

# Growth/Proliferation Marker: SignalStain® Phospho-p44/42 MAPK (Thr202/Tyr204) IHC Detection Kit

✓ 1 Kit  
(150 slides)

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orders@cellsignal.com  
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rev. 02/07/12

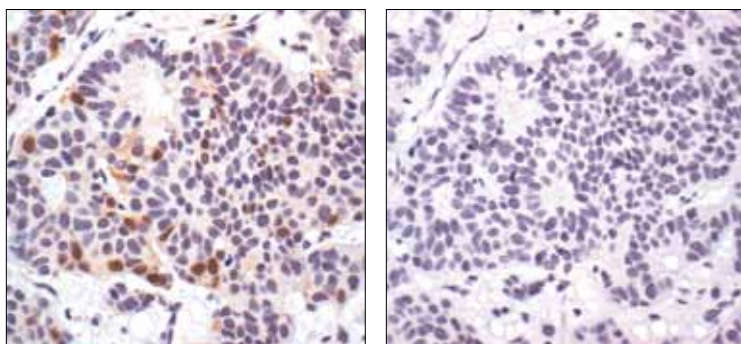
This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Kit Components	Product #	Kit Quantity	Color
Peroxidase Quench		15 ml	Orange
Blocking Solution		15 ml	Blue
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP™ Rabbit mAb #4370		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		600 µl	Red
Mixing Bottle		1	Yellow
Phospho-p44/42 MAPK (Thr202/Tyr204) Blocking Peptide (IHC Specific)	1150	100 µl	White

**Description:** CST's Growth/Proliferation Marker: SignalStain® Phospho-p44/42 MAPK (Thr202/Tyr204) IHC Detection Kit is a "ready to use" system designed to detect the activation of MAP kinase in human tissue and cell preps by immunohistochemistry. The kit utilizes the ABC immunoperoxidase method to detect endogenous levels of phosphorylated p44/42 MAP kinase. Prediluted Phospho-p44/42MAPK (Thr202/Tyr204) (20G11) Rabbit mAb (IHC Preferred) #4376 is bound by a biotinylated secondary antibody.

Avidin DH and biotinylated horseradish peroxidase are complexed by mixing in 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-p44/42 MAP Kinase localization and offers the highest sensitivity with the lowest background.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma, showing nuclear localization, using Growth/Proliferation Marker: SignalStain® Phospho-p44/42 MAPK (Thr202/Tyr204) IHC Detection Kit (left). Serial section stained with matched negative control demonstrates specificity of staining (right).

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

**Note:** Blocking solution, Prediluted Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP™ Rabbit mAb, Prediluted Negative Control and the Biotinylated Secondary Antibody contain 0.05% sodium azide.

**Reagents not supplied:** Xylene

Ethanol, 100% and 95%

Distilled water (dH2O)

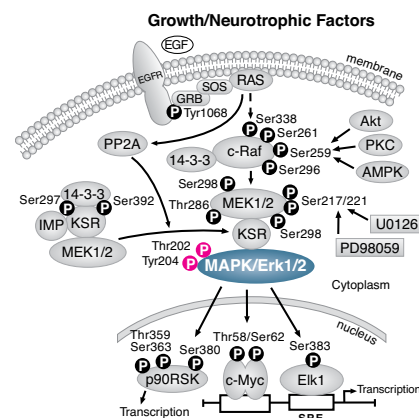
Tris-Buffered Saline + 0.1% Tween 20 (TBS/T)

Sodium citrate buffer, pH 6.0

Hematoxylin (optional)

Mounting medium

**Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.**



\* NovaRed™ is a trademark of Vector Labs.

