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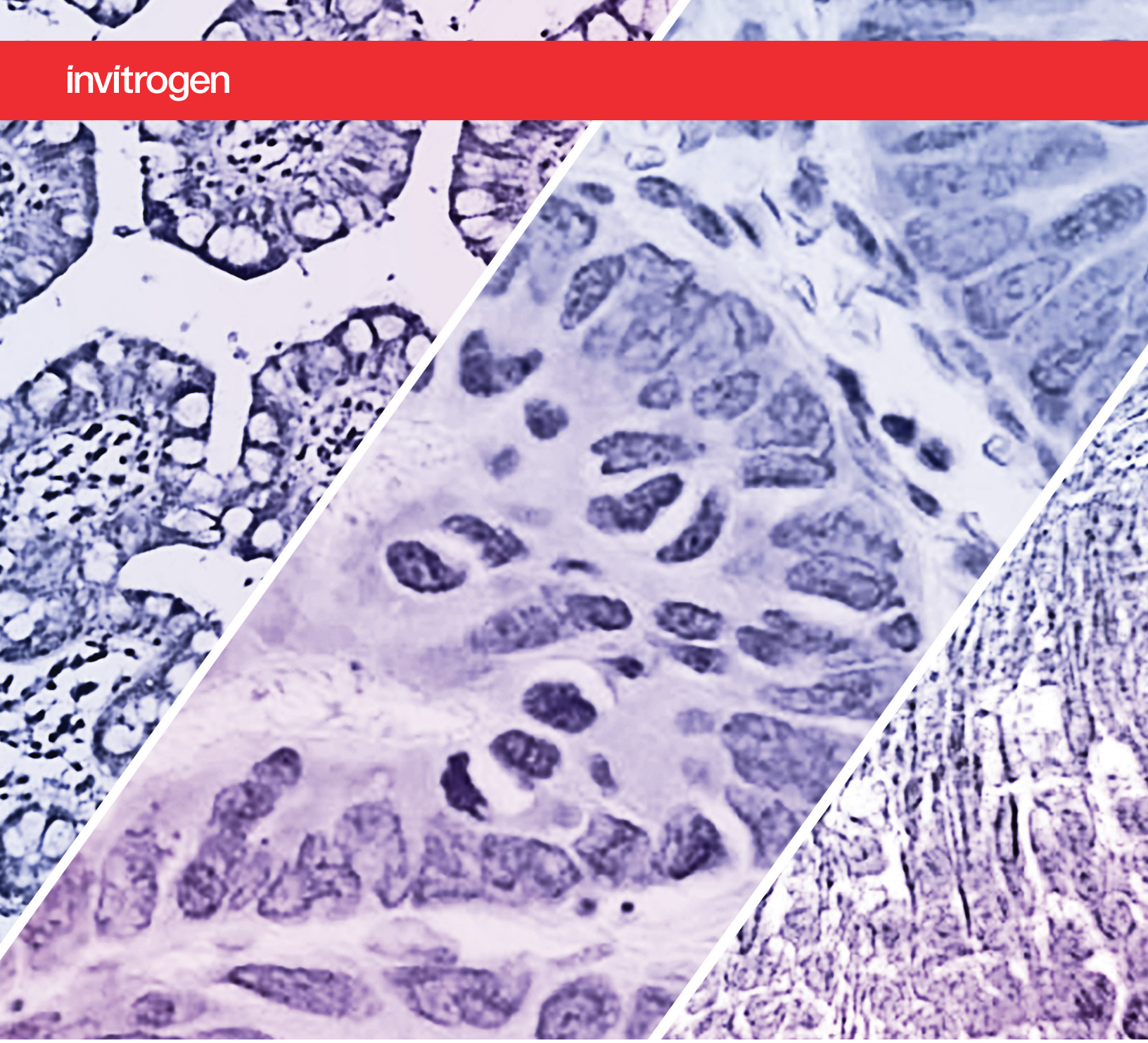

