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Immunocytochemical Methods and Protocols

Third Edition

Edited by

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Preface

Antibodies tagged with fluorescent markers have been used in histochemistry for over 50 years. Although early applications were focused on the detection of microbial antigens in tissues, the use of immunocytochemical methods now has spread to include the detection of a wide array of antigens including proteins, carbohydrates, and lipids from virtually any organism. Today, immunohistochemistry is widely used to identify, in situ, various components of cells and tissues in both normal and pathological conditions. The method gains its strength from the extremely sensitive interaction of a specific antibody with its antigen. For some scientific areas, books have been published on applications of immunocytochemical techniques specific to that area.

What distinguished *Immunocytochemical Methods and Protocols* from earlier books when it was first published was its broad appeal to investigators across all disciplines, including those in both research and clinical settings. The methods and protocols presented in the first edition were designed to be general in their application; the accompanying "Notes" provided the reader with invaluable assistance in adapting or troubleshooting the protocols. These strengths continued to hold true for the second edition and again for the third edition. Since the publication of the first edition, the application of immunocytochemical techniques in the clinical laboratory has continued to rise and this third edition provides methods that are applicable to basic research as well as to the clinical laboratory. The third edition also provides sites for resources that are available on the Internet. As with the previous editions, chapters providing overviews of selected topics related to immunocytochemistry are interspersed throughout the book.

Immunocytochemical Methods and Protocols, Third Edition, begins with an overview of the use of antibodies in immunocytochemistry followed by methods for purifying and conjugating them for use in immunostaining protocols. The next set of protocols details the fixation and preparation, including antigen retrieval, of tissues and cells for light microscopic immunostaining. Various methods for the use and detection of fluorescently labeled antibodies are then given. The confocal microscope and laser-microbeam applications are discussed in detail. This section is followed by protocols used for immunodetection by bright field microscopy. The use of enzyme-conjugated antibodies and colloidal gold to localize antigens in a variety of preparations is considered at length. The following section concentrates on the preparation and staining of cells for flow cytometry using a fluorescence-activated cell sorter (FACS). This section is followed by protocols detailing the preparation and use of colloidal gold for immunostaining samples for transmission electron microscopy. The final section of the book focuses on the clinical laboratory, where regulations and troubleshooting guidelines are discussed. Many of the special applications discussed here are normally limited to applications within a specific area, and not given within the context of a broader work devoted to immunocytochemical methods and protocols. By bringing these methods together in a single volume, it enables both researchers and clinicians to be well informed about their options when considering an immunohistochemical approach.

We are deeply indebted to the authors of the various chapters in the first and second editions as well as to the additional authors who contributed to this edition, for their interest in this work. We appreciate all of the authors' hard work, dedication, and willingness to share their expertise. The authors are experts in their respective areas and routinely use these protocols in their own laboratories. With the "Notes" that the authors have provided, they share the details of each protocol that make the method work successfully in any laboratory. We would also like to thank Dr. John Walker, *Methods in Molecular Biology* series editor, for his help and encouragement throughout the process of compiling this third edition. Final thanks go to David Casey and the staff of Humana Press for making this book a reality.

Constance Oliver
Maria Célia Jamur

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