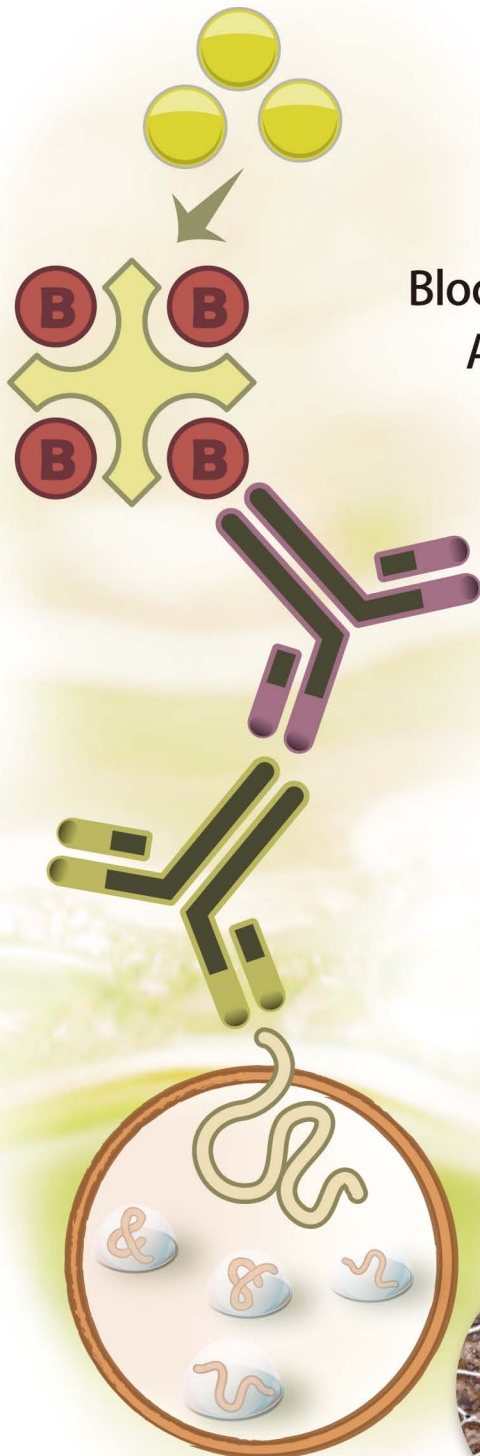




## IHC (paraffin) Troubleshooting

### 4 Steps to solve IHC high background staining



#### Substrate

# 1

- The substrate possibly reacts with endogenous enzyme.

No Staining

#### Blocking serum ABC reagent

Substrate

# 2

- Tissues are possibly bound to ABC reagent.
- Contaminants in certain grade of BSA probably cause background staining.

No Staining

#### Secondary Antibody

Blocking serum  
ABC reagent  
Substrate

# 3

- Nonspecific binding of secondary antibody.
- Secondary antibody may cross-react with endogenous immunoglobulins or other tissue proteins.
- Use the wrong species of blocking serum.

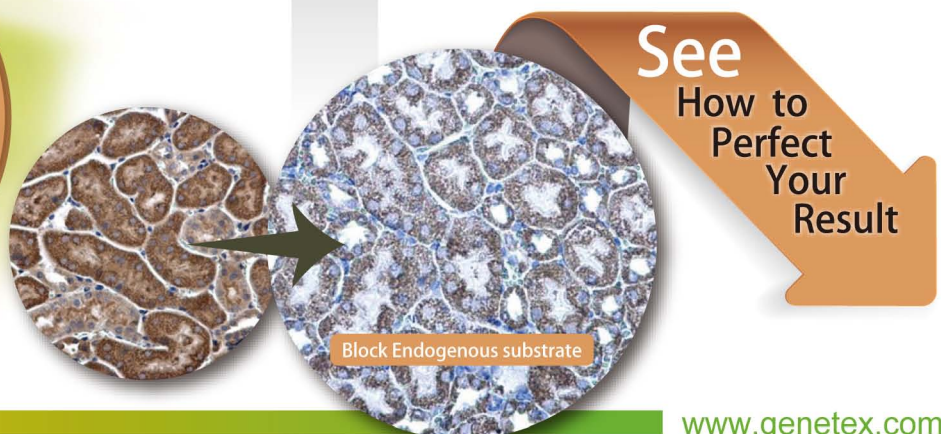
No Staining

#### Primary Antibody

Secondary Antibody  
Blocking serum  
ABC reagent  
Substrate

# 4

- Add excessive amount of primary antibody.
- Nonspecific binding of primary antibody.
- Denatured immunoglobulin might precipitates in the primary antibody if the section shows small, amorphous, punctate staining.



