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Datasheet for 600-401-268S

AKT phospho S473 Antibody

Overview

Description:	Anti-AKT pS473 (RABBIT) Antibody - 600-401-268S
Item No.:	600-401-268S
Size:	25 µL
Applications:	Dot Blot, ELISA, IF, IHC, Multiplex, WB
Reactivity:	Human, Mouse, Rat
Host Species:	Rabbit

Product Details

Background:	AKT is a component of the PI-3 kinase pathway and is activated by phosphorylation at Ser 473 and Thr 308. AKT is a cytoplasmic protein also known as AKT1, Protein Kinase B (PKB) and rac (related to A and C kinases). AKT is a key regulator of many signal transduction pathways. AKT Exhibits tight control over cell proliferation and cell viability. Overexpression or inappropriate activation of AKT is noted in many types of cancer. AKT mediates many of the downstream events of PI 3-kinase (a lipid kinase activated by growth factors, cytokines and insulin). PI 3-kinase recruits AKT to the membrane, where it is activated by PDK1 phosphorylation. Once phosphorylated, AKT dissociates from the membrane and phosphorylates targets in the cytoplasm and the cell nucleus. AKT has two main roles: (i) inhibition of apoptosis; (ii) promotion of proliferation.
Synonyms:	rabbit anti-AKT pS473 Antibody, AKT1 phospho S473, RAC-PK-alpha, Protein kinase B, PKB, C-AKT, RAC-alpha serine/threonine-protein kinase, Proto-oncogene c-Akt, AKT1, AKT 1, AKT-1
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	AKT1
Reactivity:	Human, Mouse, Rat
PTM Specificity:	Phosphorylation

Immunogen Type:	Conjugated Peptide
Immunogen:	Rabbit Anti-AKTpS473 Antibody was prepared by repeated immunizations in rabbits with a synthetic phospho-peptide corresponding to a C-terminus region near phospho Serine 473 of the human, mouse, rat and chicken AKT proteins conjugated to KLH.
Purity/Specificity:	This product was prepared from monospecific antiserum by immunoaffinity chromatography using a phospho peptide coupled to agarose beads followed by solid phase adsorption(s) against a non-phospho peptide and non-specific peptide to remove any unwanted reactivities. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-Rabbit Serum. This antibody is specific for phosphorylated human AKTpS473. Minimal reactivity occurs against non-phosphorylated AKT. Reactivity against AKT from other species may occur but has not yet been tested.
Relevant Links:	• NCBI - 62241011 • UniProtKB - P31749 • GeneID - 207 • 600-401-268 EU SDS

Application Details

Tested Applications:	Dot Blot, ELISA, IF, IHC, Multiplex, WB
Application Note:	This AKT antibody is phospho specific for pS473 and is tested in ELISA, western blotting, immunohistochemistry (formalin-fixed paraffin-embedded sections), and immunofluorescence. By immunoblot a single band of the expected apparent molecular weight ~56kDa is observed. For immunohistochemistry no pre-treatment of sample is required.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:15,000 - 1:60,000
FC:	User Optimized
IF:	User Optimized
IHC:	1:100 - 1:500
WB:	1:200 - 1:1000

Formulation

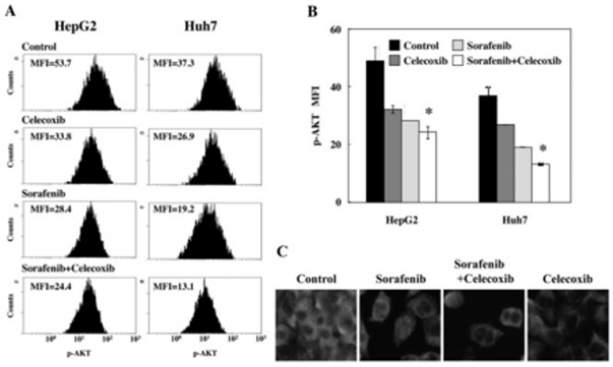
Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

