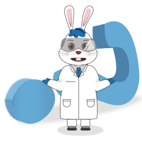


Elabscience®

Immunohistochemistry Troubleshooting Tips

Immunohistochemistry (IHC) is a technology applying the antigen-antibody specific binding principle to determine the position, qualitative and quantitative properties of intracellular antigen (polypeptide and protein) through the colorimetric chemical reaction of the labelled antibody color reagent (fluorescein, enzyme, metal ion, isotope). When the IHC staining fails, the causes should be systematically analyzed, and only one possible factor can be excluded at each time.

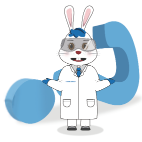
Here Elabscience lists the common Immunohistochemistry troubleshooting.

1. No staining of sample

Possible causes	Suggestions
Some reagent or procedure has been ignored, such as primary antibody, secondary antibody, substrate, addition order, incubation time, etc.	Record the experiment procedures to ensure that no operation or reagent is forgotten
The detection system and the secondary antibody are not compatible	Ensure that the detection system and the secondary antibody are compatible
The primary antibody and the secondary antibody are not compatible	Use a secondary antibody that was raised against the species in which the primary antibody was raised (e.g. primary is raised in rabbit, use anti-rabbit secondary). Be sure that the isotypes of the primary and secondary are compatible (e.g. IgM vs IgG)
The target protein is not present or low expressed in the tissue/ The antibody concentration may be too low	Choose another positive section to detect/ Increase the antibody concentration
The primary/ secondary antibody was not stored properly	Replace the antibody
The substrate is invalid	Replace the substrate
Improper pH of buffers	Ensure that the pH of buffers applied are in accordance with the experiment requirements

2. Weakly positive (In addition to the same reasons mentioned above, there are also the following situations)

Possible causes	Suggestions
Inappropriate antigen retrieval	The Paraffin-sections must be treated with heat-induced method (heat-induced epitope retrieval; HIER) or enzymatic digestion or both the two methods at the same time to make the antigen epitope exposed
Liquid on the section was not cleaned out, resulting in artificially dilution of antibody	Ensure that there is no liquid on the section before adding of antibody
The section was not placed horizontally, resulting in loss of antibody	Ensure that the section is horizontally placed during incubation
Improper fixation method	Choose a proper fixation method, ensure the quantity and quality of antigen



3. Non-specific staining

Possible causes	Suggestions
Insufficient deparaffinization of Paraffin-sections	Prolong the deparaffinization time
There is endogenous enzymes or biotin	Remove the endogenous enzymes and biotin effectively
Wrong blocking or insufficient blocking	Use proper blocking buffer or prolong the blocking time
The antibody specificity is low	Use antibody with better specificity
Insufficient washing	Operate washing in accordance with the experiment process strictly
The primary/ secondary antibody concentration may be too high	Use a lower concentration of antibody
DAB incubation time may be too long	Shorten the DAB incubation time
The sections/cells have dried out	Keep sections/cells at high humidity and do not let them dry out
There is cross-reactivity between the secondary antibody and endogenous proteins	Avoid using the secondary antibody which has the same species as the sample
Antigen translocation	Refer to relevant literatures to make sure that whether the antigen translocation is caused by the specific treatment of sample

Summary: The method and operation of IHC are not so complicated, but it is not easy to produce high quality staining results. Only to know well the principle and purpose of each operation step of IHC, we can optimize and correct the wrong operations during the experiment.

Positive and negative controls are necessary for IHC. Section which express the target antigen is usually used as positive control. Negative control is generally set by using PBS or non-primary antibody to replace the primary antibody, the other steps are the same. Positive control is used to eliminate the mistakes of experiment method and system, and negative control is used to eliminate the non-specific staining except primary antibody.