Store at 4°C

#8130

Mitotic Marker: SignalStain® Phospho-Histone H3 (Ser10) IHC Detection Kit

☑ 1 Kit
(150 slides)

Cell Signaling TECHNOLOGY®

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orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 01/17/08

This product is for *in vitro* research use only and is not intended for use in humans or animals.

Products Included	Product #	Color
Peroxidase Quench		Orange
Blocking Solution		Blue
Prediluted Phospho-Histone H3 (Ser10) Antibody		Purple
Prediluted Negative Control		Brown
Biotinylated Secondary Antibody		Green
A and B Reagents		Gray
NovaRed Substrate		Red
Phospho-Histone H3 (Ser10) Blocking Peptide	#1000	White

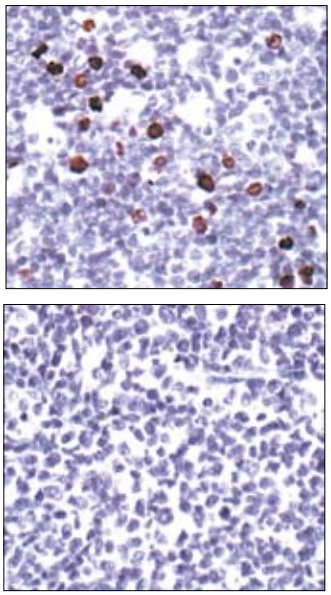
Description: CST's Mitotic Marker: SignalStain® Phospho-Histone H3 (Ser10) IHC Detection Kit is a "ready to use" system designed to detect the activation of histone H3 in human tissue and cell preps by immunohistochemistry. The kit utilizes the ABC immunoperoxidase method to detect endogenous levels of phosphorylated histone H3 protein. Prediluted Phospho-Histone H3 (Ser10) Antibody is bound by a biotinylated secondary antibody. Avidin DH and biotinylated horseradish peroxidase are complexed by mixing defined amounts prior to use, and the mixture subsequently binds the secondary antibody. The macromolecular complex is localized by incubation with NovaRed™ enzyme substrate.

The prediluted primary antibody, along with the ABC system, allows the user consistently to examine phosphorylated histone H3 localization and offers the highest sensitivity with the lowest background.

Background: Modulation of chromatin structure plays an important role in the regulation of transcription in eukaryotes. The nucleosome, made up of four core histone proteins (H2A, H2B, H3 and H4), is the primary building block of chromatin (1). The amino-terminal tails of core histones undergo various post-translational modifications, including acetylation, phosphorylation, methylation and ubiquitination (2-5). These modifications occur in response to various stimuli and have a direct effect on the accessibility of chromatin to transcription factors and, therefore, on gene expression (6). In most species, histone H2B is primarily acetylated at Lys5, 12, 15 and 20 (4,7). Histone H3 is primarily acetylated at Lys9, 14, 18 and 23 (2,3). Acetylation of H3 at Lys9 appears to have a dominant role in histone deposition and chromatin assembly in some organisms (2,3). Phosphorylation at Ser10, Ser28 and Thr11 of histone H3 is tightly correlated with chromosome condensation during both mitosis and meiosis (8-10). Phosphorylation of Thr3 of histone H3 is highly conserved among many species and is catalyzed by the kinase haspin. Immunostaining with phospho-specific antibodies in mammalian cells reveals mitotic phosphorylation of H3 Thr3 in prophase and its dephosphorylation during anaphase (11).

Specificity/Sensitivity: Mitotic Marker: SignalStain® Phospho-Histone H3 (Ser10) IHC Detection Kit detects endogenous histone H3 only when phosphorylated at serine 10. The antibody does not cross-react with other phosphorylated histones or with histone H3 modified not by phosphorylation but by acetylation. This kit was developed for and is recommended for immunohistochemistry only.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide corresponding to residues surrounding serine 10 of human histone H3. Antibodies are purified by protein A and peptide affinity chromatography.



◀ *Immunohistochemical staining of phosphorylated histone H3 in paraffin-embedded human tonsil showing nuclear localization in mitotic cells, using Mitotic Marker: SignalStain® Phospho-Histone H3 (Ser10) IHC Detection Kit (left). Serial section stained with matched negative control demonstrates specificity of staining (right).*

Storage: Store at 4°C. Components are ready to useand should not be aliquotted.

