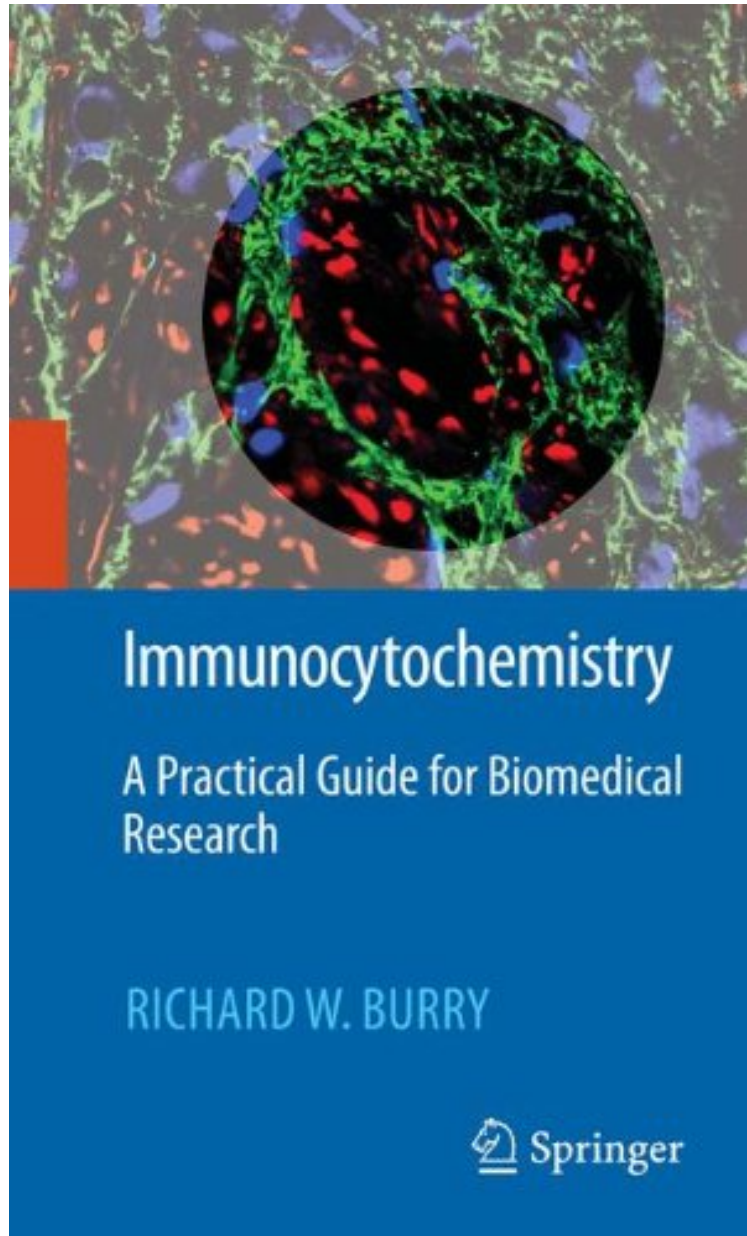


(Ebook pdf) Immunocytochemistry: A Practical Guide for Biomedical Research

Immunocytochemistry: A Practical Guide for Biomedical Research

Richard W. Burry

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Description: In biomedical research, because of a dramatic increase in productivity, immunocytochemistry has emerged as a major technique. The proposed book will provide the first practical guide to planning, performing, and evaluating immunocytochemical experiments. In todays graduate education the emphasis is on doing research and not on formal class work. Graduate students therefore lack the background in many essential techniques necessary to perform research in fields in which they were not trained. As director of a university core microscopy facility which sees students and faculty from dozens of laboratories each year, Dr. Burry has surmised the vast majority of these novice microscope users need considerable help. In an attempt to educate users, Dr. Burry has initiated immunocytochemistry seminars and workshops which serve to train people in this powerful research tool. The proposed book is an outgrowth of these presentations and conversations with, by now, hundreds of people who have asked for help. The philosophy which separates this book from other books in this field is that it is practical, rather than academic. In looking at other important immunocytochemistry titles, the predominant orientation is academic, with the author attempting to comprehensively discuss the topic. For example, one book with sample preparation lists ten fixatives which can be used; however, only two such fixatives are commonly used today. In this particular title, the detailed discussion of old methods might be seen as important in establishing the author as an expert. By contrast, the approach for Burrys book would be to discuss methods based on what works in animal research laboratories today, and focus only on the most productive methods. An additional distinction with this proposed book is the focus on animal research and not human pathology. There is a certification program for pathology technicians which requires them to learn a set body of material based on processing human tissue for examination by a pathologist. Many of the books on immunocytochemistry aim at this large pathology user base. Due to historical reasons, pathology laboratories process human tissues in a specific way and embed the tissue in paraffin, as has been done for over a century. In the last ten years, the power of immunocytochemistry in clinical diagnosis has become clear and has accordingly been adapted to pathology. However, the extensive processing needed for paraffin sections is not needed if the tissues are from research animals. Processing for animal-based tissues takes about a third of the time and results in higher quality images. The focus of this book is on processing these animal research tissues for immunocytochemistry. Today, there are no technique books which are aimed at this user base. As a subject matter expert in the area of the proposed book, Dr. Burry will make recommendations and offer opinions. Because this field is new and is emerging, there are numerous advantages of specific methods over other, more generalized methods. The purpose of this book is to show a novice how to do immunocytochemistry without engaging in a discussion of possible advanced methods. For the advanced user, there are several good books which discuss the unusual methods, yet for the novice there are currently none. Main Author : Richard W. Burry, The Ohio State University (United States). The Outline of the Book : Each chapter supplies a set of important principals and steps necessary for good immunocytochemistry. The information is distilled down to include only the most important points and does not attempt to