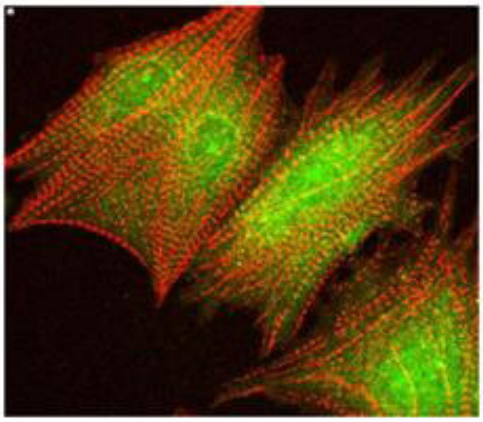
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.
Expiration:	Expiration date is one (1) year from date of receipt.

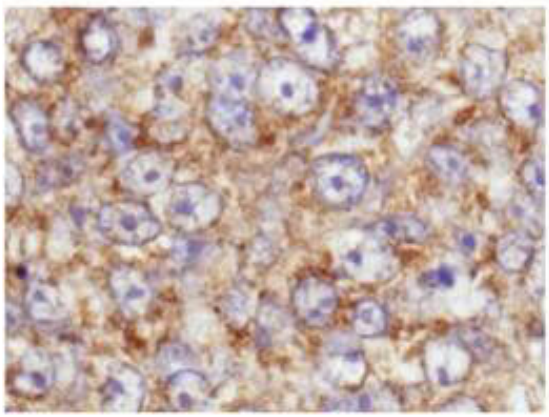
Images



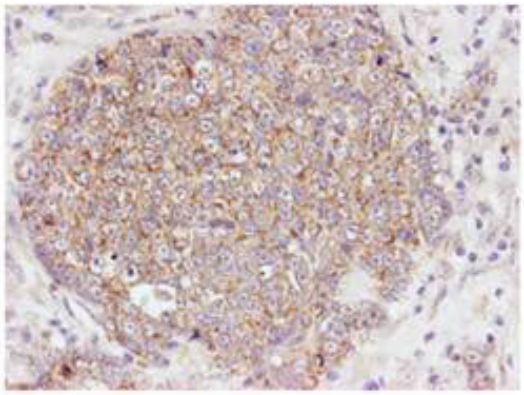
Flow Cytometry
Flow cytometric analysis of phospho-AKT expression in HCC cells. A; Representative flow cytometric data of phospho-AKT staining in HepG2 (left) and Huh7 (right) cells. B; Semi-quantitation of p-AKT levels in HepG2 and Huh7 cells. Phospho-AKT levels were determined using flow cytometry as described in Materials and Methods. Columns represent the mean± standard deviation of three samples in each group. *Statistically significant difference from the control, sorafenib-alone, or celecoxib-alone ($p<0.01$). C; Phospho-AKT immunostaining in HCC cells. HepG2 cells were treated with sorafenib, celecoxib, or a combination of these for 48 h, then stained with an antibody against p-AKT (×400). Fig 4.
PMID: 23564777



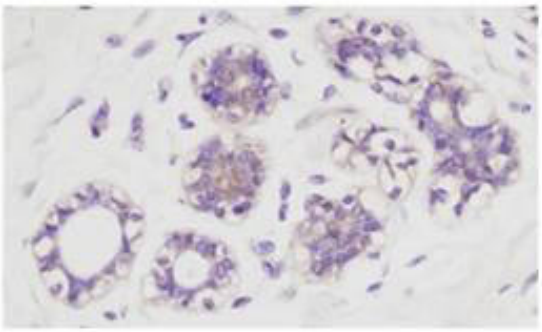
Immunofluorescence Microscopy
Immunofluorescence Confocal Microscopy of Rabbit anti-AKT pS473 antibody. Tissue: cardiomyocytes infected with adenovirus expressing with wild-type AKT. Fixation: 0.5% PFA. Antigen retrieval: not required. Primary antibody: AKT pS473 antibody at 1:40 for 1 h at RT. Secondary antibody: texas-red conjugated rabbit secondary antibody at 1:10,000 for 45 min at RT. Localization: AKT pS473 is nuclear. Staining: AKT pS473 as green fluorescent signal with texas-red conjugated phalloidin (red) to label filamentous actin.