**Background:** Mitogen-activated protein kinases (MAPKs) are a widely conserved family of serine/threonine protein kinases involved in many cellular programs such as cell proliferation, differentiation, motility, and death. The p44/42 MAPK (ERK1/2) signaling pathway can be activated in response to a diverse range of extracellular stimuli including mitogens, growth factors, and cytokines (1-3) and is an important target in the diagnosis and treatment of cancer (4). Upon stimulation, a sequential three-part protein kinase cascade is initiated, consisting of a MAP kinase kinase kinase (MAPKKK), a MAP kinase kinase (MAPKK), and a MAP kinase. While multiple ERK1/2 MAP3Ks have been identified, including the Raf family, Mos, and Tpl2/Cot, MEK1 and MEK2 are the primary MAPKKs in this pathway (5,6). MEK1 and MEK2 activate ERK1/p44 and ERK2/p42 through phosphorylation of activation loop residues Thr202/Tyr204 and Thr185/Tyr187, respectively. Several downstream targets of ERK1/2 have been identified, including p90RSK (7) and the transcription factor Elk-1 (8,9). ERK1/2 are negatively regulated by a family of dual-specificity (Thr/Tyr) MAPK phosphatases, known as DUSPs or MKPs (10), along with MEK inhibitors such as U0126 and PD98059.

**Specificity/Sensitivity:** Growth/Proliferation Marker: SignalStain® Phospho-p44/42 MAPK (Thr202/Tyr204) IHC Detection Kit recognizes endogenous levels of p42 and p44 MAP kinase (Erk1 and Erk2) only when phosphorylated at Thr202 and Tyr204 of Erk1 (Thr183 and Tyr185 of Erk2). The antibody does not cross-react with the corresponding phosphorylated residues of either SAPK/JNK or p38 MAP kinase. This kit was developed for and is recommended for immunohistochemistry only.

**Source/Purification:** Monoclonal antibody is produced by immunizing animals with a synthetic phospho-peptide corresponding to residues surrounding Thr202/Tyr204 of human p44/42 MAP kinase.

**Background References:**

- (1) Roux, P.P. and Blenis, J. (2004) *Microbiol Mol Biol Rev* 68, 320–44.
- (2) Baccarini, M. (2005) *FEBS Lett* 579, 3271–7.
- (3) Meloche, S. and Pouyssegur, J. (2007) *Oncogene* 26, 3227–39.
- (4) Roberts, P.J. and Der, C.J. (2007) *Oncogene* 26, 3291–310.
- (5) Rubinfeld, H. and Seger, R. (2005) *Mol Biotechnol* 31, 151–74.
- (6) Murphy, L.O. and Blenis, J. (2006) *Trends Biochem Sci* 31, 268–75.
- (7) Dalby, K.N. et al. (1998) *J Biol Chem* 273, 1496–505.
- (8) Marais, R. et al. (1993) *Cell* 73, 381–93.
- (9) Kortjenann, M. et al. (1994) *Mol Cell Biol* 14, 4815–24.
- (10) Owens, D.M. and Keyse, S.M. (2007) *Oncogene* 26, 3203–13.