Immunohistochemistry: five steps to publication-quality images

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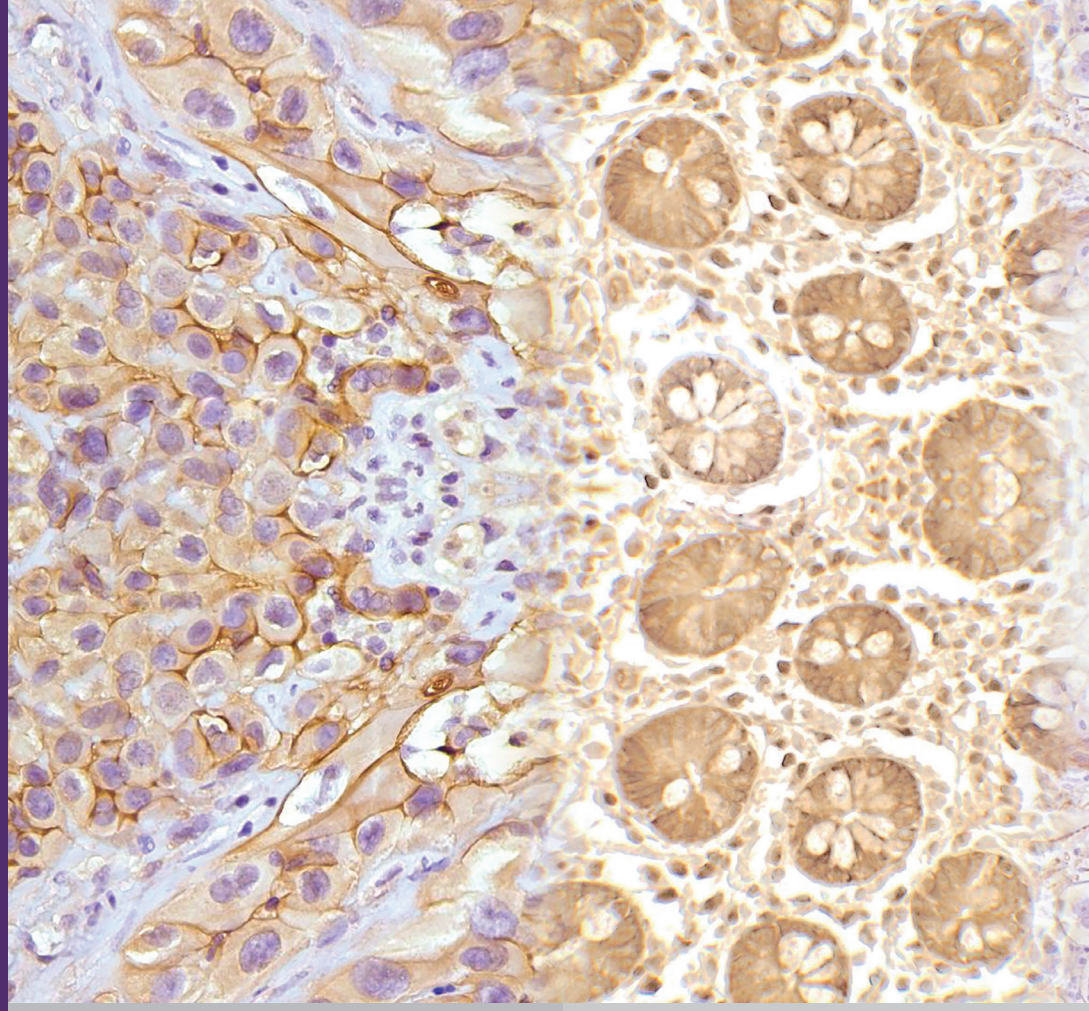
Introduction

The discovery and use of antibodies in life science research has been critical to many advancements across all disease areas. In antibody research, the development of tissue fixation to specifically identify and quantitate proteins is an additional step forward that supports clinical pathology. Immunohistochemistry (IHC) is a critical technique to complement other methods that are used to define normal and disease processes in cells, and help in the discovery of potential ways to treat and resolve conditions.

We support discovery research with a variety of tools developed, manufactured, and produced to a high standard of quality. We develop and verify procedures that can help scientists achieve results and obtain answers to their specific questions.

While targeting the antigen with the right antibody and amplification of the resulting signal are crucial for optimal visualization, all the steps in the IHC workflow are critical in order to obtain high-quality, publication-ready images.

