

Apoptosis Marker: SignalStain® Cleaved Caspase-3 (Asp175) IHC Detection Kit

✓ 1 Kit
(150 slides)

Orders ■ 877-616-CELL (2355)
orders@cellsignaling.com

Support ■ 877-678-TECH (8324)
info@cellsignaling.com

Web ■ www.cellsignaling.com

rev. 01/20/12

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Kit Components	Product #	Kit Quantity	Color
Peroxidase Quench		19.5 ml	Orange
Blocking Solution		15 ml	Blue
Prediluted Cleaved Caspase-3 (D175) Antibody #9661		15 ml	Purple
Prediluted Negative Control		15 ml	Brown
Biotinylated Secondary Antibody		15 ml	Green
Reagent A		850 µl	Gray
Reagent B		850 µl	Gray
NovaRed Substrate 1		600 µl	Red
NovaRed Substrate 2		600 µl	Red
NovaRed Substrate 3		600 µl	Red
NovaRed Substrate 4		900 µl	Red
Mixing Bottle		1	Yellow
Cleaved Caspase-3 Blocking Peptide	1050	100 µl	Dark Blue

Description: CST's Apoptosis Marker: SignalStain® Cleaved Caspase-3 (Asp175) IHC Detection Kit is a "ready to use" system designed to detect the activation of caspase-3 in human tissue and cell preps by immunohistochemistry. The kit utilizes the ABC immunoperoxidase method to detect endogenous levels of caspase-3 protein. Prediluted Cleaved Caspase-3 (Asp175) 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 to examine caspase-3 localization consistently and offers the highest sensitivity with the lowest background.

Background: Caspase-3 (CPP-32, Apoptain, Yama, SCA-1) is a critical executioner of apoptosis, as it is either partially or totally responsible for the proteolytic cleavage of many key proteins such as the nuclear enzyme poly(ADP-ribose) polymerase (PARP) (1). Activation of caspase-3 requires proteolytic processing of its inactive zymogen into activated p17 and p12 fragments. Cleavage of caspase-3 requires aspartic acid at the P1 position (2).

Specificity/Sensitivity: Apoptosis Marker: SignalStain® Cleaved Caspase-3 (Asp175) IHC Detection Kit detects endogenous levels of the large fragment (17/19 kDa) of activated caspase-3 resulting from cleavage adjacent to Asp175. This antibody does not recognize full length caspase-3 or other cleaved caspases. This kit was developed for and is recommended for immunohistochemistry only.

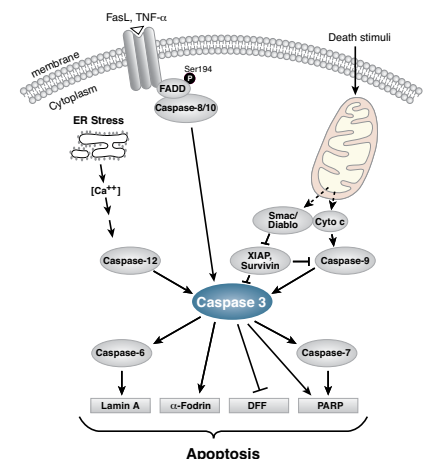
Storage: Store kit at 4°C. Components are ready to use and should not be aliquotted.

Note: Blocking solution, Prediluted Phospho44/42 MAPK (Thr202/Tyr204) (20G11) Rabbit mAb (IHC Preferred), Prediluted Negative Control and the Biotinylated Secondary Antibody contain 0.05% sodium azide.

Reagents not supplied:

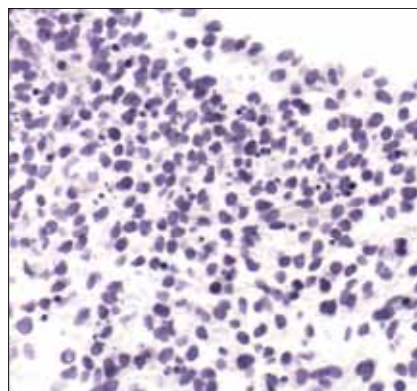
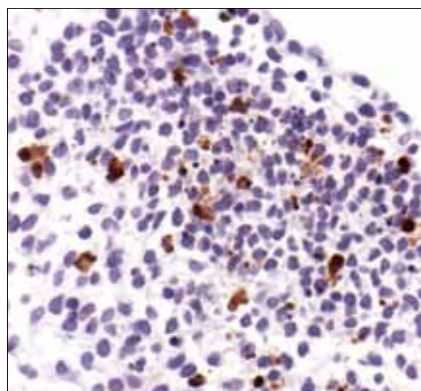
Xylene
Ethanol, 100% and 95%
Distilled water (dH₂O)
Phosphate-Buffered Saline+0.1% Tween -20 (PBS/T)
Sodium citrate buffer, pH 6.0
Hematoxylin (optional)
Mounting medium

Please visit www.cellsignaling.com for a complete listing of recommended companion products.