See  
How to  
Perfect  
Your  
Result

# Weak/No signal

## Problem

## Possible Solution(s)

- |   |  |
|---|--|
| ○ 1 <sup>st</sup> antibody and 2 <sup>nd</sup> antibody may not match.                        | ○ 2 <sup>nd</sup> antibody be against the species in which the 1 <sup>st</sup> was raised.   |
| ○ The antibody 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 primary antibody.  | ○ Increase concentration ; incubate for longer at 4°C ; lengthen incubation time.  |
| ○ Target protein is not abundantly present in the tissue.                                     | ○ Run a positive control. Utilizing an amplification step can assist in amplifying the signal. Use a lower dilution of primary antibody. |
| ○ Deparaffinization may be insufficient.  | ○ Deparaffinize sections longer. Use freshly prepared xylene.  |
| ○ Fixatives (formalin & paraformaldehyde) may be modifying the epitope.                       | ○ Use antigen retrieval method to unmask the epitope. Fix for less time.   |
| ○ Inadequate fixation.  | ○ Avoid delay of fixation or over-fixation.  |
| ○ The protein of interest is a nuclear protein and the antibody cannot penetrate the nucleus. | ○ To facilitate penetration of the nucleus, add a permeabilizing agent to the blocking buffer and the antibody dilution buffer.          |
| ○ Permeabilization has damaged the membrane and removed the membrane protein.                 | ○ Remove permeabilizing agent from your buffers.   |

# Signal Amplification

## Polymer HRP Detection System

Product name	Application	Cat. No.
OneStep Polymer HRP anti-mouse/rat Detection System (Ready-to-Use)	IHC, WB	GTX83400
OneStep Polymer HRP anti-rabbit Detection System (Ready-to-Use)	IHC, WB	GTX83399

## Biotin Blocker

Product name	Application	Cat. No.
Avidin/Biotin blocking kit	ICC/IF, IHC, WB	GTX30966
Streptavidin/Biotin blocking kit	ICC/IF, IHC, WB	GTX30965

## Labeled StreptAvidin Biotin (LSAB)

Product name	Application	Cat. No.
Streptavidin (HRP)	ELISA, IHC, WB	GTX85912
Streptavidin (AP)	ELISA, IHC, WB	GTX85909
Streptavidin (APC)	FACS, ICC/IF	GTX85908
Streptavidin (Cy3)	FACS, ICC/IF	GTX85902
Streptavidin (FITC)	FACS, ICC/IF	GTX85911
Streptavidin (Texas Red™)	FACS, ICC/IF	GTX85907
Streptavidin (PE)	FACS, ICC/IF	GTX85910

## PAP pen

Product name	Application	Cat. No.
PAP pen	IHC	GTX22601

## Counterstain

© Citation

Product name	Application	Cat. No.
© Aqueous Hematoxylin	IHC-P	GTX73341
© Nuclear Fast Red	IHC-P	GTX73309
© Light Green counterstain	IHC-P	GTX73306
Methylene Blue counterstain	IHC-P	GTX73307

## Mounting

© Citation

Product name	Application	Cat. No.
© Immuno Mount™	IHC	GTX30928
© FluoroGel mounting medium	H, ICC/IF, IHC, IHC-Fr, IHC-P	GTX28214
ImmunoHistoMount™	IHC	GTX30922
Clear Mount mounting medium-general purpose	IHC	GTX30703

# Poor Morphology

## Problem

## Possible Solution(s)

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

# Antigen Retrieval

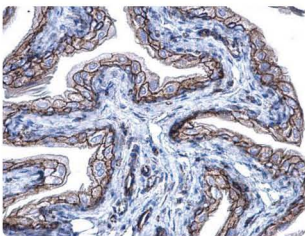
## Heat-Induced Epitope Retrieval (HIER)

Product name	Application	Cat. No.
Citrate buffer, 10X pH6.0	IHC-P	GTX30936
EDTA buffer, 10X pH 8.0	IHC-P	GTX30937
Antigen retrieval solution	IHC-P	GTX30709

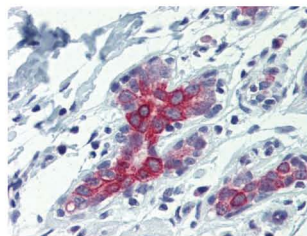
## Protease-Induced Epitope Retrieval (PIER)

© Citation

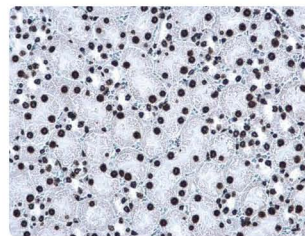
Product name	Application	Cat. No.
Trypsin Antigen Retrieval Kit	IHC-P	GTX30934
Pepsin Reagent (ready to use)	IHC-P	GTX30935
Ⓢ Pronase reagent	IHC-P	GTX73181
Ⓢ Antigen Retrieval Tablets	IHC	GTX28212



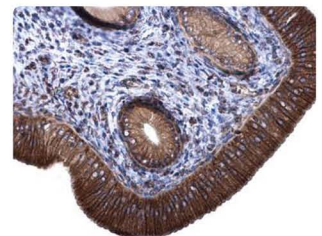
$\beta$ -Catenin antibody (GTX101435)