Consider these five steps for superior IHC images:



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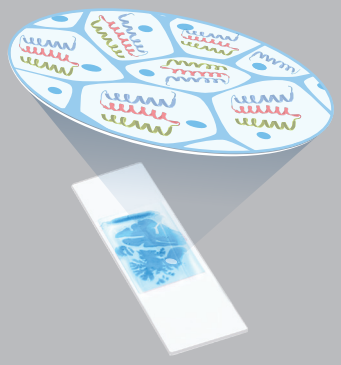
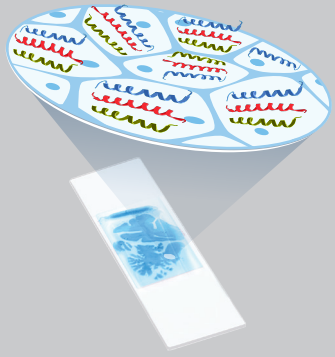
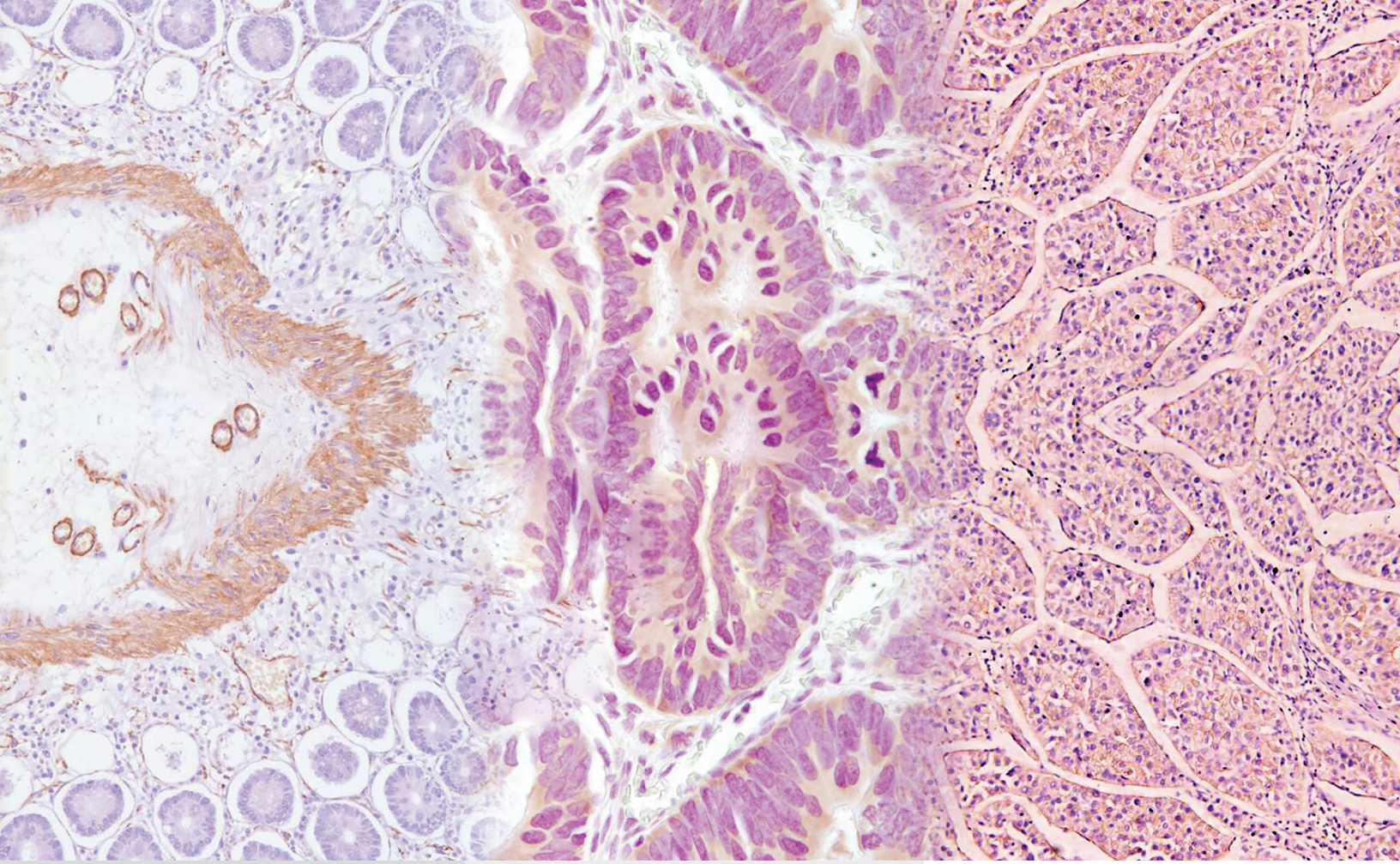


