

Table of Contents

- Abbreviations p. xi
- Preface p. xiii
- Key to symbols p. xv
- 1. Introduction p. 1
 - Definition p. 1
 - History and development p. 1
 - References p. 3
- 2. Production of Antibodies p. 5
 - Immunization p. 5
 - Testing p. 6
 - Region-specific antibodies p. 6
 - Monoclonal antibodies p. 8
 - Selection of antibodies by phage display p. 9
 - Characteristics of a 'good' antibody p. 10
 - References p. 11
- 3. Preparation of Tissue for Immunocytochemistry p. 13
 - Fixation p. 13
 - Cross-linking fixatives p. 14
 - Precipitant fixatives p. 15
 - Combination fixatives p. 15
 - Fixed, paraffin-embedded tissue p. 16
 - Fresh material--frozen sections and cell preparations p. 16
 - Frozen sections p. 17
 - Whole cell preparations p. 18
 - Pre-fixed, non-embedded tissue p. 20
 - Pre-fixed frozen sections p. 20
 - Pre-fixed Vibratome sections p. 21
 - Whole-mounts p. 21
 - Permeabilization p. 21
 - Freeze-dried tissue p. 22
 - Tissue storage p. 22
 - Paraffin blocks and sections p. 22
 - Frozen blocks and sections p. 23
 - Cell preparations p. 23
 - Adherence of sections and cell preparations to slides p. 24
 - Antigen retrieval in fixed tissues p. 24
 - Washing p. 24
 - Colour plate section p. 33
 - Protease treatment p. 24
 - Heat-mediated antigen retrieval p. 26
 - References p. 29
- 4. Visualizing the End-product of Reaction p. 45
 - Fluorescent labels p. 46
 - Advantages p. 46

- Disadvantages p. 46
- Uses of immunofluorescence p. 46
- Fluorescein p. 47
- Rhodamine p. 47
- Phycoerythrin p. 48
- AMCA p. 48
- Other fluorophores p. 48
- Fluorescent counterstains p. 48
- Enzyme labels p. 49
- Peroxidase p. 49
- Alkaline phosphatase p. 52
- Glucose oxidase p. 52
- [beta]-d-Galactosidase p. 53
- Gold labels p. 53
- Colloidal gold p. 53
- Nanogold p. 54
- Other labels p. 55
- Biotin p. 55
- Haptens p. 55
- Radioisotopes p. 55
- References p. 56
- 5. Non-specific Staining due to Tissue Factors p. 59
 - Causes of non-specific binding p. 60
 - Charged sites p. 60
 - Hydrophobic attraction p. 60
 - Fc receptors p. 60
 - Prevention of non-specific binding p. 60
 - Other problems p. 61
 - Endogenous enzymes p. 61
 - Endogenous biotin p. 61
 - Autofluorescence p. 61
 - References p. 62
- 6. Methods p. 63
 - General considerations p. 63
 - Buffers p. 63
 - Antibody diluent and storage p. 64
 - Antibody dilution relative to reaction time, temperature and technique p. 65
 - Methods p. 67
 - Nature of antibodies (IgG) p. 67
 - Application of antibodies to preparations p. 69
 - Direct (one-step) method p. 70
 - Indirect (two-step) method p. 72
 - Three-layer methods p. 73
 - Avidin-biotin methods p. 76
 - References p. 79
- 7. Testing Antibodies: Specificity and Essential Controls p. 81

