

Survival Marker: Signal Stain® Phospho-Akt (Ser473) IHC Detection Kit

✓ 1 Kit
(150 slides)



Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

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		19.5 ml	Orange
Blocking Solution		15 ml	Blue
Prediluted Phospho-Akt (Ser473) Antibody		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		800 µl	Red
Mixing Bottle			Yellow
Phospho-Akt (Ser473) Blocking Peptide	#1140	100 µg	Dark Blue

Description: CST's Survival Marker: Signal Stain® Phospho-Akt (Ser473) IHC Detection Kit is a "ready to use" system designed to detect the activation of Akt in human tissue and cell preparations using immunohistochemistry. The kit utilizes the ABC immunoperoxidase method to detect endogenous levels of phosphorylated Akt protein. Prediluted Phospho-Akt (Ser473) 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 consistently examine phosphorylated-Akt localization and offers the highest sensitivity with the lowest background.

Background: Akt, also referred to as PKB or Rac, plays a critical role in controlling survival and apoptosis (1-3). This protein kinase is activated by insulin and various growth and survival factors to function in a wortmannin-sensitive pathway involving PI3 kinase (2,3). Akt is activated by phospholipid binding and activation loop phosphorylation at Thr308 by PDK1 (4) and by phosphorylation within the carboxy terminus at Ser473. The previously elusive PDK2 responsible for phosphorylation of Akt at Ser473 has been identified as mammalian target of rapamycin (mTOR) in a rapamycin-insensitive complex with rictor and Sin1 (5,6). Akt promotes cell survival by inhibiting apoptosis through phosphorylation and inactivation of several targets, including Bad (7), forkhead transcription factors (8), c-Raf (9), and caspase-9. PTEN phosphatase is a major negative

regulator of the PI3 kinase/Akt signaling pathway (10). LY294002 is a specific PI3 kinase inhibitor (11). Another essential Akt function is the regulation of glycogen synthesis through phosphorylation and inactivation of GSK-3 α and β (12,13). Akt may also play a role in insulin stimulation of glucose transport (12). In addition to its role in survival and glycogen synthesis, Akt is involved in cell cycle regulation by preventing GSK-3 β -mediated phosphorylation and degradation of cyclin D1 (14) and by negatively regulating the cyclin dependent kinase inhibitors p27 Kip (15) and p21 Waf1/CIP1 (16). Akt also plays a critical role in cell growth by directly phosphorylating mTOR in a rapamycin-sensitive complex containing raptor (17). More importantly, Akt phosphorylates and inactivates tuberlin (TSC2), an inhibitor of mTOR within the mTOR-raptor complex (18,19).

Specificity/Sensitivity: Survival Marker: Signal Stain® Phospho-Akt (Ser473) IHC Detection Kit detects Akt1 only when phosphorylated at serine 473, and Akt2 and Akt3 only when phosphorylated at equivalent sites. The antibody does not detect Akt phosphorylated at other sites or related kinases such as PKC or p70 S6 kinase. This kit was developed for and is recommended for immunohistochemistry only.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues around Ser473 of mouse Akt. Antibodies are purified by protein A and peptide affinity chromatography.

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

Note: Note: Blocking Solution, Prediluted Phospho-Akt(Ser473) Antibody, Prediluted Negative Control and Biotinylated Secondary Antibody contain 0.05% sodium azide. Blocking Solution Blue

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 for a complete listing of recommended companion products.

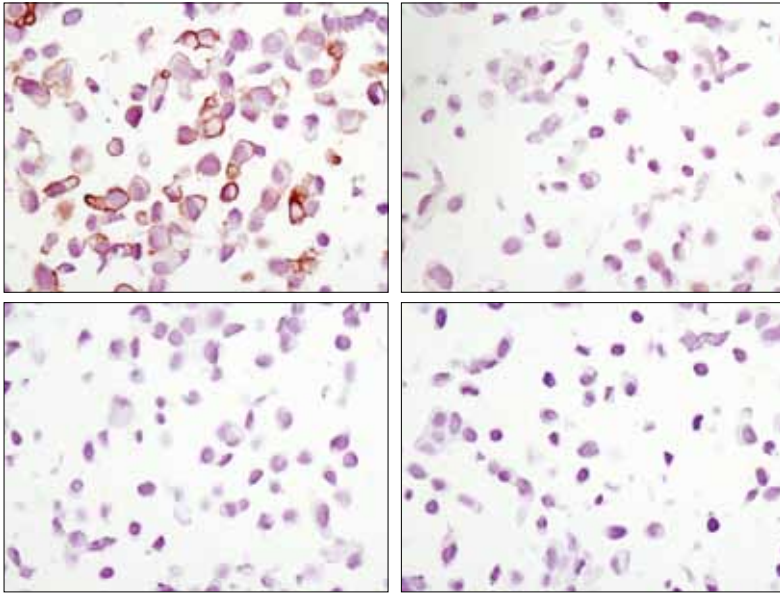
Background References:

