

IHC Troubleshooting Guide

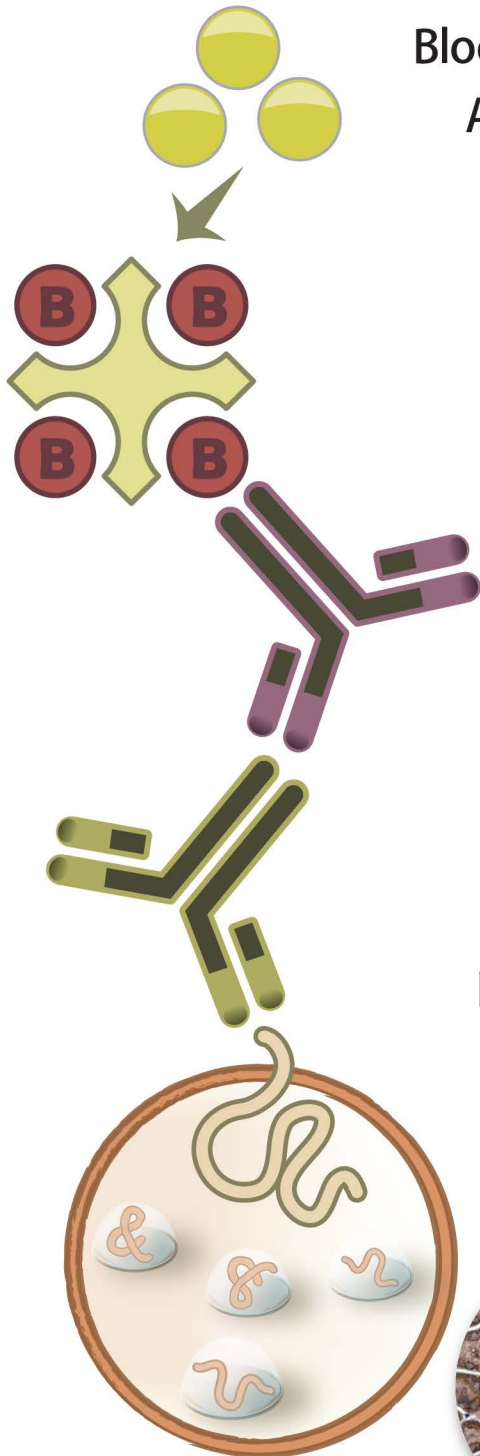
Your Expertise

Our Antibodies

Accelerated Discovery

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4 Steps to solve IHC high background staining



Blocking serum

ABC reagent

Substrate

Secondary Antibody

Blocking serum

ABC reagent

Substrate

Primary Antibody

Secondary Antibody

Blocking serum

ABC reagent

Substrate

Sample preparation

4

- Wrong species of blocking serum is used?
- Tissue binds to ABC reagent directly?
- The substrate reacts with endogenous enzyme directly?

3

- Non-specific binding of secondary antibody?
- Secondary antibody cross-reactivity?

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- Excessive amount of primary antibody is added?
- Non-specific binding of primary antibody?
- Primary antibody precipitates in the solution?

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- Sections dried out?
- Poor fixation?



See How to Perfect Your Results

Poor Morphology

Problem	Possible Solution(s)
<ul style="list-style-type: none"> ○ Tissue section falling off slide 	<ul style="list-style-type: none"> ○ Use freshly prepared, adequately charged slides
<ul style="list-style-type: none"> ○ Tissue section appears torn or folded; Air bubbles under section 	<ul style="list-style-type: none"> ○ Re-cut sections using a sharp blade ○ Avoid damaged areas while analyzing the results
<ul style="list-style-type: none"> ○ Poor resolution of tissue morphology 	<ul style="list-style-type: none"> ○ Prepare thinner sections
<ul style="list-style-type: none"> ○ Underfixation has physically damaged the tissue during the staining process 	<ul style="list-style-type: none"> ○ Increase fixation time and/or add a post-fixation step ○ Increase the fixative/tissue ratio ○ Prepare smaller tissue blocks for more thorough immersion fixation
<ul style="list-style-type: none"> ○ Antigen retrieval methods may be too harsh 	<ul style="list-style-type: none"> ○ Empirically determine the conditions that preserve tissue morphology while restoring the immunoreactivity of the antigen

Antigen Retrieval

Recombinant
Citation Support
Comparable Abs
Orthogonal Validation
Protein Overexpression

Heat-Induced Epitope Retrieval (HIER)

Product name	Application	Package	Cat. No.
Antigen retrieval solution	IHC-P	100 ml	GTX30709
Citrate buffer, 10X pH 6.0	IHC-P	100 ml	GTX30936
EDTA buffer, 10X pH 8.0	IHC-P	100 ml	GTX30937

Protease-Induced Epitope Retrieval (PIER)

Product name	Application	Package	Cat. No.
+ Pepsin (ready to use)	IHC-P	50 ml	GTX28194
+ Pronase reagent (ready to use)	IHC	100 ml	GTX73181
+ Trypsin Antigen Retrieval Kit	IHC-P	1 kit	GTX30934

