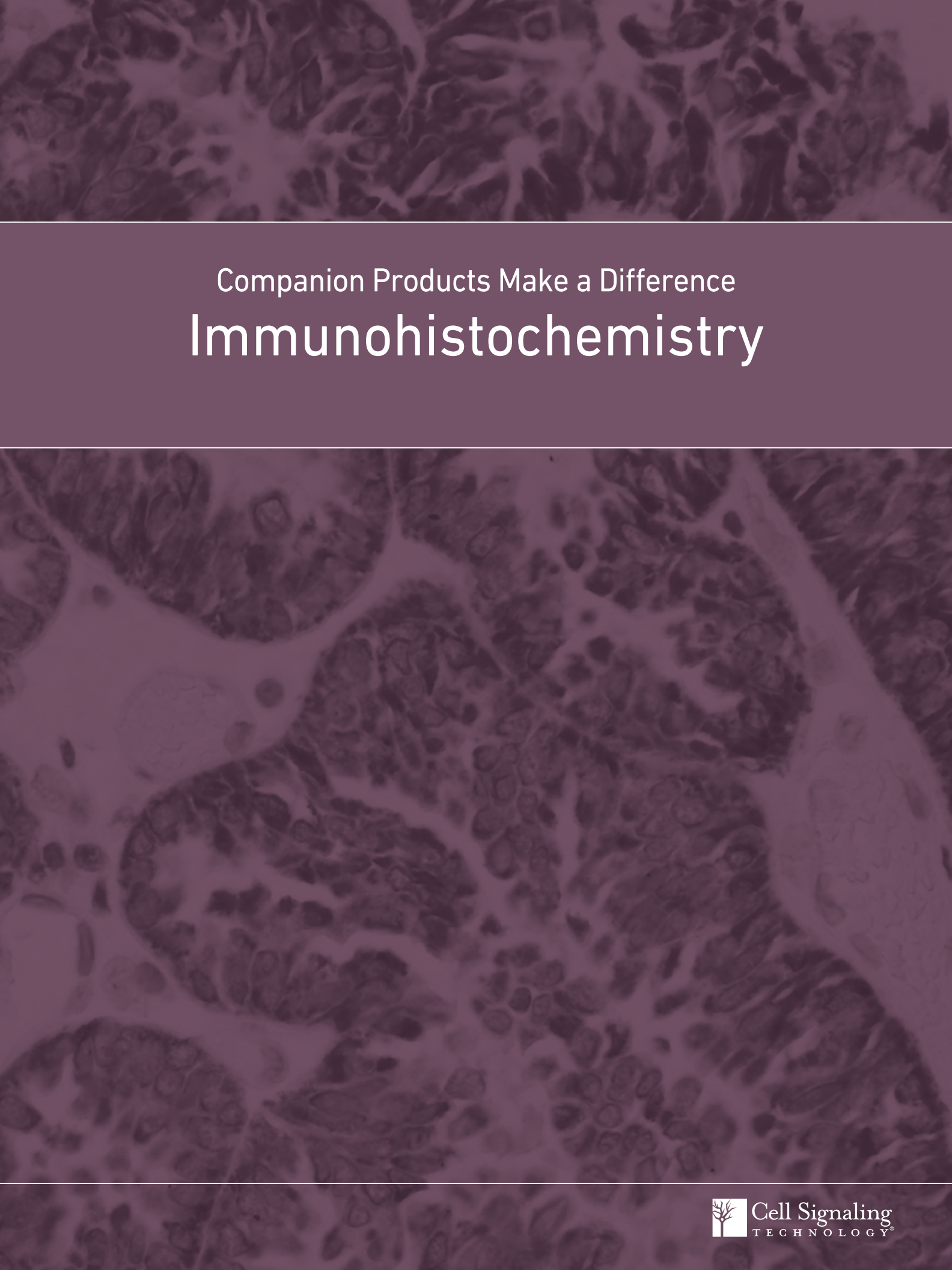


Companion Products Make a Difference

Immunohistochemistry

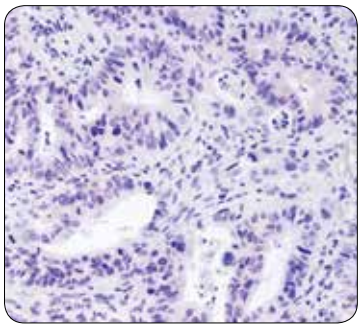
Cell Signaling TECHNOLOGY®

See for yourself

Using the optimal reagents and protocol for your antibody can greatly improve your results.

