First Set	128
(J) Incubate Second 1° Antibody	128
(K) Rinse After Second 1° Antibody	129
(L) Incubate Second 2° Antibody	129
(M) Rinse After Second 2° Antibody	129
(N) Mount Coverslip	129
(O) Examine in Microscope	129
(P) Evaluate Results	129
Combine Two 1° Antibodies from the Same	
Species with Zenon	130
Experimental Design Chart for the Same Species with Zenon	130
Design the Antibody Control for the Same Species with Zenon	133
Final Procedure for Two 1° Antibody from the Same Species	
with Zenon	135
(A) Prepare Cell Culture	135
(B) Fix Culture	135
(C) Block and Permeabilize	136
(D) Rinse after Block and Permeabilize	136
(E) Prepare the Zenon Reagents	136
(F) Incubate with Labeled Antibody(ies)	136
(G) Rinse After Antibody Incubation	136
(H) Fix with 4% Paraformaldehyde	136
(I) Rinse after Antibody Incubation	137
(J) Mount Coverslip	137
(K) Examine in Microscope	137
(L) Evaluate Results	137

13	Fluorescent Microscopy and Imaging	139
	Introduction	139
	Filter Sets in Fluorescence Microscopy	140
	Fluorescent Bleed-Through	142
	Fluorescence Quench and Photobleach	145
	Image Parameters – Contrast and Pixel Saturation	146
	Ethics of Image Manipulation	148
	Do	149
	Do Not	149
14	Troubleshooting	151
	Introduction	151
	Procedural Errors	152
	Method of Troubleshooting	152
	Case No. 1	153
	Case No. 2	156
	Case No. 3	158
	Case No. 4	164
	Case No. 5	167
	Troubleshooting Unique to Multiple Primary Antibodies	173
	Bad Antibodies	173
	Bad 1° Antibodies	173
	Bad 2° Antibodies	174
15	Electron Microscopic Immunocytochemistry	175
	Protocol – Pre-embedding Electron Microscopic Immunocytochemistry	175
	Introduction	175
	Need for Electron Microscopic Immunocytochemistry	176
	Pre-embedding Electron Microscopic Immunocytochemistry	178
	Postembedding Electron Microscopic Immunocytochemistry	181
	Choice of a Method	185
	Advantages and Disadvantages	185
	Protocol – Pre-embedding Electron Microscopic Immunocytochemistry	185
	Solutions	186
	Stock Solutions to Make Ahead and Store	186
	Solutions Made on the First Day of the Experiment	187
	NPG Silver Enhancement Solution and Silver Lactate	188
	Test Strip	189
	Appendix	191
	References	199
	Glossary	203
	Index	213



<http://www.springer.com/978-1-4419-1303-6>

Immunocytochemistry

A Practical Guide for Biomedical Research

Burry, R.W.

2010, XV, 223 p., Hardcover

ISBN: 978-1-4419-1303-6