Preventing Non-Specific Staining

Blocking Reagent

Product name	Application	Package	Cat. No.
+ Trident Universal Protein Blocking Reagent (animal serum free)	WB, ICC/IF, IHC-Fr, ELISA, IHC	100 ml	GTX30963

Endogenous Enzyme Interference

Product name	Application	Package	Cat. No.
Avidin/Biotin blocking kit	WB, ICC/IF, IHC-P, IHC-Fr	1 kit	GTX30966
+ Endogenous peroxidase blocking kit	ICC/IF, IHC	15 ml	GTX30967
Endogenous Alkaline phosphatase blocking kit	IHC	15 ml	GTX30968
+ Streptavidin/Biotin blocking kit	WB, ICC/IF, IHC	1 kit	GTX30965

Mouse on Mouse

Product name	Application	Package	Cat. No.
Mouse on Mouse Polymer HRP Detection System (ready to use)	IHC	1 kit	GTX83396

Anti-Fluorescent Protein Antibodies

Product name	Clonality	Application	Package	Cat. No.
+ GFP antibody	Rb pAb	WB, ICC/IF, IHC-P, IHC-Fr, IHC-Wm, IP, Dot, ELISA, IHC	25 ul, 100 ul	GTX113617
+ GFP antibody [GT859]	Ms mAb	WB, ICC/IF, IHC-P, IHC-Fr, IHC-Wm, IP, ELISA, Sandwich ELISA	25 ul, 100 ul	GTX628528
+ GFP antibody [HL1845]	Rec Rb mAb	WB, ICC/IF, IHC-P (cell pellet)	25 ul, 100 ul	GTX637570
+ mCherry antibody	Rb pAb	WB, ICC/IF, IHC-Fr, IHC-Wm, IP, PLA	25 ul, 100 ul	GTX128508
+ mCherry antibody [GT857]	Ms mAb	WB, ICC/IF, IHC-Wm, IHC	25 ul, 100 ul	GTX630189
+ mCherry antibody [HL1482]	Rec Rb mAb	WB, ICC/IF, IHC-P (cell pellet)	25 ul, 100 ul	GTX636956
+ RFP antibody	Rb pAb	WB, ICC/IF, IP	25 ul, 100 ul	GTX127897
+ RFP antibody [GT1610]	Ms mAb	WB, ICC/IF, IP	25 ul, 100 ul	GTX628545
+ RFP antibody [HL2028]	Rec Rb mAb	WB, ICC/IF	25 ul, 100 ul	GTX637929

PAP Pen (GTX22601)

- Keep staining buffers localized
- Allows use of less reagents
- Insoluble in alcohol and acetone



Non-specific staining / High background

Problem

Possible Solution(s)

Autofluorescence

- | | |
|--|---|
| <ul style="list-style-type: none"> ○ Fixation method may cause autofluorescence | <ul style="list-style-type: none"> ○ Try aldehyde versus non-aldehyde fixatives ○ Treat the tissue sample with dyes that quench selective autofluorescence ○ Switch sample embedding method from paraffin to frozen sections (OCT or gelatin) ○ Choose a fluorescence that will not compete with the autofluorescence |
|--|---|

Other

- | | |
|---|--|
| <ul style="list-style-type: none"> ○ Primary/secondary antibody issues:
- Concentration of the antibodies may be too high
- Non-specific binding of antibodies | <ul style="list-style-type: none"> ○ Decrease antibody concentration ○ Decrease antibody incubation time ○ Increase blocking buffer concentration ○ Conduct staining with Mouse-on-Mouse IHC staining kit (GTX83396) |
| <ul style="list-style-type: none"> ○ Incubation temperature may be too high | <ul style="list-style-type: none"> ○ Incubate sections at 4°C |
| <ul style="list-style-type: none"> ○ Endogenous enzymes (peroxidases or alkaline phosphatase activity) | <ul style="list-style-type: none"> ○ Use enzyme inhibitors:
- H₂O₂ (0.3% v/v) for peroxidase
- Levamisol (2 mM) for alkaline phosphatase |
| <ul style="list-style-type: none"> ○ Endogenous biotin | <ul style="list-style-type: none"> ○ Block endogenous biotin by incubation with Avidin |

Signal Amplification

Polymer HRP Detection System

Product name	Application	Package	Cat. No.
Signal+ for Immunostaining	ICC/IF, IHC	1 kit	GTX49998

DyLight Conjugated Secondary Antibodies

Product name	Clonality	Application	Package	Cat. No.
Goat Anti-Rabbit IgG antibody (DyLight488)	Gt pAb	WB, ICC/IF, IHC-P, IHC-Fr, IHC-Wm, FACS	500 ul	GTX213110-04
Goat Anti-Rabbit IgG antibody (DyLight594)	Gt pAb	WB, ICC/IF, IHC-P, IHC-Fr, FACS	500 ul	GTX213110-05
Goat Anti-Mouse IgG antibody (DyLight488)	Gt pAb	WB, ICC/IF, IHC-P, IHC-Fr, FACS	500 ul	GTX213111-04
Goat Anti-Mouse IgG antibody (DyLight594)	Gt pAb	WB, ICC/IF, IHC-P, IHC-Fr, FACS	500 ul	GTX213111-05