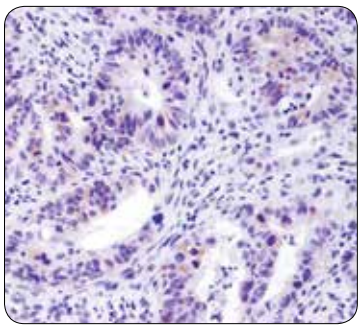
Companion products can have a significant effect on the strength of your IHC signal. That's why at CST our antibodies, our reagents and our protocols are optimized to provide you with the information and tools you need to set up successful experiments.

PLK1 (208G4) Rabbit mAb #4513 staining on colon carcinoma.
Shown below, step by step reagent substitution, without altering primary antibody concentration, demonstrates the impact ancillary reagents have upon staining results.



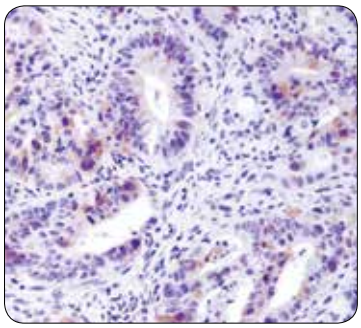
Staining results are poor when a protocol including the following reagents is used.

Diluent: **TBST/5% NGS**
Detection: **biotin-based**
Chromogen: **NovaRed™**



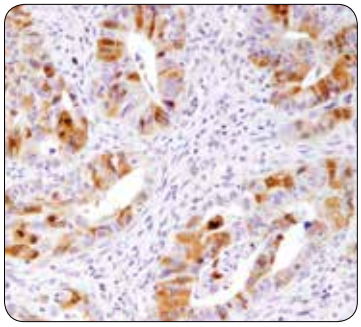
Incorporation of #8112 into the protocol results in a slightly improved signal, though still insufficient to be useful for tissue analysis.

Diluent: **SignalStain® Antibody Diluent #8112**
Detection: **biotin-based**
Chromogen: **NovaRed™**



Use of #8114, a sensitive polymer-based detection system, further improves the signal. Based on our rigorous standards, however, this signal does not warrant an IHC recommendation for this antibody.

Diluent: **SignalStain® Antibody Diluent #8112**
Detection: **SignalStain® Boost IHC Detection Reagent (HRP, Rabbit) #8114**
Chromogen: **NovaRed™**



Finally, incorporation of #8059 into the protocol along with #8112 and #8114, results in a robust signal warranting an IHC recommendation for this antibody.

Diluent: **SignalStain® Antibody Diluent #8112**
Detection: **SignalStain® Boost IHC Detection Reagent (HRP, Rabbit) #8114**
Chromogen: **SignalStain® DAB Substrate Kit #8059**

Solutions for consistent results

Antibodies are only one of the factors responsible for experimental success. It is just as important to select companion products that help the antibody perform at its best. The companion products offered by Cell Signaling Technology (CST) are the same reagents our scientists use to validate our primary antibodies for immunohistochemistry (IHC). These products have been thoroughly optimized to work with our primary antibodies and protocols, so you can acheive consistent, reliable results with your experiments.

In addition, we determine the optimal diluent, unmasking, and detection conditions, so you can start your experiment without stopping to test your reagents. These specifications are generated and supported by our scientists and are provided as product-specific protocols on the website.



Antibodies are only one of the factors responsible for experimental success.

Our companion products help our antibodies perform at their best.

Technical Support

At CST, providing exceptional customer service and technical support are top priorities. Our scientists work at the bench daily to produce and validate our antibodies, so they have hands-on experience and in-depth knowledge of each antibody's performance. In the process, these same scientists generate valuable reference information that they use to answer your questions and help troubleshoot your experiment by phone or email.

Technical Support:
www.cellsignal.com/support

Immunohistochemistry Application Solutions Kit (Rabbit) #13079

Each Immunohistochemistry Application Solutions Kit contains the pre-made buffers and reagents you'll need for each step of your protocol. At CST, the companion products we offer are the same ones you'll find on our lab benches. If we don't use it, we won't offer it to you. So you can be sure our companion products will help you get the best results from your experiments every time.

