

Application Solutions for
Immunohistochemistry



Cell Signaling
TECHNOLOGY®

Immunohistochemistry

Companion Products

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 achieve consistent, reliable results from 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 on the website and on the datasheet that accompanies every antibody.

Antibodies are only one of the factors responsible for experimental success. Our companion products help our antibodies perform at their best.



Immunohistochemistry Application Solutions Kit #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
- #5425 Normal Goat Serum
- #8114 SignalStain® Boost IHC Detection Reagent (HRP, Rabbit)
- #9997 TBST (10x)
- #8112 SignalStain® Antibody Diluent

CST Technical Support

At CST, providing exceptional customer service and technical support are our 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.

www.cellsignal.com/support (USA & Europe)

www.cst-c.com.cn/support (China)

www.cstj.co.jp/support (Japan)

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:

www.cellsignal.com/ihccontrols

www.cellsignal.com/ihccompanion

Immunohistochemistry

How does using the right companion products affect your IHC results?

See for yourself

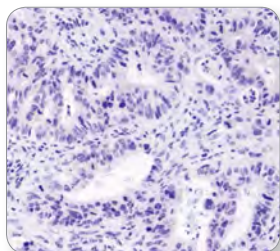
Companion Products Make a Difference

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

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

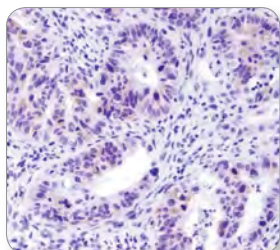
PLK1 (208G4) Rabbit mAb #4513:

IHC analysis of paraffin-embedded human colon carcinoma using #4513 with step-by-step reagent substitution, as indicated, demonstrating that reagents can make the difference between poor staining and publishable results.



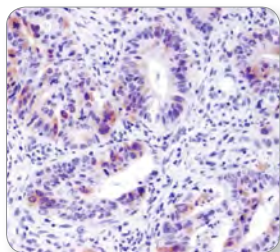
PLK1 (208G4) Rabbit mAb #4513: When #4513 was first released several years ago, our IHC group could not recommend it for IHC based on its lack of staining. At that time, the standard reagents for IHC analysis were:

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



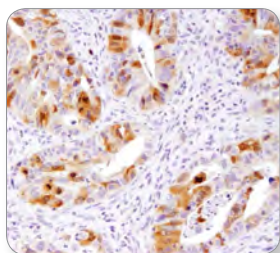
SignalStain® Antibody Diluent #8112: When #8112 was later developed, we observed a slightly improved signal as compared with our original conditions.

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



SignalStain® Boost IHC Detection Reagent (HRP, Rabbit) #8114: Changing the detection system to #8114 further improved the signal. However, based upon our rigorous standards, even a signal at this level did not warrant an IHC recommendation for this antibody.

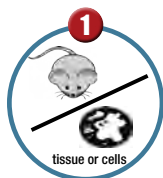
Diluent: **SignalStain® Antibody Diluent #8112**
Detection: **SignalStain® Boost IHC Detection Reagent (HRP, Rabbit) #8114**
Chromogen: **NovaRed™**



SignalStain® DAB Substrate Kit #8059: Finally, when we changed the chromogen to #8059, we were able to produce a robust signal using this antibody, thus warranting an IHC recommendation. The improvement in signal strength was achieved without altering the original antibody dilution.

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

Reliable reagents to support your protocol



1
SLIDE
PREPARATION



2
DEPARAFFINIZATION
/REHYDRATING



3
ANTIGEN RETRIEVAL

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

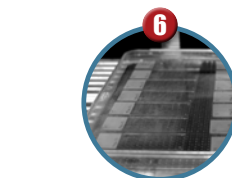


4
QUENCHING

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



5
BLOCKING



6
PRIMARY Ab INCUBATION

- #9808 Phosphate Buffered Saline (PBS-20X)
- #5425 Normal Goat Serum
- #9997 Tris Buffered Saline with Tween® 20 (TBST-10X)
- #12498 Tris Buffered Saline (TBS-10X)
- #8112 SignalStain® Antibody Diluent

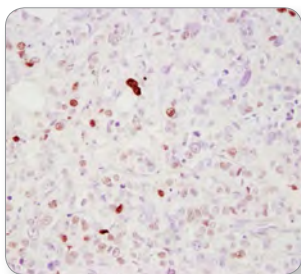
Normal Goat Serum #5425

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

SignalStain® Antibody Diluent #8112

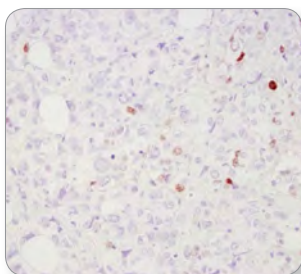
yields superior staining when used as the diluent for recommended antibodies. Please consult the antibody datasheet to determine the recommended diluent for your product of interest.