Labeled StreptAvidin Biotin (LSAB)

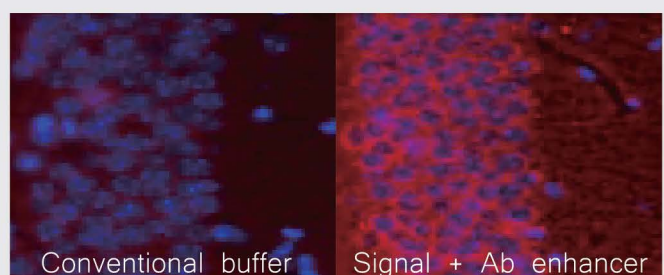
Product name	Application	Cat. No.
Streptavidin (AP)	WB, ELISA, IHC	GTX85909
Streptavidin (APC)	ICC/IF, FACS	GTX85908
Streptavidin (FITC)	ICC/IF, IHC-P, FACS	GTX85911
Streptavidin (HRP)	WB, ELISA, IHC	GTX85912
Streptavidin (PE)	ICC/IF, FACS	GTX85910
Streptavidin (TxRd)	ICC/IF, FACS	GTX85907

Counterstain

Product name	Application	Cat. No.
Aqueous Hematoxylin	IHC, IHC-P	GTX73341
Light Green counterstain	IHC	GTX73306
Methylene Blue counterstain	IHC	GTX73307
Nuclear Fast Red counterstain	IHC-P	GTX73305

Signal+ for Immunostaining (GTX49998)

- Enhances signals
- Reduces background
- Works with any systems
- Compatible with ABC or polymer complex
- Ready-to-use

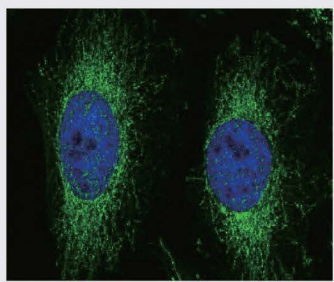


Weak/No signal

<i>Problem</i>	<i>Possible Solution(s)</i>
Antibody potency	
<ul style="list-style-type: none"> Primary and secondary antibody may not match 	<ul style="list-style-type: none"> Use secondary antibody generated against the species in which the primary was raised
<ul style="list-style-type: none"> Antibody used may not be suitable for IHC 	<ul style="list-style-type: none"> Test the antibody on a non-denatured and denatured western blot to make sure the antibody is recognizing non-denatured antigen
<ul style="list-style-type: none"> Not enough antibody added 	<ul style="list-style-type: none"> Increase antibody concentration ; lengthen incubation time
Enzyme-substrate reactivity	
<ul style="list-style-type: none"> Chromogenic solution lost effect <ul style="list-style-type: none"> - Enzyme inhibitor present in the substrate buffer - pH of the substrate buffer may not be appropriate 	<ul style="list-style-type: none"> Test the enzyme-substrate reaction by placing a drop of the enzyme into the prepared substrate solution
Sample preparation	
<ul style="list-style-type: none"> Insufficient de-paraffinization 	<ul style="list-style-type: none"> De-paraffinize sections longer; Use freshly prepared xylene
<ul style="list-style-type: none"> Fixatives (formalin & paraformaldehyde) may be modifying the epitope 	<ul style="list-style-type: none"> Use antigen retrieval methods to unmask the epitope Decrease fixation duration
<ul style="list-style-type: none"> Inadequate fixation 	<ul style="list-style-type: none"> Avoid delay of fixation or over-fixation
Sample property	
<ul style="list-style-type: none"> Target protein may not abundantly present in the tissue 	<ul style="list-style-type: none"> Run a positive control Use more primary antibody Use amplification steps to amplify the signal
<ul style="list-style-type: none"> Target protein is a nuclear protein and the antibody cannot penetrate the nucleus 	<ul style="list-style-type: none"> Add permeabilizing reagent into the blocking buffer and antibody dilution buffer to facilitate antibody penetration into the nucleus
<ul style="list-style-type: none"> Target protein is a membrane protein and epitope may be damaged or removed by permeabilization 	<ul style="list-style-type: none"> Decrease or remove permeabilizing reagent from buffers

Mounting

Product name	Application	Cat. No.
FluoroGel mounting medium	ICC/IF, IHC, IHC-Fr, IHC-P	GTX28214
Fluoroshield™ with DAPI	ICC/IF, IHC, IHC-Fr	GTX30920
Fluoroshield™ with PI	ICC/IF, IHC	GTX30921



Fluoroshield™ Mounting Medium with DAPI (GTX30920)

- Fortified with DAPI for DNA & RNA staining
- Preserve fluorescence during prolonged storage
- Prevent photobleaching
- Compatible with various fluorophore