KIT INCLUDES

#8059	SignalStain® DAB Substrate Kit
#8114	SignalStain® Boost IHC Detection Reagent (HRP, Rabbit)
#8112	SignalStain® Antibody Diluent
#15019	Animal-Free Blocking Solution (5X) #15019

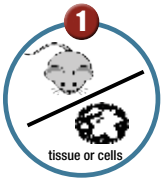
IHC
tools for success

Control Slides

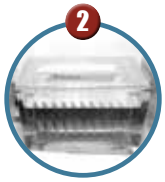
Each slide contains formalin-fixed, paraffin-embedded cell pellets that can be used to assess the performance of staining reagents and methods. Learn more at:

www.cellsignal.com/ihccontrols

Reliable reagents to support your protocol



SLIDE PREPARATION



DEPARAFFINIZATION /REHYDRATING



ANTIGEN RETRIEVAL

#14746	SignalStain® Citrate Unmasking Solution (10X)
#14747	SignalStain® EDTA Unmasking Solution (10X)

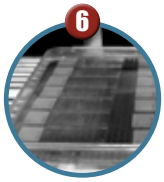


QUENCHING



BLOCKING

#5425	Normal Goat Serum
#9997	Tris Buffered Saline with Tween® 20 (TBST-10X)
#15019	Animal-Free Blocking Solution (5X)

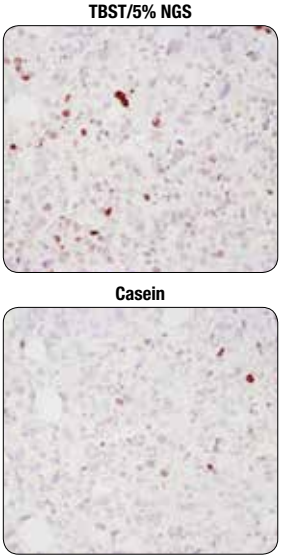


PRIMARY AB INCUBATION

#8112	SignalStain® Antibody Diluent
#5425	Normal Goat Serum
#9997	Tris Buffered Saline with Tween® 20 (TBST-10X)
#9809	Phosphate Buffered Saline with Tween® 20 (PBST-20X)

Normal Goat Serum #5425

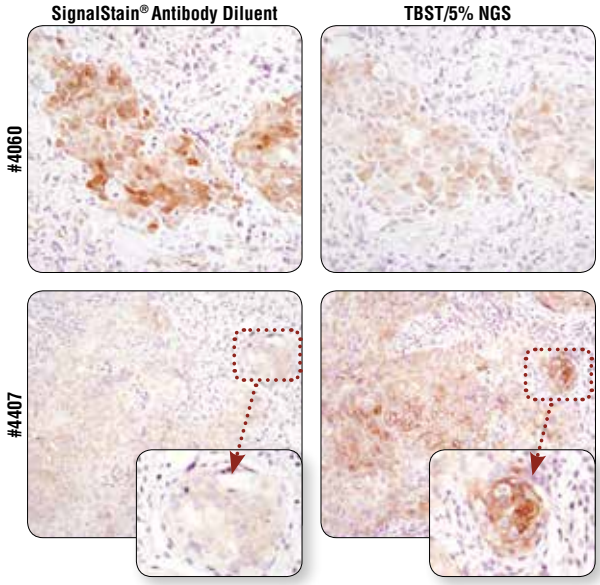
is an ideal blocking reagent for chromogenic and fluorescent IHC applications.



TBST/5% NGS is the better choice for blocking reagent when using phospho-specific antibodies. IHC analysis of paraffin-embedded human breast carcinoma using Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb #9718 after blocking with TBST/5% NGS (top) or a casein-based blocking solution (bottom). As shown, casein block produces a lower overall signal compared with TBST/5% NGS.

SignalStain® Antibody Diluent #8112

yields superior staining when used as the diluent for recommended antibodies. Please consult the antibody datasheet to determine the recommended diluent for your product of interest.