- (1) Franke, T.F. et al. (1997) *Cell* 88, 435-7.
- (2) Burgering, B.M. and Coffey, P.J. (1995) *Nature* 376, 599-602.
- (3) Franke, T.F. et al. (1995) *Cell* 81, 727-36.
- (4) Alessi, D.R. et al. (1996) *EMBO J* 15, 6541-51.
- (5) Sarbassov, D.D. et al. (2005) *Science* 307, 1098-101.
- (6) Jacinto, E. et al. (2006) *Cell* 127, 125-37.
- (7) Cardone, M.H. et al. (1998) *Science* 282, 1318-21.
- (8) Brunet, A. et al. (1999) *Cell* 96, 857-68.
- (9) Zimmermann, S. and Moelling, K. (1999) *Science* 286, 1741-4.
- (10) Cantley, L.C. and Neel, B.G. (1999) *Proc Natl Acad Sci USA* 96, 4240-5.
- (11) Vlahos, C.J. et al. (1994) *J Biol Chem* 269, 5241-8.
- (12) Hajduch, E. et al. (2001) *FEBS Lett* 492, 199-203.
- (13) Cross, D.A. et al. (1995) *Nature* 378, 785-9.
- (14) Diehl, J.A. et al. (1998) *Genes Dev* 12, 3499-511.
- (15) Gesbert, F. et al. (2000) *J Biol Chem* 275, 39223-30.
- (16) Zhou, B.P. et al. (2001) *Nat Cell Biol* 3, 245-52.
- (17) Navé, B.T. et al. (1999) *Biochem J* 344 Pt 2, 427-31.
- (18) Inoki, K. et al. (2002) *Nat Cell Biol* 4, 648-57.
- (19) Manning, B.D. et al. (2002) *Mol Cell* 10, 151-62.

* NovaRed™ is a trademark of Vector Labs.

Untreated

LY294002-treated



Immunohistochemical analysis on #8101 SignalSlide® Phospho-Akt (Ser473) IHC Controls (paraffin-embedded LNCaP cells, untreated (left) or LY294002-treated (right)) using Survival Marker: SignalStain® Phospho-Akt (Ser473) IHC Detection Kit (upper). A serial section stained with a matched negative control demonstrates specificity of staining (lower).

SignalStain® Protocol

Reagents Not Supplied:

Xylene
 Ethanol, 100% and 95%
 Distilled water (dH₂O)
 Tris-Buffered Saline + 0.1% Tween-20 (TBS/T): To prepare 1 liter: Add 2.42g Trizma Base, (C₄H₁₁NO₃) and 8g sodium chloride (NaCl) to 800 ml dH₂O. 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 (C₆H₅Na₃O₇•2H₂O) to 800ml dH₂O. 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. dH ₂ O, 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):	Apply 1–2 drops Peroxidase Quench to slide, completely covering tissue. Wash in two changes dH ₂ O and one change TBS/T.	10 minutes, 25°C 3 minutes each
Block (blue cap):	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):	Apply 1–3 drops Negative Control to a separate slide, completely covering tissue. Wash in TBS/T, 3 changes.	Overnight, 4°C 5 minutes each
Biotinylated Secondary Antibody (green cap):	Apply 1–3 drops Biotinylated Secondary Antibody to slide, completely covering tissue. Prepare AB Reagent as directed below. Wash in TBS/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):	Add 1–3 drops premixed AB Reagent to slide, completely covering tissue. Wash in TBS/T, 3 changes	30 minutes, 25°C 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 ₂ O in the clean mixing bottle. Mix well. Apply 1–3 drops Substrate-Chromagen mixture to slide, completely covering tissue. Monitor staining and immerse in dH ₂ O 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 ₂ CO ₃) 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.