Prepare the sample—
minimize nonspecific signals

2



Retrieve the antigen—
unmask the epitope



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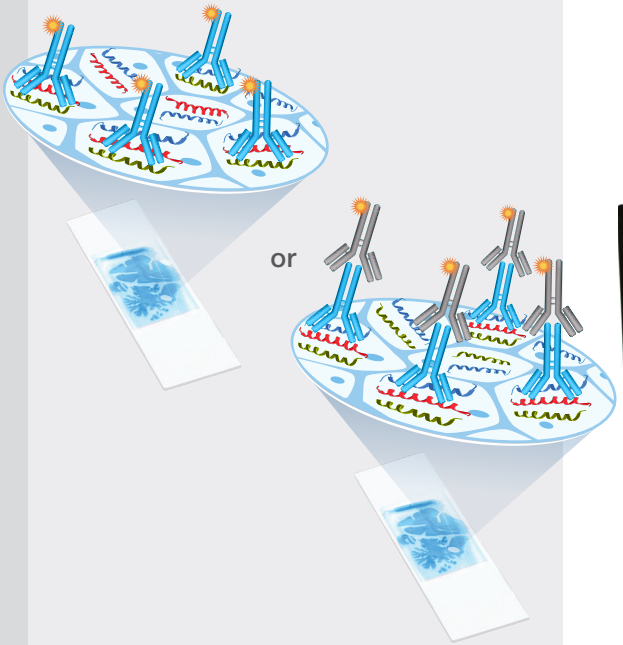
Block the background—
minimize nonspecific signals

4



Detect the target—
detect the target antigen
with an antibody

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Visualize the sample—
capture tissue images on
a microscope

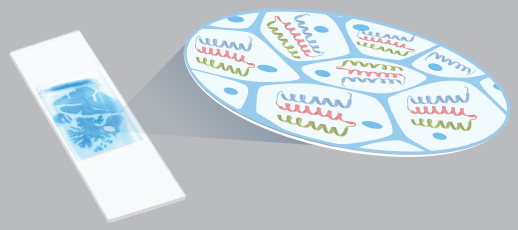
1 Step 1: Prepare the sample

This step determines the tissue structure and the techniques to detect antigens by antibodies. Choose the right fixation method based on the type of tissue and the experimental requirements. Frozen, acetone-fixed tissue is the best choice when expression of the antigen is high. On the other hand, formalin-fixed, paraffin-embedded (FFPE) samples give the best results when cell morphology is to be preserved. Whether you are fixing tissue through freezing or paraffin-embedding, we have a reagent solution and a variety of slide choices to help you prepare your sample for optimal protein visualization by IHC.

To learn more, go to **thermofisher.com/ihc5steps**

Product highlights

- Thermo Scientific™ Spin Tissue Processor Microm STP 120
- Thermo Scientific™ Richard-Allan Scientific™ Signature Series™ Pen-Fix™ Fixative
- Thermo Scientific™ Superfrost™ Excell™ Microscope Slides
- Thermo Scientific™ Polysine™ Microscope Adhesion Slides
- Invitrogen™ Image-iT™ Fixation/Permeabilization Kit



Prepare the frozen or paraffin-embedded slides for detection and visualization.

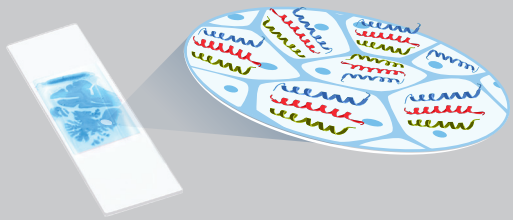
2 Step 2: Retrieve the antigen

Antigen unmasking (or retrieval) is a necessary step for FFPE samples prior to antibody labeling, as the tissue-fixation process typically causes crosslinking of proteins. This step re-exposes the epitopes on the antigen and allows antibody binding. This step is not necessary for frozen or fresh tissue. Antigen masking in formalin fixation of cells is due to the nature of the chemistry but can easily be reversed with heat (most commonly), simple buffer treatment, or protease digestion. The choice of treatment will depend on the tissue preparation technique and the antibody used for detection.

To learn more, go to **thermofisher.com/ihc5steps**

Product highlights

- Thermo Scientific™ Lab Vision™ PT Module™ Deparaffinization and Heat-Induced Epitope Retrieval Solutions (100X)
- Thermo Scientific™ Lab Vision™ Protease XXV for Enzyme-Induced Epitope Retrieval
- Thermo Scientific™ Lab Vision™ Pepsin Solution for Enzyme-Induced Epitope Retrieval
- Invitrogen™ eBioscience™ IHC Antigen Retrieval Solution – High pH (10X)
- Invitrogen™ eBioscience™ IHC Antigen Retrieval Solution – Low pH (10X)



It is essential to expose the antigen epitopes to enable the antibodies to bind.

