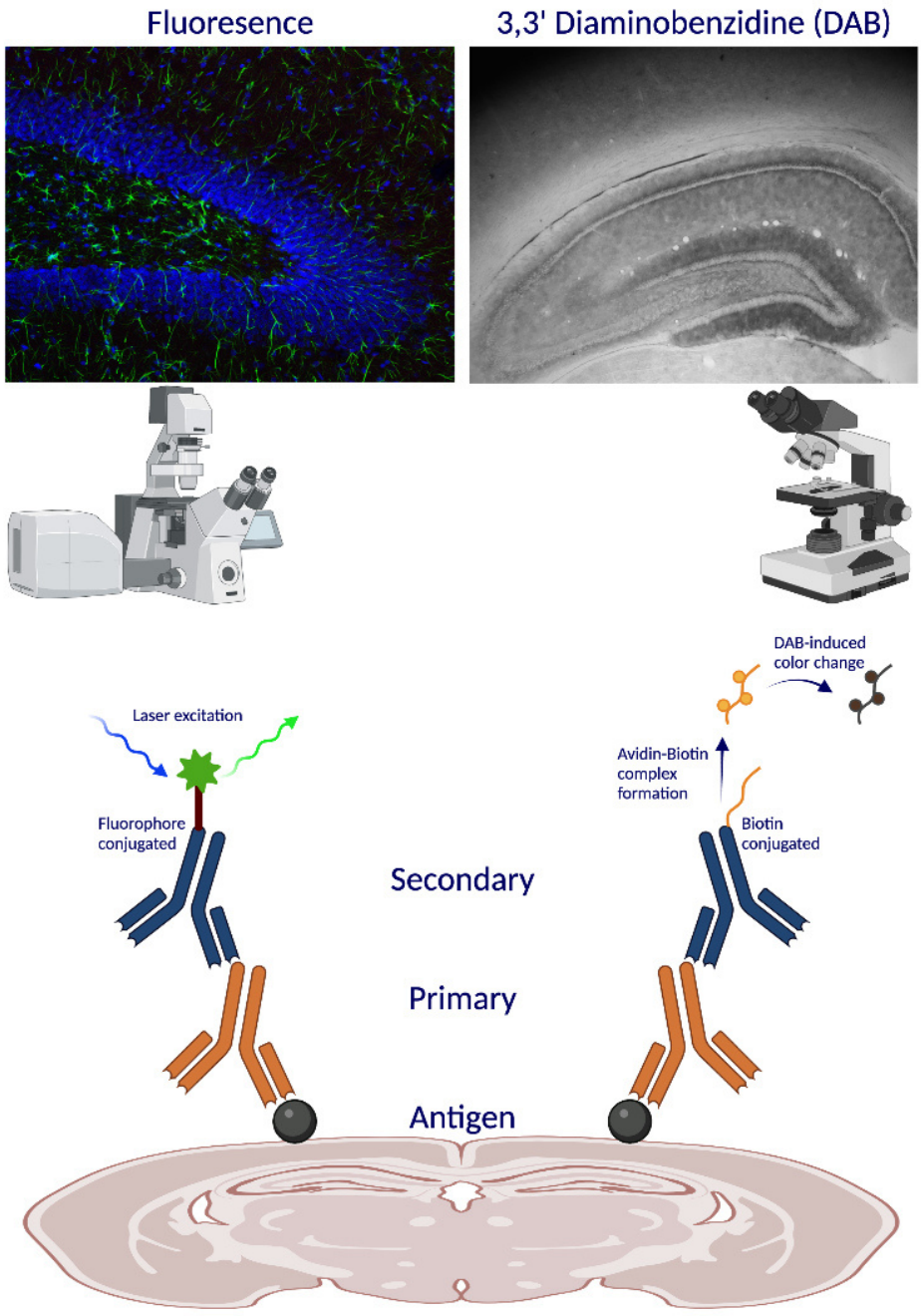
# Guide to Immunohistochemistry (IHC)

## What is Immunohistochemistry?

IHC is a popular technique used to determine the existence and abundance of target molecules in tissue. A protein or other antigen of interest can be visualized via microscopy by the binding of a series of antibodies. In some cases, multiple proteins can be probed simultaneously to assess their colocalization.




## Antigen Retrieval

Fixing brain slices with paraformaldehyde can cause cross-linking that obscures binding sites for your protein of interest. Heating slices in a high pH citrate buffer releases these bonds.

## Blocking

No antibody binds perfectly. Blocking with serum helps to prevent non-specific binding and lowers background signal. *Make sure to match your serum host to the secondary host*

## Primary Antibody

Specifically binds to your protein of interest. Often there can be multiple antibodies for the same protein, so make sure to pick the one that can be used for IHC.

## Secondary Antibody

A more general antibody that binds to any primary sharing the same host. It is attached to a reporter molecule that aids in visualization of the antigen. We have two main types:

- **Fluorescent tagged:** light sensitive and will fluoresce at a specific wavelength during imaging.
- **Biotinylated:** conjugated to biotin, which has many binding sites and is used to amplify signal when imaging. Further steps are needed to visualize, often a fluorescent tertiary or DAB staining

## DAB Staining

After application of a biotinylated secondary, the slices undergo a further incubation to form a complex between biotin and an avidin detection enzyme. When the chemical substrate DAB binds to our detection enzyme, it causes a color change, darkening where the antigen is detected. This non-light sensitive method allows for images to be taken via light microscopy.



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