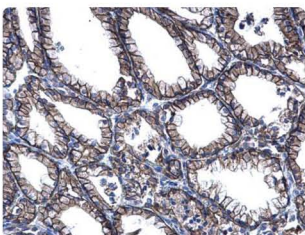
BAK antibody (GTX100063)



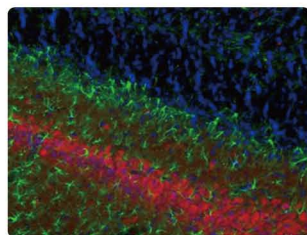
Histone H3 (k27me3) antibody (GTX129774)



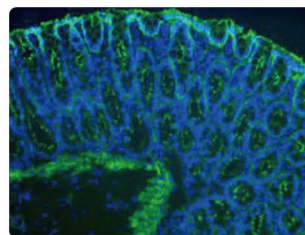
HPRT antibody (GTX113466)



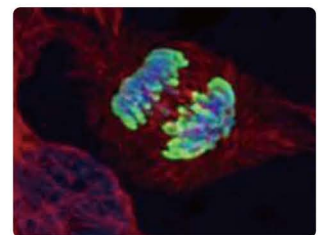
PRRG2 antibody (GTX114668)



GFAP antibody (GTX108711)



Claudin 1 antibody (GTX15098)



Histone H3S10ph antibody (GTX128116)

# Non-specific staining / High background

## Problem

## Possible Solution(s)

- |  |  |
|--|--|
| <ul style="list-style-type: none"> <li>Fixation procedures (using formalin or paraformaldehyde fixatives) are causing autofluorescence.</li> </ul> | <ul style="list-style-type: none"> <li>Formalin/PFA usually autofluorescence in the green spectrum, so try a fluorophore in the red range.</li> </ul>                                    |
| <ul style="list-style-type: none"> <li>The concentration of the 1<sup>st</sup> or 2<sup>nd</sup> antibody may be too high.</li> </ul>              | <ul style="list-style-type: none"> <li>Try decreasing the antibody concentration and/or the incubation period.</li> </ul>  |
| <ul style="list-style-type: none"> <li>Incubation temperature may be too high.</li> </ul>  | <ul style="list-style-type: none"> <li>Incubate sections or cells at 4°C.</li> </ul>   |
| <ul style="list-style-type: none"> <li>Endogenous Peroxidases or Alkaline Phosphatase Activity.</li> </ul>   | <ul style="list-style-type: none"> <li>Use enzyme inhibitors such as H<sub>2</sub>O<sub>2</sub> (0.3% v/v) for peroxidase or Levamisol (2 mM) for alkaline phosphatase.</li> </ul>       |
| <ul style="list-style-type: none"> <li>Endogenous Biotin.</li> </ul>   | <ul style="list-style-type: none"> <li>Incubation with avidin effectively blocks endogenous biotin.</li> </ul>   |
| <ul style="list-style-type: none"> <li>The primary antibody is raised against the same species as the tissue stained.</li> </ul>                   | <ul style="list-style-type: none"> <li>Use a primary antibody against a different species than your tissue. Conduct staining with Mouse-on-Mouse IHC staining kit (GTX83396).</li> </ul> |

## Preventing Non-Specific Staining

### Blocking Reagent

 Citation

Product name	Application	Cat. No.
<ul style="list-style-type: none"> <li>Universal Protein Blocking Reagent (animal serum free)</li> </ul>	ELISA, ICC/IF, IHC, IHC-P, WB	GTX30963
Blocking Solution with Bovine Serum (ready to use)	ELISA, ICC/IF, IHC, WB	GTX30970
Blocking Solution with Chicken Serum (ready to use)	ELISA, ICC/IF, IHC, WB	GTX30971
Blocking Solution with Donkey Serum (ready to use)	ELISA, ICC/IF, IHC, WB	GTX30972
<ul style="list-style-type: none"> <li>Blocking Solution with Goat Serum (ready to use)</li> </ul>	ELISA, ICC/IF, IHC, WB	GTX30973
Blocking Solution with Horse Serum (ready to use)	ELISA, ICC/IF, IHC, WB	GTX30974
Blocking Solution with Rabbit Serum (ready to use)	ELISA, ICC/IF, IHC, WB	GTX30975
Blocking Solution with Fish Serum (ready to use)	ICC/IF, IHC, WB	GTX85478

### Endogenous Enzyme Interference

 Citation

Product name	Application	Cat. No.
<ul style="list-style-type: none"> <li>Endogenous peroxidase blocking kit</li> </ul>	ICC/IF, IHC, IHC-P	GTX30967
Endogenous Alkaline phosphatase blocking kit	IHC	GTX30968

### Mouse-on-Mouse IHC Kit

Product name	Application	Cat. No.
Mouse on Mouse Polymer HRP Detection System (Ready-to-Use)	ICC/IF, IHC	GTX83396

Quality Antibodies • Quality Results

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