## SignalStain® Protocol

<b>Reagents Not Supplied</b>	<ul style="list-style-type: none"> <li>• Xylene</li> <li>• Ethanol, 100% and 95%</li> <li>• Distilled water (dH<sub>2</sub>O)</li> <li>• Tris-Buffered Saline + 0.1% Tween-20 (TBS/T): To prepare 1 liter: Add 2.42g Trizma Base, (C<sub>4</sub>H<sub>11</sub>NO<sub>3</sub>) and 8g sodium chloride (NaCl) to 800ml dH<sub>2</sub>O. Adjust pH to 7.6 with concentrated HCl. Bring volume to 1 liter and add 1ml Tween-20. Mix well.</li> <li>• 0.01M Sodium Citrate Buffer, pH 6.0: To prepare 1 liter: Add 2.94g sodium citrate trisodium salt dihydrate (C<sub>6</sub>H<sub>5</sub>Na<sub>3</sub>O<sub>7</sub>•2H<sub>2</sub>O) to 800ml dH<sub>2</sub>O. Adjust pH to 6.0, then bring volume to 1 liter.</li> <li>• Hematoxylin (optional)</li> <li>• Mounting medium</li> </ul>	
<b>Deparaffinization</b>	<ul style="list-style-type: none"> <li>• Xylene, 3 changes.</li> </ul>	5 minutes each
<b>Rehydration</b>	<ul style="list-style-type: none"> <li>• 100% ethanol, 2 changes and 95% ethanol, 2 changes.</li> <li>• dH<sub>2</sub>O, 2 changes.</li> </ul>	10 minutes each 5 minutes each
<b>Antigen Unmasking</b>	<ul style="list-style-type: none"> <li>• 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.</li> </ul>	
<b>Peroxidase Quench (orange cap)</b>	<ul style="list-style-type: none"> <li>• Apply 1–2 drops <b>Peroxidase Quench</b> to slide, completely covering tissue.</li> <li>• Wash in two changes dH<sub>2</sub>O and one change TBS/T.</li> </ul>	10 minutes, 25C 3 minutes each
<b>Block (blue cap)</b>	<ul style="list-style-type: none"> <li>• Apply 1–3 drops <b>Blocking Solution</b> to slide, completely covering tissue.</li> <li>• Prepare Peptide Block if desired, as directed below.</li> </ul>	60 minutes, 25C.
<b>Peptide Blocking (optional)</b>	<ul style="list-style-type: none"> <li>• Combine 3 drops prediluted primary antibody and 5 µl blocking peptide. Incubate for at least 1 hour at 4C.</li> </ul>	60 minutes, 4C.
<b>Primary Antibody (purple cap)</b>	<ul style="list-style-type: none"> <li>• Apply 1–3 drops <b>Primary Antibody</b> or prepared peptide blocking solution to slide, completely covering tissue.</li> </ul>	Overnight, 4C
<b>Negative Control (brown cap)</b>	<ul style="list-style-type: none"> <li>• Apply 1–3 drops <b>Negative Control</b> to a separate slide, completely covering tissue.</li> <li>• Wash in TBS/T, 3 changes.</li> </ul>	Overnight, 4C 5 minutes each
<b>Biotinylated Secondary Antibody (green cap)</b>	<ul style="list-style-type: none"> <li>• Apply 1–3 drops <b>Biotinylated Secondary Antibody</b> to slide, completely covering tissue.</li> <li>• Prepare <b>AB Reagent</b> as directed below.</li> <li>• Wash in TBS/T, 3 changes.</li> </ul>	30 minutes, 25C 5 minutes each
<b>Prepare AB Reagent (gray cap)</b>	<ul style="list-style-type: none"> <li>• Add 1 drop <b>Reagent A</b> and 1 drop <b>Reagent B</b> to 2.5ml dH<sub>2</sub>O in <b>mixing bottle</b> (yellow cap). Mix well.</li> </ul>	30 minutes, 25C
<b>AB Reagent (gray cap)</b>	<ul style="list-style-type: none"> <li>• Add 1–3 drops premixed <b>AB Reagent</b> to slide, completely covering tissue.</li> <li>• Wash in TBS/T, 3 changes</li> </ul>	30 minutes, 25C 5 minutes each
<b>Substrate-Chromagen (red cap)</b>	<ul style="list-style-type: none"> <li>• Rinse <b>mixing bottle</b> well. Combine 1 drop each <b>Substrate</b> reagents 1, 2, 3 and 4 in 2.5ml dH<sub>2</sub>O in the clean <b>mixing bottle</b>. Mix well.</li> <li>• Apply 1–3 drops <b>Substrate-Chromagen</b> mixture to slide, completely covering tissue.</li> <li>• Monitor staining and immerse in dH<sub>2</sub>O when sections turn red-brown in color.</li> <li>• Note: Prolonged incubation of NovaRed™ in alcohol or use of alcohol-based differentiating solutions may decrease sensitivity.</li> <li>• Note: Excess dilute working solutions of NovaRed™ may be decomposed with a solution of 3% potassium permanganate (KMnO<sub>4</sub>), 2% sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) in deionized or distilled water.</li> <li>• Dispose excess substrate in accordance with local regulations.</li> </ul>	2–10 minutes
<b>Counterstain (optional)</b>	<ul style="list-style-type: none"> <li>• Counterstain slides in hematoxylin per manufacturer's recommendations.</li> </ul>	
<b>Dehydration</b>	<ul style="list-style-type: none"> <li>• Dehydrate sides in 2 changes 95% ethanol and 2 changes 100% ethanol, then clear in 2 changes of xylene.</li> </ul>	10 seconds each
<b>Mount Coverslips</b>	<ul style="list-style-type: none"> <li>• Apply permanent mounting medium to slide and mount with coverslip.</li> </ul>	

# 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](http://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.