- Testing a new primary antibody p. 81
- A primary antibody with a known localization p. 81
- A primary antibody with an unknown localization p. 85
- Negative control for polyclonal antibodies--normal serum p. 85
- Negative controls for monoclonal antibodies p. 85
- Testing for non-specific binding of second and third reagents p. 86
- Non-specific or unwanted specific staining due to antibody factors p. 86
- Unwanted specific staining of unknown antigens p. 86
- Non-specific binding of antisera to basic proteins p. 86
- Unwanted specific cross-reactivity of anti-immunoglobulins p. 87
- Cross-reactivity of the primary antibody with related antigens p. 87
- Remedies for non-specificity due to heterogeneity of the antibody p. 89
- Remedies for non-specificity due to tissue factors p. 89
- Blocking binding sites with normal serum p. 89
- Absorption with tissue powder p. 89
- Dilution p. 89
- Affinity purification p. 89
- Remedies for non-specificity due to cross-reactivity p. 90
- Essential staining controls p. 90
- Negative controls p. 90
- Positive controls p. 90
- Experimental controls p. 91
- References p. 91
- 8. Increasing Sensitivity and Enhancing Standard Methods p. 93
- Increasing sensitivity p. 93
- Immunogold with silver enhancement p. 93
- Build-up methods p. 94
- Tyramine signal amplification (TSA) p. 97
- Intensification of the peroxidase/DAB/H₂O₂ product p. 100
- Post-reaction intensification p. 100
- Intensification during the peroxidase reaction p. 101
- References p. 101
- 9. Multiple Immunostaining p. 103
- Double direct immunostaining with separately labelled primary antibodies p. 103
- Double immunostaining with primary antibodies raised in different species, or of different immunoglobulin sub-class p. 104
- Double immunoenzymatic method p. 104
- Double immunofluorescence method p. 106
- Triple immunostaining p. 106
- Unlabelled primary antibodies from the same species p. 106
- The problem p. 106
- Elution methods p. 107
- Indirect double immunostaining without elution p. 109
- References p. 113
- 10. Immunocytochemistry for the Transmission Electron Microscope p. 115
- Principles p. 115

- Fixation p. 115
- Pre-embedding immunocytochemistry p. 116
- Non-embedding immunocytochemistry p. 116
- Processing to resin p. 117
- Labels p. 118
- Sectioning resin blocks p. 119
- Pre-treatment p. 120
- Immunolabelling procedure p. 121
- Immunolabelling with peroxidase p. 121
- Amplification p. 122
- Contrasting p. 122
- Radioimmunoassay p. 126
- Multiple labelling p. 122
- References p. 123
- 11. In Vitro Methods for Testing Antigen-Antibody Reactions p. 125
 - Enzyme-linked immunosorbent assay (ELISA) p. 126
 - Western blotting p. 127
 - Dot blots p. 127
 - References p. 128
- 12. Applications of Immunocytochemistry p. 129
 - Histopathological diagnosis p. 129
 - Controls p. 130
 - Choice of antibody p. 131
 - Tips for diagnostic laboratories p. 131
 - Flow cytometry and fluorescent antibody cell sorting (FACS) p. 135
 - Research p. 133
 - Quantification p. 133
 - Confocal microscopy p. 134
 - Simpler methods of quantification p. 135
 - Non-immunocytochemical uses of labelled probes p. 136
 - Receptor localization p. 137
 - Lectin histochemistry p. 137
 - In situ hybridization of nucleic acids p. 138
 - References p. 138
- Appendix Technical Notes p. 141
 - Buffers for diluting antibodies and rinsing p. 141
 - Phosphate-buffered normal saline (PBS) p. 141
 - Tris-buffered normal saline (TBS) p. 141
 - Antibody diluent and storage of antibodies p. 142
 - Double dilutions p. 142
 - Adherence of preparations to slides p. 143
 - Coating slides with poly-L-lysine p. 143
 - Coating slides with silane p. 144
 - Blocking endogenous peroxidase reaction p. 144
 - Paraffin sections p. 144
 - Milder methods for cryostat sections and whole-cell preparations p. 145

- Blocking endogenous biotin p. 146
- Enzyme pre-treatment p. 146
- Trypsin p. 146
- Protease p. 147
- Pepsin p. 147
- Neuraminidase p. 148
- Heat-mediated antigen retrieval using a microwave oven p. 148
- Enzyme development methods p. 150
- Peroxidase p. 150
- Alkaline phosphatase p. 153
- Glucose oxidase p. 154
- During development p. 156
- [beta]-D-Galactosidase p. 155
- Intensifying the peroxidase/DAB reaction product p. 156
- Following standard development p. 156
- Immunostaining methods p. 158
- Initial procedures p. 158
- Immunostaining--all preparations p. 160
- Immunogold staining with silver enhancement p. 161
- Silver acetate auto-metallography p. 163
- Double immunoenzymatic staining p. 164
- Absorption specificity control (liquid phase) p. 168
- Primary antibodies from different species p. 164
- Primary antibodies from the same species, heat-blocking method p. 165
- Post-embedding electron microscopical immunocytochemistry using epoxy resin-embedded tissue and an indirect immunogold method p. 166
- References p. 169
- Index p. 171