Antibody diluent can dramatically affect signal strength. IHC analysis of paraffin-embedded human breast carcinoma (top) and HCC827 xenograft (bottom) using Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb #4060 or Phospho-EGF Receptor (Tyr1173) (53A5) Rabbit mAb #4407 after dilution in either SignalStain® Antibody Diluent (left) or TBST/5% NGS (right). As shown, a superior signal is achieved when #4060 is diluted in SignalStain® Antibody Diluent compared with TBST/5% NGS. In contrast, #4407 performs better when diluted in TBST/5% NGS. Always check the product datasheet for the recommended diluent for your specific antibody.



IHC Application Guide

Helpful tips and explanations to support your IHC experiments are available in "A Guide to Successful Immunohistochemistry."
Learn more at:

www.cellsignal.com/ihcsuccess

Blocking Peptides

CST offers blocking peptides for target specificity analysis.
Learn more at:

www.cellsignal.com/blockingpeptides



WASHING

#9997 Tris Buffered Saline with Tween® 20 (TBST-10X)



DETECTION

#8125 SignalStain® Boost IHC Detection Reagent (HRP, Mouse)

#8114 SignalStain® Boost IHC Detection Reagent (HRP, Rabbit)

#8059 SignalStain® DAB Substrate Kit



WASHING

#9997 Tris Buffered Saline with Tween® 20 (TBST-10X)



COUNTERSTAINING AND DEHYDRATING

#14166 Hematoxylin



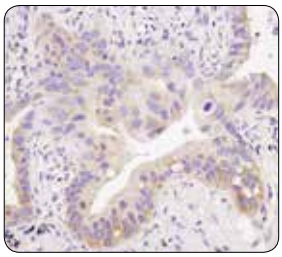
MOUNTING & EXAMINATION

#14177 SignalStain® Mounting Medium

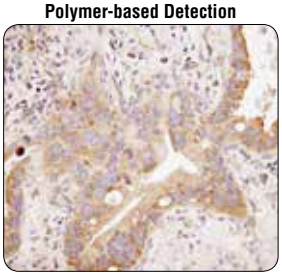
SignalStain® Boost IHC Detection Reagents #8125 and #8114

are highly sensitive, biotin-free, polymer-based detection reagents that avoid background staining caused by endogenous biotin. SignalStain® Boost IHC Detection Reagent (HRP, Mouse) #8125 and SignalStain® Boost IHC Detection Reagent (HRP, Rabbit) #8114 can be used in IHC assays to detect mouse or rabbit primary antibodies, respectively.

Biotin-based Detection



Polymer-based Detection

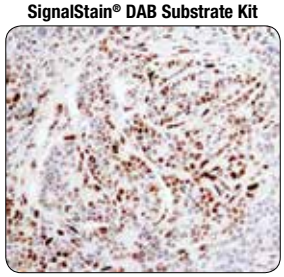


Polymer-based detection is more sensitive than biotin-based systems. IHC analysis of paraffin-embedded human lung carcinoma using S6 Ribosomal Protein (54D2) Mouse mAb #2317 and either biotin-based detection (left) or polymer-based detection (SignalStain® Boost IHC Detection Reagent #8125; right). As shown, polymer-based detection offers enhanced sensitivity and results in more robust staining.

SignalStain® DAB Substrate Kit #8059

offers high levels of sensitivity and works optimally with our primary antibodies.

SignalStain® DAB Substrate Kit



Other Company's DAB Substrate



Not all DAB substrates perform equally. IHC analysis of paraffin-embedded human breast carcinoma using Phospho-Stat3 (Tyr705) (D3A7) XP® Rabbit mAb #9145. Chromogenic detection was performed using SignalStain® DAB Substrate Kit #8059 (upper), or DAB supplied by another company (lower). The SignalStain® DAB Substrate Kit produces a much stronger signal than the DAB supplied by another company.



IHC Tips and Techniques Video

This video demonstrates how protocol variations affect your results and how to verify specific staining in your IHC experiments.

www.cellsignal.com/ihctips



IHC Protocol Video for Paraffin-embedded Tissue Sections

This video walks you through each step of our optimized IHC protocol for formalin-fixed, paraffin-embedded tissue samples.

www.cellsignal.com/ihcwatch

Cell Signaling TECHNOLOGY®

Cell Signaling Technology (CST) is a private, family-owned research organization founded by scientists and dedicated to providing high-quality research tools to the biomedical research community. Our employees operate worldwide from our US headquarters in Massachusetts and our offices in the Netherlands, China, and Japan.

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