



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 today's 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 Burry's 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 field?enzyme chromogen

Choice

Problems

11. Images

Size, intensity, and pixels

Manipulation?what is ethical?

Manuscript Figures

11. Planning and Troubleshooting

Scheme for decision-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

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Steven Cordell:

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