

IHC Troubleshooting Guide

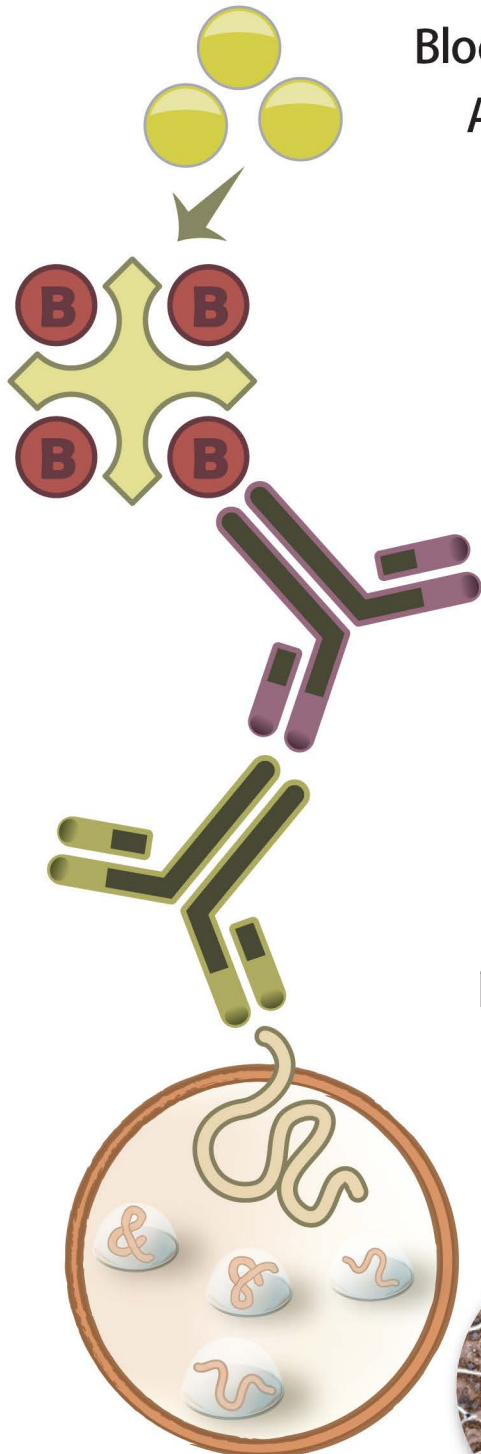
Your Expertise

Our Antibodies

Accelerated Discovery

www.genetex.com

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

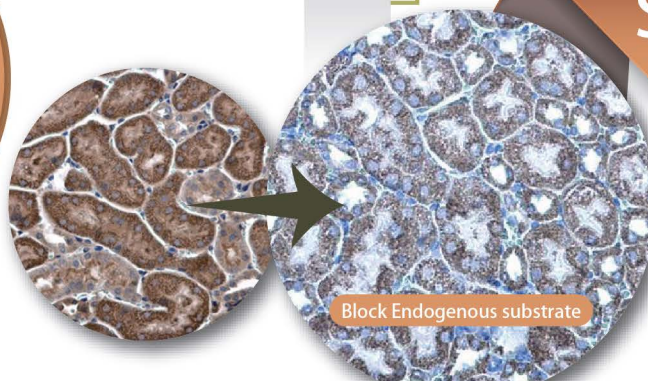
- ☐ Non-specific binding of secondary antibody?
- ☐ Secondary antibody cross-reactivity?

2

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

1

- ☐ Sections dried out?
- ☐ Poor fixation?



**See
How to
Perfect
Your
Results**

Poor Morphology

Problem	Possible Solution(s)
○ Tissue section falling off slide ○ Tissue section appears torn or folded; Air bubbles under section ○ Poor resolution of tissue morphology ○ Underfixation has physically damaged the tissue during the staining process ○ Antigen retrieval methods may be too harsh	○ Use freshly prepared, adequately charged slides ○ Re-cut sections using a sharp blade ○ Avoid damaged areas while analyzing the results ○ Prepare thinner sections ○ Increase fixation time and/or add a post-fixation step ○ Increase the fixative/tissue ratio ○ Prepare smaller tissue blocks for more thorough immersion fixation ○ 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