Note: Blocking Solution, Prediluted Phospho-Histone H3 (Ser10) Antibody, Prediluted Negative Control and Biotinylated Secondary Antibody contain 0.05% sodium azide.

Reagents Not Supplied:

Xylene
Ethanol, 100% and 95%
Distilled water (dH2O)
Tris-Buffered Saline + 0.1% Tween 20 (TBS/T)
Sodium citrate buffer, pH 6.0
Hematoxylin (optional)
Mounting medium

Companion Products:

Phospho-Histone H3 (Ser10) Blocking Peptide #1000
Phospho-Histone H3 (Ser10) Antibody #9701

Background References:

(1) Workman, J.L. and Kingston, R.E. (1998) *Annu. Rev. Biochem.* 67, 545–579.
(2) Hansen, J.C. et al. (1998) *Biochemistry* 37, 17637–17641.
(3) Strahl, B.D. and Allis, C.D. (2000) *Nature* 403, 41–45.
(4) Cheung, P. et al. (2000) *Cell* 103, 263–271.
(5) Bernstein, B.E. and Schreiber, S.L. (2002) *Chem. Biol.* 9, 1167–1173.
(6) Jaskelioff, M. and Peterson, C.L. (2003) *Nat. Cell Biol.* 5, 395–399.
(7) Thorne, A.W. et al. (1990) *Eur. J. Biochem.* 193, 701–713.
(8) Hendzel, M.J. et al. (1997) *Chromosoma* 106, 348–360.
(9) Goto, H. et al. (1999) *J. Biol. Chem.* 274, 25543–25549.
(10) Preuss, U. et al. (2003) *Nucleic Acids Res.* 31, 878–885.
(11) Dai, J. et al. (2005) *Genes Dev.* 19, 472–488.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **IC**—Immunocytochemistry **IF**—Immunofluorescence **F**—Flow cytometry **E**—ELISA **D**—DELFIA®

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **X**—Xenopus **Z**—zebra fish **B**—bovine **All**—all species expected

Species enclosed in parentheses are predicted to react based on 100% sequence homology.

SignalStain® Protocol

Reagents Not Supplied:	Xylene	
	Ethanol, 100% and 95%	
	Distilled water (dH_2O)	
	Tris-Buffered Saline + 0.1% Tween-20 (TBS/T): To prepare 1 liter: Add 2.42g Trizma Base, ($C_4H_{11}NO_3$) and 8g sodium chloride (NaCl) to 800 ml dH_2O. Adjust pH to 7.6 with concentrated HCl. Bring volume to 1 liter and add 1 ml Tween-20. Mix well.	
	0.01 M Sodium Citrate Buffer, pH 6.0: To prepare 1 liter: Add 2.94g sodium citrate trisodium salt dihydrate ($C_6H_5Na_3O_7 \bullet 2H_2O$) to 800ml dH_2O. Adjust pH to 6.0, then bring volume to 1 liter.	
	Hematoxylin (optional)	
	Mounting medium	
Deparaffinization:	Xylene, 3 changes.	5 minutes each
Rehydration:	100% ethanol, 2 changes and 95% ethanol, 2 changes.	10 minutes each
	dH_2O, 2 changes.	5 minutes each
Antigen Unmasking:	Immerse slides in 0.01M sodium citrate buffer (pH 6.0) and bring the solution to a boil. Maintain at a sub-boiling temperature for 10 minutes. Cool slides in buffer on the bench for 30 minutes.	
Peroxidase Quench (orange cap):	Apply 1–2 drops Peroxidase Quench to slide, completely covering tissue.	10 minutes, 25°C
	Wash in two changes dH_2O and one change TBS/T.	3 minutes each
Block (blue cap):	Apply 1–3 drops Blocking Solution to slide, completely covering tissue.	60 minutes, 25°C.
	Prepare Peptide Block if desired, as directed below.	
Peptide Blocking (optional):	Combine 3 drops prediluted primary antibody and 5 ml blocking peptide. Incubate for at least 1 hour at 4°C.	60 minutes, 4°C.
Primary Antibody (purple cap):	Apply 1–3 drops Primary Antibody or prepared peptide blocking solution to slide, completely covering tissue.	Overnight, 4°C
Negative Control (brown cap):	Apply 1–3 drops Negative Control to a separate slide, completely covering tissue.	Overnight, 4°C
	Wash in TBS/T, 3 changes.	5 minutes each
Biotinylated Secondary Antibody (green cap):	Apply 1–3 drops Biotinylated Secondary Antibody to slide, completely covering tissue.	30 minutes, 25°C
	Prepare AB Reagent as directed below.	
	Wash in TBS/T, 3 changes.	5 minutes each
Prepare AB Reagent (gray cap):	Add 1 drop Reagent A and 1 drop Reagent B to 2.5ml dH_2O in mixing bottle (yellow cap). Mix well.	30 minutes, 25°C
AB Reagent(gray cap):	Add 1–3 drops premixed AB Reagent to slide, completely covering tissue.	30 minutes, 25°C
	Wash in TBS/T, 3 changes	5 minutes each
Substrate-Chromagen(red cap):	Rinse mixing bottle well. Combine 1 drop each Substrate reagents 1, 2, 3 and 4 in 2.5ml dH_2O in the clean mixing bottle. Mix well.	2–10 minutes
	Apply 1–3 drops Substrate-Chromagen mixture to slide, completely covering tissue.	
	Monitor staining and immerse in dH_2O when sections turn red-brown in color.	
	Note: Prolonged incubation of NovaRed™ in alcohol or use of alcohol-based differentiating solutions may decrease sensitivity.	
	Note: Excess dilute working solutions of NovaRed™ may be decomposed with a solution of 3% potassium permanganate ($KMnO_4$), 2% sodium carbonate (Na_2Co_3) in deionized or distilled water.	
	Dispose excess substrate in accordance with local regulations.	
Counterstain (optional):	Counterstain slides in hematoxylin per manufacturer's recommendations.	
Dehydration:	Dehydrate sides in 2 changes 95% ethanol and 2 changes 100% ethanol, then clear in 2 changes of xylene.	10 seconds each
Mount Coverslips:	Apply permanent mounting medium to slide and mount with coverslip.	