cover infrequently used procedures or reagents. At the end of most chapters is a section on trouble-shooting many of the common problems using the Sherlock Holmes method. Each chapter also includes specific protocols which can be used. The goal of each chapter is to present the reader with enough information to successfully design experiments and solve many of the problems one may encounter. Using immunocytochemical protocols without the understanding of their workings is not advised, as the user will need to evaluate his or her results to determine whether the results are reliable. Such evaluation is extremely important for users who need reliable images which will clearly answer important scientific questions. 1. Introduction Definitions (immunocytochemistry and immunohistochemistry) Scope: animal research and not human pathology, paraffin sections, epitope retrieval, or immunohistochemistry Focus: fluorescence and enzyme detection Why do immunocytochemistry? Immunocytochemistry "individual study" rather than "population study" Example of a two-label experiment What is included in these chapters? Overview of the theory Background with enough information to help solve common problems. Advantages and disadvantages of different options Opinions and suggestions 2. Fixation and Sectioning Chemistry of fixation Denaturing vs cross-linking fixatives Application of fixative Perfusion, drop-in, cultures, fresh-frozen Selection of sample section type Sectioning tissue Rapid freezing, cryostat, freezing microtome, vibratome Storage of tissue Protocols 3. Antibodies Introduction Isoforms, structure, reactivity Generation Polyclonal vs monoclonal Antibodies as reagents Antibody specificity and sources Storage and handling 4. Labels for antibodies Fluorescence, enzymes and particulates Fluorescence theory Fluorescent labels - four generations Enzymes theory Selecting enzymes vs. fluorescence Selecting a label- advantages and disadvantages Protocols 5. Methods of applying antibodies Direct method Indirect method Antibody amplification methods ABC TSA Protocols 6. Blocking and Permeability Theory of blocking Theory of detergents Protocols 7. Procedure- Single

primary antibody Planning steps Sample, fixation, sectioning Vehicle Antibody dilutions Controls Protocols 8. Multiple primary antibodies - primary antibodies of different species Procedure Controls Protocols 9. Multiple primary antibodies-primary antibodies of same species Block-between Zenon HRP-chromogen development High-titer incubations Controls Protocols 10. Microscopy Wide-field fluorescence microscope Confocal microscope Bright fieldenzyme chromogen Choice Problems 11. Images Size, intensity, and pixels Manipulationwhat is ethical? Manuscript Figures 11. Planning and Troubleshooting Scheme for discussion-making in planning experiments Case studies with Sherlock Holmes detective work 12. So you want to do electron microscopic ICC? Criteria in decision-making Summary of the two techniques

From the Back CoverImmunocytochemistry is a microscopic method that uses antibodies to localize proteins in tissue sections and cell cultures. Dr. Burrys book is the first practical guide to planning, performing, and evaluating immunocytochemical experiments designed for novice immunocytochemists. This books predominant orientation is academic, focusing only on the most productive methods, with a primary emphasis on animal-based research tissues. The clear descriptions of the methods are easily followed and the specific design charts show how to plan an experiment. Included are a series of experimental cases exploring actual mistakes that demonstrate the approaches presented. Immunocytochemistry is no longer considered a specialized technique - Immunocytochemistry: A Practical Guide for Biomedical Research showcases this mainstream morphological approach in an accessible and exciting manner.