3 Step 3: Block the background

The presence in tissue of endogenous enzymes such as peroxidase and alkaline phosphatase and endogenous antibodies can result in false-positive staining. Perform this step to minimize such background staining that can mask the detection of the target antigen. Furthermore, nonspecific binding of secondary antibodies can also be reduced by adding a blocking step.

Samples are incubated with a buffer that blocks the nonspecific sites to which the primary or secondary antibodies may otherwise bind.

To learn more, go to **thermofisher.com/ihc5steps**

Product highlights

- Thermo Scientific™ Blocker™ FL Fluorescent Blocking Buffer
- Invitrogen™ eBioscience™ IHC/ICC Blocking Buffer – Low Protein
- Invitrogen™ eBioscience™ IHC/ICC Blocking Buffer – High Protein
- Invitrogen™ Avidin/Biotin Blocking Kit
- Invitrogen™ CAS-Block™ Histochemical Reagent

Add blocking buffer to avoid nonspecific binding of primary and/or secondary antibodies.

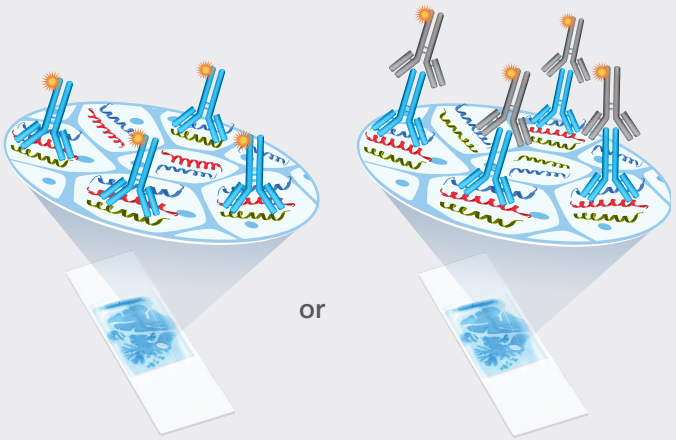
4 Step 4: Detect the target

Detection is the step of using specific antibodies to label the target proteins in the tissue. Direct detection uses conjugated primary antibodies, while indirect detection uses primary antibodies followed by labeled secondary antibodies to amplify the signal. The choice of direct detection is largely dependent on the abundance of target protein in the tissue slide and whether the primary antibody is available in the desired conjugated format. Invitrogen™ primary and secondary antibodies are available unconjugated as well as in multiple conjugated configurations for both colorimetric and fluorescent IHC. Additionally, the variety of antibody labels allows for multiplex IHC.

Explore our portfolio of primary and secondary antibodies at **thermofisher.com/antibodies**

Product highlights

- Invitrogen primary and secondary antibodies for IHC



Use direct or indirect detection, depending on target abundance and availability of the labeled primary antibody.

Performance guaranteed

Terms and conditions apply. For complete details, go to **thermofisher.com/antibody-performance-guarantee**

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Step 5: Visualize the sample

Prior to visualization, seal the sample by mounting a coverslip with an appropriate mountant solution (organic or aqueous).

For both colorimetric and fluorescent IHC, a specialized platform is helpful in capturing high-quality images. Designed to eliminate the complexities of microscopy without compromising on performance, the Invitrogen™ EVOS™ line of imaging systems is now accessible to almost every lab and budget. The EVOS FL Auto 2 Imaging System can image quickly and stitch together multiple images to create a large, high-resolution image of the entire sample.

For simple visualization of chromogenic stains, we recommend either the EVOS XL Core or EVOS XL Imaging System.

For simple visualization of fluorescent stains, we recommend the EVOS FL Imaging System.

For visualization and image analysis of both chromogenic and fluorescent stains, we recommend the EVOS FL Auto 2 Imaging System.

Product highlights

- EVOS FL Auto 2 Imaging System
- EVOS XL Core Imaging System
- EVOS XL Imaging System

Explore the EVOS lineup at **thermofisher.com/evos**

Visualize your sample using the appropriate platform.

Whether you're new to IHC or an experienced researcher wanting to confirm your knowledge, consider these five proven steps to help ensure that your images are publication ready.

For additional information, including troubleshooting tips for every step, go to **thermofisher.com/ihc5steps**

Find out more at **thermofisher.com/ihc5steps**

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