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Immunocytochemical Methods and Protocols

Second Edition

Edited by

Lorette C. Javois

Catholic University of America, Washington, DC

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Dedication

To Larry, who wonders whether this "counts" as another publication, and to Alexandra, who decided to skip the midnight sessions down the home stretch this time.

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Cover illustration: The head and upper body column of the freshwater coelenterate, *Hydra oligactis*, labeled with the head-specific monoclonal antibody CP8, using the immunofluorescent technique described in Chapter 18 (ref. 10). Labeling on the whole mount preparation is restricted to the hypostome (mouth) and surrounding tentacles but not the body column beneath. Photograph by Daniel R. Bessette.

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Preface

The principle that antibodies can be used as cytochemical agents provided they are tagged with suitable markers has been evident for over 55 years. During this time the use of immunocytochemical methods has spread to a wide array of biological disciplines. Early applications focused on the detection of microbial antigens in tissues while more recent applications have used monoclonal antibodies to study cell differentiation during embryonic development. For a select few disciplines volumes have been published focusing on the specific application of immunocytochemical techniques to that discipline.

What distinguished *Immunocytochemical Methods and Protocols* from earlier books when it was first published four years ago was its broad appeal to researchers in all disciplines, including those in both research and clinical settings. The methods and protocols presented here are designed to be general in their application and the accompanying “Notes” provide invaluable assistance in adapting or troubleshooting the protocols. These strengths hold true for the second edition. In addition, the last four years have seen the ever-expanding application of immunocytochemical techniques in the clinical laboratory and the widespread use of immunocytochemistry in combination with *in situ* hybridization. Both of these subjects are given more detailed treatment in this second edition. As with the first edition, interspersed throughout the book are chapters providing overviews of select topics related to immunocytochemistry.

Immunocytochemical Methods and Protocols, Second Edition begins with a series of protocols geared to purify and prepare antibodies for use in subsequent staining procedures. The next series of protocols focuses on the fixation and preparation of tissues for light microscopic analyses, including antigen retrieval methods. Then, the use of fluorescent- and enzyme-conjugated antibodies to localize antigens in a variety of preparations is considered at length. The use of special instrumentation such as the confocal microscope and laser microbeam workstation, is also discussed. The next section focuses on the preparation and staining of cells for fluorescence activated cell sorter (FACS) analysis and assays which measure various cell parameters using FACS. This is followed by protocols detailing the fixation and staining of

samples for electron microscopic evaluation of antibody labeling using colloidal gold. Applications combining immunocytochemistry with *in situ* hybridization are organized into a new section while the final section of the book focuses on additional applications relevant to the clinical laboratory. In particular, regulations and troubleshooting guidelines are discussed. Many of these special applications have never been presented within the context of a volume devoted to immunocytochemistry methods and protocols, and the desire is for researchers and clinicians considering an immunocytochemical approach to be as well informed about their options as possible.

I am deeply indebted to the authors of the various chapters in the first edition for their continued interest and suggestions for improving the volume. I appreciate all the authors' hard work, dedication, and willingness to share their expertise. The protocols presented here are routinely used in their laboratories as the extensive "Notes" they have provided will attest to. Once again, the high quality of the manuscripts made my job as an editor an easy one. I would also like to thank Dr. John Walker, *Methods in Molecular Biology* series editor, for his encouragement throughout the process of compiling this second edition. Final thanks go to Thomas Lanigan, Fran Lipton, and the staff of Humana Press for making this book a reality.

Lorette C. Javois, PhD

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