Quality Antibodies · Quality Results

GeneTex

IHC Troubleshooting Guide

Your Expertise
Our Antibodies
Accelerated Discovery

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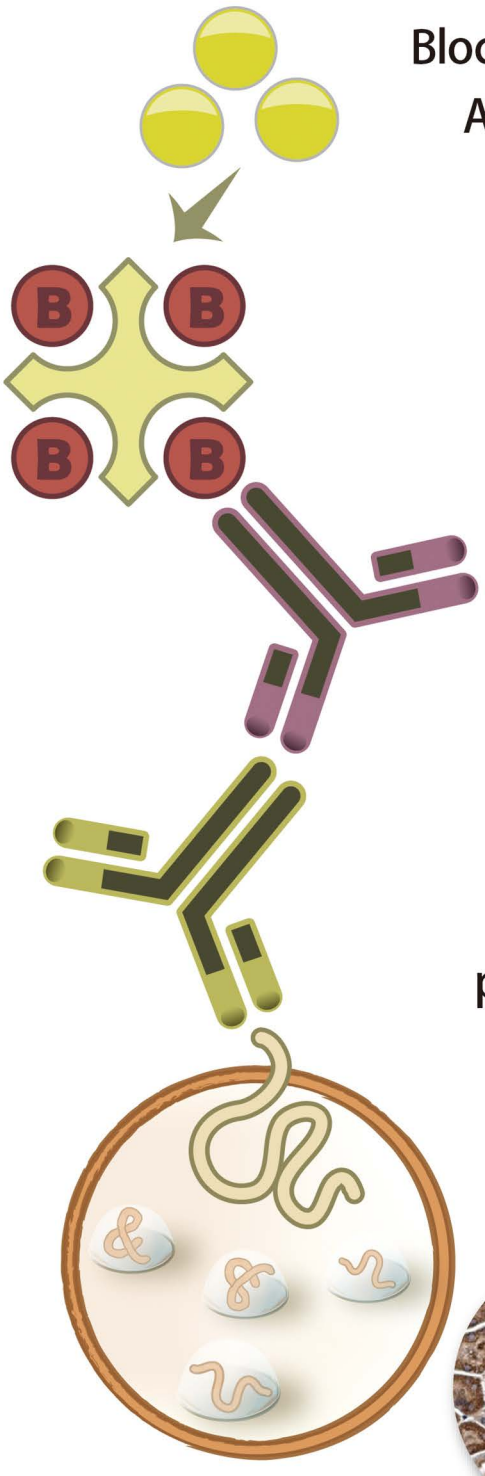
4 Steps to solve IHC high background staining



Blocking serum
ABC reagent
Substrate

1

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

Secondary Antibody
Blocking serum
ABC reagent
Substrate

2

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

Primary Antibody
Secondary Antibody
Blocking serum
ABC reagent
Substrate

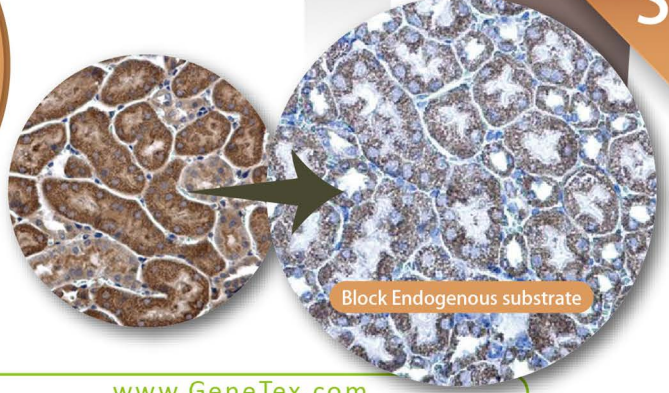
3

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

Sample preparation

4

- ☐ Sections dried out?
- ☐ Poor fixation?



See How to Perfect Your Results

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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-parafinization	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

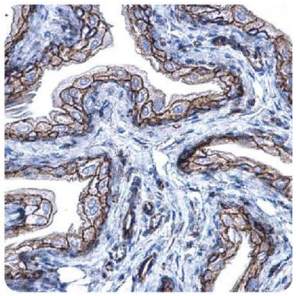
Antigen Retrieval

Heat-Induced Epitope Retrieval (HIER)

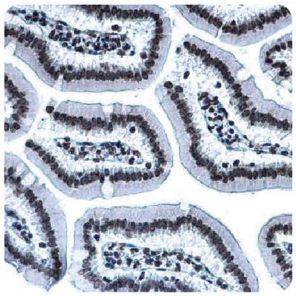
Product name	Application	Cat. No.
Citrate buffer, 10X pH 6.0	IHC-P	GTX30936
EDTA buffer, 10X pH 8.0	IHC-P	GTX30937
Antigen retrieval solution,10X pH 10.0	IHC-P	GTX30709

Protease-Induced Epitope Retrieval (PIER)