Material Safety Data Sheet (MSDS) for SignalStain® IHC Detection Kit

New 06/09

I. IDENTIFICATION:

Product name: SignalStain® IHC Detection Kit
Product Catalog Number: #8100, 8110, 8120, 8130
Manufacturer Supplier: Cell Signaling Technology®
3 Trask Lane
Danvers, MA 01923 USA
1-978-867-2300 TEL
1-978-867-2400 FAX
1-978-578-6737 Emergency Phone

II. COMPOSITION/INFORMATION ON INGREDIENTS:

Substance Name: SignalStain® IHC Detection Kit

CAS#: None

Please see the individual material safety data sheets which can be found on the CST website **www.cellsignal.com/support/msds.html** for hazard information specific to kit components.

- Peroxidase Quench MSDS
- Blocking Solution MSDS
- Prediluted Cleaved Antibody (covered by "Antibodies" MSDS)
- Prediluted Negative Control (covered by "Antibodies" MSDS)
- Biotinylated Secondary Antibody (covered by "Antibodies" MSDS)
- Reagent A+B MSDS
- NovaRed Substrate 1, 2, 3, 4 MSDS
- Blocking Peptide MSDS

III. HAZARD IDENTIFICATION:

Flammable. Irritant.

HMIS Rating: Health: 2 Flammability: 3 Reactivity: 1

VII. HANDLING AND STORAGE:

Storage: Store kit in tightly closed container at 4°C.

VIII-XIII: Refer to individual MSDS for kit components for Sections 8-13 information: Exposure Controls/Personal Protection, Physical and Chemical Properties, Stability and Reactivity, Toxicological Information, Ecological information, Disposal Considerations.

XIV. TRANSPORT INFORMATION:

D.O.T. and IATA
Proper Shipping Name: None
Non hazardous for transport

XV. REGULATORY INFORMATION:

EU Regulations/Classifications/Labeling Information:

Risk Phrases: Irritant. Irritating to eyes and skin. Harmful if swallowed.

Safety Phrases: In case of contact wash with water and seek medical attention.

US Regulatory Information: Irritating to eyes, respiratory system and skin.

Sara Listed: No.

XVI. OTHER INFORMATION:

This product is not intended for use in humans. To the best of our knowledge, this document is accurate. It is intended to serve as a guide for safe use of this product in a laboratory setting by experienced personnel. The burden of safe use of this material rests entirely with the user. The above information is believed to be accurate but is not necessarily all-inclusive and shall be used only as a guide. Cell Signaling Technology, Inc., shall not be held liable for any damage resulting from the handling of or from contact with the above product.