TBST/5% NGS

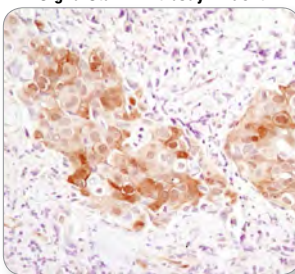


TBST/5% NGS is the better choice for blocking reagent when using phospho-specific antibodies. IHC analysis of paraffin-embedded human breast carcinoma using Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb #9718 after blocking with TBST/5% NGS (top) or a casein-based blocking solution (bottom). As shown,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

Casein

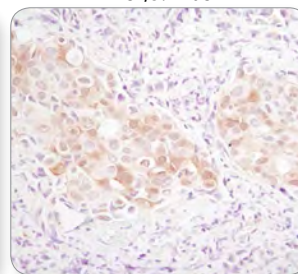


SignalStain® Antibody Diluent



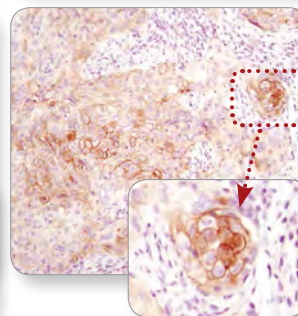
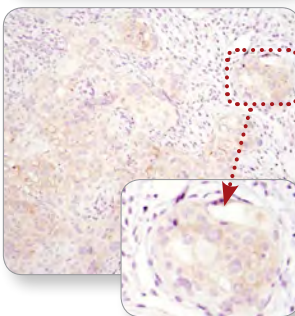
#4060

TBST/5% NGS



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 as 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.

#4407



IHC Application Guide

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

www.cellsignal.com/ihcsuccess

Blocking Peptides

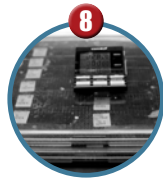
CST offers blocking peptides for target specificity analysis. Learn more:

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)



DEHYDRATING



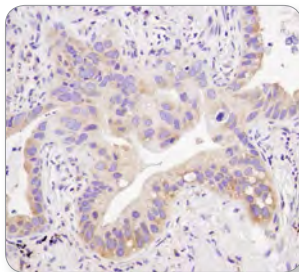
MOUNTING & EXAMINATION

#14177 SignalStain® Mounting Medium

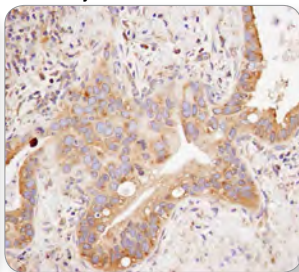
SignalStain® Boost IHC Detection Reagents #8125 and #8114

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

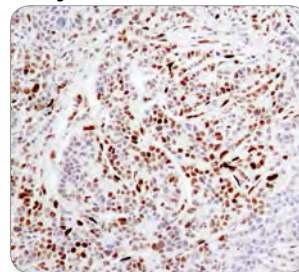


Polymer-based detection is more sensitive than biotin-based systems. IHC analysis of paraffin-embedded human lung carcinoma using S6 Ribosomal Protein (54D2) Mouse mAb #2317 and either biotin-based detection (left) or polymer-based detection (SignalStain® Boost IHC Detection Reagent #8125; right). As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

SignalStain® DAB Substrate Kit #8059

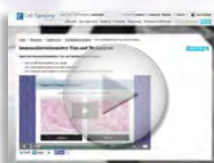
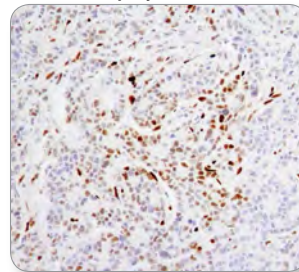
offers high levels of sensitivity and works optimally with our primary antibodies.

SignalStain® DAB Substrate Kit



Not all DAB substrate performs 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.

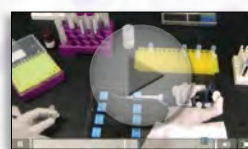
Other Company's DAB Substrate



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 company, founded by scientists and dedicated to providing high quality research tools to the biomedical research community. Our employees operate worldwide from our U.S. headquarters in Massachusetts and our offices in the Netherlands, China, and Japan.

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