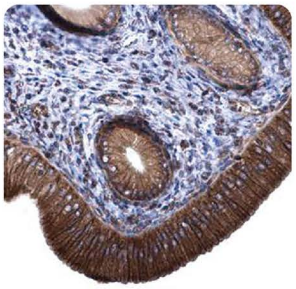
Product name	Application	Cat. No.
Trypsin Antigen Retrieval Kit - for cytosolic antigen	IHC-P	GTX30934
Pepsin Reagent (ready to use) - for ECM antigen	IHC-P	GTX30935
Pronase reagent	IHC-P	GTX73181



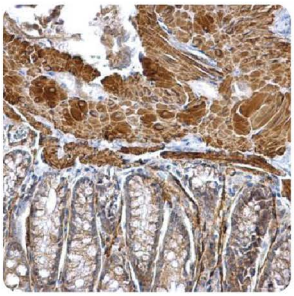
β-Catenin antibody (GTX101435)



Histone H3K27me3 (trimethyl Lys27) antibody (GTX121184)



HPRT antibody (GTX113466)



alpha Smooth Muscle Actin antibody (GTX100034)

Poor Morphology

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

Signal Amplification

Citation Support | KO/KD Validation | Comparable Abs | Orthogonal Validation | Protein Overexpression

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
OneStep Polymer HRP anti-mouse/rat/rabbit	IHC, IHC-P, WB	GTX83398

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)

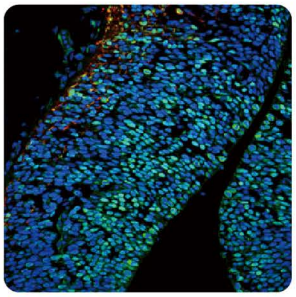
Product name	Application	Cat. No.
Streptavidin (HRP)	ELISA, IHC, WB	GTX85912
Streptavidin (AP)	ELISA, IHC, WB	GTX85909
Streptavidin (APC)	FACS, ICC/IF	GTX85908
Streptavidin (Cy3)	FACS, ICC/IF	GTX85902
Streptavidin (FITC)	FACS, ICC/IF	GTX85911
Streptavidin (Texas Red™)	FACS, ICC/IF	GTX85907
Streptavidin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

Counterstain

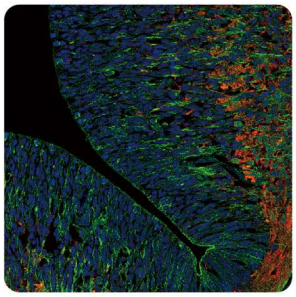
Product name	Application	Cat. No.
Aqueous Hematoxylin	IHC, IHC-P	GTX73341
Nuclear Fast Red	IHC	GTX73309
Light Green counterstain	IHC	GTX73306
Methylene Blue counterstain	IHC	GTX73307

Mounting

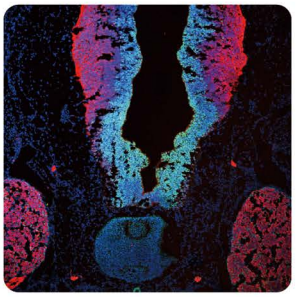
Product name	Application	Cat. No.
Clear Mount mounting medium-general purpose	H, IHC-Fr, IHC-P	GTX30703
FluoroGel mounting medium	ICC/IF, IHC, IHC-Fr, IHC-P	GTX28214
Fluoroshield™ with DAPI	ICC/IF, IHC, IHC-Fr	GTX30920
ImmunoHistoMount™	IHC	GTX30922
Immuno Mount™	IHC	GTX30928



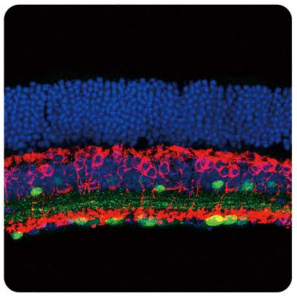
Brn2 antibody (GTX114650)



Caspr2 antibody [N2N3] (GTX109389)



SOX2 antibody [N1C3] (GTX101507)



Calretinin antibody (GTX103261)

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**	Decrease antibody concentration Decrease antibody incubation time Increase blocking buffer concentration Conduct staining with Mouse-on-Mouse IHC staining kit (GTX83396)
Incubation temperature may be too high	Incubate sections at 4°C
Endogenous enzymes (peroxidases or alkaline phosphatase activity)	Use enzyme inhibitors: - H2O2 (0.3% v/v) for peroxidase - Levamisol (2 mM) for alkaline phosphatase
Endogenous biotin	Block endogenous biotin by incubation with Avidin

Preventing Non-Specific Staining

Blocking Reagent

Citation Support

Product name	Application	Cat. No.
Universal Protein Blocking Reagent (animal serum free)	ELISA, ICC/IF, IHC, IHC-P, WB	GTX30963
Blocking Buffer, PBS with 1% BSA (10X)	ELISA, IHC, WB	GTX48881
Blocking Buffer, TBS with 1% BSA (10X)	ELISA, IHC, WB	GTX48882
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
Blocking Solution with Goat Serum (ready to use)	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

Product name	Application	Cat. No.
Endogenous peroxidase blocking kit	ICC/IF, IHC, IHC-P	GTX30967
Endogenous Alkaline phosphatase blocking kit	IHC	GTX30968

Mouse on Mouse

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

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