Store at 4°C

#8100

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

☑ 1 Kit
(150 slides)

Cell Signaling TECHNOLOGY®

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Products Included	Product #	Color
Peroxidase Quench		Orange
Blocking Solution		Blue
Prediluted Phospho-Akt(Ser473) Antibody		Purple
Prediluted Negative Control		Brown
Biotinylated Secondary Antibody		Green
A and B Reagents		Gray
NovaRed® Substrate		Red
Phospho-Akt(Ser473) Blocking Peptide	#1000	White

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 by phosphorylating and inactivating 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-3α 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-3β 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 tuberin (TSC2), an inhibitor of mTOR within the mTOR-raptor complex (18). Inhibition of mTOR stops the protein synthesis machinery due to inactivation of its effector, p70 S6 kinase and activation of the eukaryotic initiation factor 4E binding protein 1 (4E-EP1), an inhibitor of translation (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 rabbits with a synthetic phospho-peptide (KLH-coupled) 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: 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-Akt (Ser473) Blocking Peptide #1140

Phospho-Akt (Ser473) (736E11) Rabbit mAb (IHC Specific) #3787

SignalSlide™ Phospho-Akt (Ser473) IHC Controls #8101

Background References:

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(12) Hajduch, E. et al. (2001) *FEBS Lett* 492, 199–203.
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(16) Zhou, B.P. et al. (2001) *Nat Cell Biol* 3, 245–52.
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(18) Inoki, K. et al. (2002) *Nat Cell Biol* 4, 648-57.
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Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E**—ELISA **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**—zebra fish **B**—bovine **Dg**—Dog **Pg**—Pig **Sc**—S. cerevisiae **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.	