- Fixation method may cause autofluorescence
 - 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

- Primary/secondary antibody issues:
 - Concentration of the antibodies may be too high
 - Non-specific binding of antibodies
- Incubation temperature may be too high
 - Decrease antibody concentration
 - Decrease antibody incubation time
 - Increase blocking buffer concentration
 - Conduct staining with Mouse-on-Mouse IHC staining kit (GTX83396)
- Endogenous enzymes (peroxidases or alkaline phosphatase activity)
 - Incubate sections at 4°C
 - Use enzyme inhibitors:
 - H₂O₂ (0.3% v/v) for peroxidase
 - Levamisole (2 mM) for alkaline phosphatase
- Endogenous biotin
 - 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 µl	GTX213110-04
Goat Anti-Rabbit IgG antibody (DyLight594)	Gt pAb	WB, ICC/IF, IHC-P, IHC-Fr, FACS	500 µl	GTX213110-05
Goat Anti-Mouse IgG antibody (DyLight488)	Gt pAb	WB, ICC/IF, IHC-P, IHC-Fr, FACS	500 µl	GTX213111-04
Goat Anti-Mouse IgG antibody (DyLight594)	Gt pAb	WB, ICC/IF, IHC-P, IHC-Fr, FACS	500 µl	GTX213111-05

Labeled StreptAvidin Biotin (LSAB)

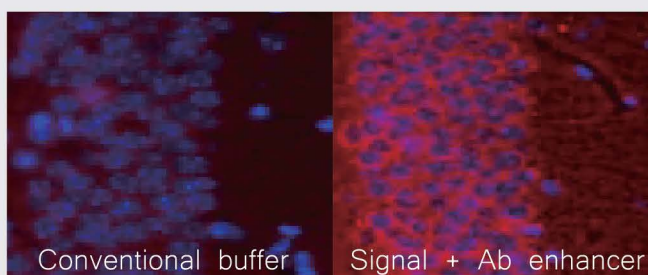
Product name	Application	Cat. No.
Streptavidin (AP)	WB, ELISA, IHC	GTX85909
Streptavidin (APC)	ICC/IF, FACS	GTX85908
Streptavidin (FITC)	ICC/IF, IHC-P, FACS	GTX85911
Streptavidin (HRP)	WB, ELISA, IHC	GTX85912
Streptavidin (PE)	ICC/IF, FACS	GTX85910
Streptavidin (TxRd)	ICC/IF, FACS	GTX85907

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

Problem

Possible Solution(s)

Antibody potency

- Primary and secondary antibody may not match
 - Use secondary antibody generated against the species in which the primary was raised
- Antibody used may not be suitable for IHC
 - Test the antibody on a non-denatured and denatured western blot to make sure the antibody is recognizing non-denatured antigen
- Not enough antibody added
 - Increase antibody concentration ; lengthen incubation time

Enzyme-substrate reactivity

- Chromogenic solution lost effect
 - Enzyme inhibitor present in the substrate buffer
 - pH of the substrate buffer may not be appropriate
 - Test the enzyme-substrate reaction by placing a drop of the enzyme into the prepared substrate solution

Sample preparation

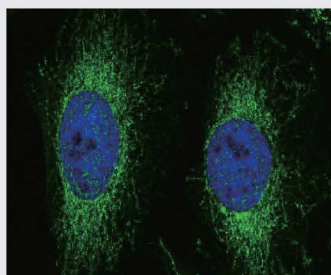
- Insufficient de-paraffinization
 - De-paraffinize sections longer; Use freshly prepared xylene
- Fixatives (formalin & paraformaldehyde) may be modifying the epitope
 - Use antigen retrieval methods to unmask the epitope
 - Decrease fixation duration
- Inadequate fixation
 - Avoid delay of fixation or over-fixation

Sample property

- Target protein may not abundantly present in the tissue
 - Run a positive control
 - Use more primary antibody
 - Use amplification steps to amplify the signal
- Target protein is a nuclear protein and the antibody cannot penetrate the nucleus
 - Add permeabilizing reagent into the blocking buffer and antibody dilution buffer to facilitate antibody penetration into the nucleus
- Target protein is a membrane protein and epitope may be damaged or removed by permeabilization
 - 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

