

The background of the cover is a fluorescence microscopy image showing a complex network of green and red signals against a dark background. The green signals form a dense, branching network, while the red signals appear as more localized, bright spots and lines. The overall appearance is that of a biological specimen, possibly a neural or vascular network, stained with fluorescent dyes.

Introduction to

Immunocytochemistry

3rd Edition

J.M. Polak and S. Van Noorden

Abbreviations	xi
Preface	xiii
Key to symbols	xv

1. Introduction	1
------------------------	----------

Definition	1
History and development	1
References	3

2. Production of Antibodies	5
------------------------------------	----------

Immunization	5
Testing	6
Region-specific antibodies	6
Monoclonal antibodies	8
Selection of antibodies by phage display	9
Characteristics of a 'good' antibody	10
References	11

3. Preparation of Tissue for Immunocytochemistry	13
---	-----------

Fixation	13
Cross-linking fixatives	14
Precipitant fixatives	15
Combination fixatives	15
Fixed, paraffin-embedded tissue	16
Fresh material – frozen sections and cell preparations	16
Frozen sections	17
Whole cell preparations	18
Pre-fixed, non-embedded tissue	20
Pre-fixed frozen sections	20
Pre-fixed Vibratome sections	21
Whole-mounts	21
Permeabilization	21

Freeze-dried tissue	22
Tissue storage	22
Paraffin blocks and sections	22
Frozen blocks and sections	23
Cell preparations	23
Adherence of sections and cell preparations to slides	24
Antigen retrieval in fixed tissues	24
Washing	24
Protease treatment	24
Heat-mediated antigen retrieval	26
References	29

Colour plate section	33
-----------------------------	-----------

4. Visualizing the End-product of Reaction	45
---	-----------

Fluorescent labels	46
Advantages	46
Disadvantages	46
Uses of immunofluorescence	46
Fluorescein	47
Rhodamine	47
Phycoerythrin	48
AMCA	48
Other fluorophores	48
Fluorescent counterstains	48
Enzyme labels	49
Peroxidase	49
Alkaline phosphatase	52
Glucose oxidase	52
β -D-Galactosidase	53
Gold labels	53
Colloidal gold	53
Nanogold	54
Other labels	55
Biotin	55
Haptens	55
Radioisotopes	55
References	56

5. Non-specific Staining due to Tissue Factors	59
---	-----------

Causes of non-specific binding	60
Charged sites	60
Hydrophobic attraction	60
Fc receptors	60
Prevention of non-specific binding	60

Other problems	61
Endogenous enzymes	61
Endogenous biotin	61
Autofluorescence	61
References	62
<hr/>	
6. Methods	63
<hr/>	
General considerations	63
Buffers	63
Antibody diluent and storage	64
Antibody dilution relative to reaction time, temperature and technique	65
Methods	67
Nature of antibodies (IgG)	67
Application of antibodies to preparations	69
Direct (one-step) method	70
Indirect (two-step) method	72
Three-layer methods	73
Avidin-biotin methods	76
References	79
<hr/>	
7. Testing Antibodies: Specificity and Essential Controls	81
<hr/>	
Testing a new primary antibody	81
A primary antibody with a known localization	81
A primary antibody with an unknown localization	85
Negative control for polyclonal antibodies – normal serum	85
Negative controls for monoclonal antibodies	85
Testing for non-specific binding of second and third reagents	86
Non-specific or unwanted specific staining due to antibody factors	86
Unwanted specific staining of unknown antigens	86
Non-specific binding of antisera to basic proteins	86
Unwanted specific cross-reactivity of anti-immunoglobulins	87
Cross-reactivity of the primary antibody with related antigens	87
Remedies for non-specificity due to tissue factors	89
Blocking binding sites with normal serum	89
Absorption with tissue powder	89
Remedies for non-specificity due to heterogeneity of the antibody	89
Dilution	89
Affinity purification	89
Remedies for non-specificity due to cross-reactivity	90
Essential staining controls	90

Negative controls	90
Positive controls	90
Experimental controls	91
References	91
<hr/>	
8. Increasing Sensitivity and Enhancing Standard Methods	93
<hr/>	
Increasing sensitivity	93
Immunogold with silver enhancement	93
Build-up methods	94
Tyramine signal amplification (TSA)	97
Intensification of the peroxidase/DAB/H ₂ O ₂ product	100
Post-reaction intensification	100
Intensification during the peroxidase reaction	101
References	101
<hr/>	
9. Multiple Immunostaining	103
<hr/>	
Double direct immunostaining with separately labelled primary antibodies	103
Double immunostaining with primary antibodies raised in different species, or of different immunoglobulin sub-class	104
Double immunoenzymatic method	104
Double immunofluorescence method	106
Triple immunostaining	106
Unlabelled primary antibodies from the same species	106
The problem	106
Elution methods	107
Indirect double immunostaining without elution	109
References	113
<hr/>	
10. Immunocytochemistry for the Transmission Electron Microscope	115
<hr/>	
Principles	115
Fixation	115
Pre-embedding immunocytochemistry	116
Non-embedding immunocytochemistry	116
Processing to resin	117
Labels	118
Sectioning resin blocks	119
Pre-treatment	120
Immunolabelling procedure	121
Immunolabelling with peroxidase	121
Amplification	122
Contrasting	122

Multiple labelling	122
References	123
<hr/>	
11. <i>In Vitro</i> Methods for Testing Antigen–Antibody Reactions	125
<hr/>	
Radioimmunoassay	126
Enzyme-linked immunosorbent assay (ELISA)	126
Western blotting	127
Dot blots	127
References	128
<hr/>	
12. Applications of Immunocytochemistry	129
<hr/>	
Histopathological diagnosis	129
Controls	130
Choice of antibody	131
Tips for diagnostic laboratories	131
Research	133
Quantification	133
Confocal microscopy	134
Flow cytometry and fluorescent antibody cell sorting (FACS)	135
Simpler methods of quantification	135
Non-immunocytochemical uses of labelled probes	136
Receptor localization	137
Lectin histochemistry	137
<i>In situ</i> hybridization of nucleic acids	138
References	138
<hr/>	
Appendix: Technical Notes	141
<hr/>	
Buffers for diluting antibodies and rinsing	141
Phosphate-buffered normal saline (PBS)	141
Tris-buffered normal saline (TBS)	141
Antibody diluent and storage of antibodies	142
Double dilutions	142
Adherence of preparations to slides	143
Coating slides with <i>poly-L</i> -lysine	143
Coating slides with silane	144
Blocking endogenous peroxidase reaction	144
Paraffin sections	144
Milder methods for cryostat sections and whole-cell preparations	145
Blocking endogenous biotin	146
Enzyme pre-treatment	146
Trypsin	146

Protease	147
Pepsin	147
Neuraminidase	148
Heat-mediated antigen retrieval using a microwave oven	148
Enzyme development methods	150
Peroxidase	150
Alkaline phosphatase	153
Glucose oxidase	154
β -D-Galactosidase	155
Intensifying the peroxidase/DAB reaction product	156
Following standard development	156
During development	156
Immunostaining methods	158
Initial procedures	158
Immunostaining – all preparations	160
Immunogold staining with silver enhancement	161
Silver acetate auto-metallography	163
Double immunoenzymatic staining	164
Primary antibodies from different species	164
Primary antibodies from the same species, heat-blocking method	165
Post-embedding electron microscopical immunocytochemistry using epoxy resin-embedded tissue and an indirect immunogold method	166
Absorption specificity control (liquid phase)	168
References	169
Index	171