◀ Immunohistochemical analysis of paraffin-embedded mouse embryo, using Apoptosis Marker: SignalStain® Cleaved Caspase-3 (Asp175) IHC Detection Kit (left) or matched negative control (right).

* NovaRed™ is a trademark of Vector Labs.



Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide

Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine

Dg—dog Pg—pig Sc—S. cerevisiae All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to amino-terminal residues surrounding to Asp175 in human caspase-3. Antibodies are purified by protein A and peptide affinity chromatography.

Background References:

- (1) Fernandes-Alnemri, T. et al. (1994) *J. Biol. Chem.* 269, 30761–30764.
- (2) Nicholson, D. W. et al. (1995) *Nature* 376, 37–43.

SignalStain® Protocol

Reagents Not Supplied	<ul style="list-style-type: none"> • Xylene • Ethanol, 100% and 95% • Distilled water (dH₂O) • Phosphate Buffered Saline + 0.1% Tween-20 (PBS/T): To prepare 1 liter: Add 8g sodium chloride (NaCl), 0.2g potassium chloride (KCl), 1.44g sodium phosphate, dibasic (Na₂HPO₄) and 0.24g potassium phosphate, monobasic (KH₂PO₄) to 1L dH₂O. Adjust pH to 7.4. Add 1mL Tween-20. Mix well. • 0.01M Sodium Citrate Buffer, pH 6.0: To prepare 1 liter: Add 2.94g sodium citrate trisodium salt dihydrate (C₆H₅Na₃O₇•2H₂O) to 800ml dH₂O. Adjust pH to 6.0, then bring volume to 1 liter. • Hematoxylin (optional) 	
Deparaffinization	• Xylene, 3 changes.	5 minutes each
Rehydration	<ul style="list-style-type: none"> • 100% ethanol, 2 changes and 95% ethanol, 2 changes. • dH₂O, 2 changes. 	10 minutes each 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)	<ul style="list-style-type: none"> • Apply 1–2 drops Peroxidase Quench to slide, completely covering tissue. • Wash in two changes dH₂O and one change PBS/T. 	10 minutes, 25°C 3 minutes each
Block (blue cap)	<ul style="list-style-type: none"> • Apply 1–3 drops Blocking Solution to slide, completely covering tissue. • Prepare Peptide Block if desired, as directed below. 	60 minutes, 25°C.
Peptide Blocking (optional)	• Combine 3 drops prediluted primary antibody and 5 µl 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)	<ul style="list-style-type: none"> • Apply 1–3 drops Negative Control to a separate slide, completely covering tissue. • Wash in PBS/T, 3 changes. 	Overnight, 4°C 5 minutes each
Biotinylated Secondary Antibody (green cap)	<ul style="list-style-type: none"> • Apply 1–3 drops Biotinylated Secondary Antibody to slide, completely covering tissue. • Prepare AB Reagent as directed below. • Wash in PBS/T, 3 changes. 	30 minutes, 25°C 5 minutes each
Prepare AB Reagent (gray cap)	• Add 1 drop Reagent A and 1 drop Reagent B to 2.5ml dH ₂ O in mixing bottle (yellow cap). Mix well.	30 minutes, 25°C
AB Reagent (gray cap)	<ul style="list-style-type: none"> • Add 1–3 drops premixed AB Reagent to slide, completely covering tissue. • Wash in PBS/T, 3 changes 	30 minutes, 25°C 5 minutes each
Substrate-Chromagen (red cap)	<ul style="list-style-type: none"> • Rinse mixing bottle well. Combine 1 drop each Substrate reagents 1, 2, 3 and 4 in 2.5ml dH₂O in the clean mixing bottle. Mix well. • Apply 1–3 drops Substrate-Chromagen mixture to slide, completely covering tissue. • Monitor staining and immerse in dH₂O 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 (KMnO₄), 2% sodium carbonate (Na₂CO₃) in deionized or distilled water. • Dispose excess substrate in accordance with local regulations. 	2–10 minutes
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.