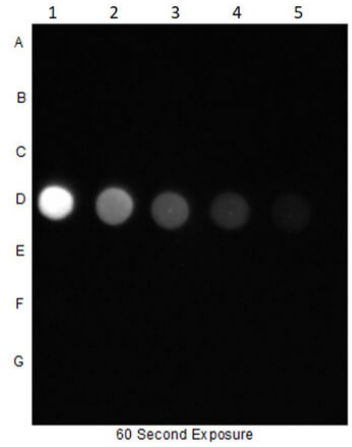
Immunohistochemistry
Immunohistochemistry at higher magnification of Rabbit Anti-Akt pS473 antibody. Tissue: human breast carcinoma. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: Akt pS473 antibody at 100 dilution for 1 h at RT. Secondary antibody: Dako's Techmate streptavidin-biotin reagents at 1:10,000 for 45 min at RT. Localization: Akt pS473 is nuclear and occasionally cytoplasmic.



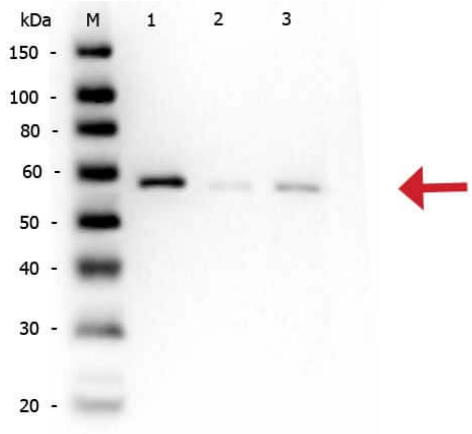
Immunohistochemistry
Immunohistochemistry of Rabbit Anti-Akt pS473 antibody. Tissue: human breast carcinoma. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: Akt pS473 antibody at 100 dilution for 1 h at RT. Secondary antibody: Dako's Techmate streptavidin-biotin reagents at 1:10,000 for 45 min at RT. Localization: Akt pS473 is nuclear and occasionally cytoplasmic.



Immunohistochemistry
Immunohistochemistry of Rabbit anti-AKT pS473 antibody. Tissue: human breast carcinoma. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: AKT pS473 antibody at 1:100 for 1 h at RT. Secondary antibody: Dako's Techmate streptavidin-biotin reagents at 1:10,000 for 45 min at RT. Localization: AKT pS473 is nuclear and occasionally cytoplasmic.



Dot Blot
Dot Blot of Rabbit Anti-AKT pS473 Antibody. Dilutions in Columns: (1) 100ng, (2) 33.33ng, (3) 11.11ng, (4) 3.7ng, (5) 1.23ng. Tested BSA Peptide Reactivity in Rows: (A) AKT1-BSA, (B) AKT1 pT308-BSA, (C) AKT1 S473-BSA, (D) AKT1 pS473-BSA, (E) CDC27 T244-BSA, (F) CDC27 pT244-BSA, (G) BSA control. Primary Antibody: Anti-AKTpS473 at 1µg/mL overnight at 2-8°C. Secondary Antibody: Goat anti-Rabbit IgG HRP (p/n 611-103-122) at 1:70,000 at RT for 30mins. Block: BlockOut Buffer (p/n MB-073).



Western Blot
Western Blot of Rabbit anti-AKT pS473 antibody. Lane 1: AKT1 Recombinant Protein (p/n 009-001-P21). Lane 2: AKT1 Mutant Human Recombinant Protein (p/n 009-001-P22). Lane 3: AKT1 (phosphatase treated) Human Recombinant Protein (p/n 009-001-I51). Load: 50 ng per lane. Primary antibody: AKT pS473 antibody at 1:1,000 for overnight at 4°C. Secondary antibody: Peroxidase rabbit secondary antibody (p/n 611-103-122) at 1:40,000 for 30 min at RT. Block: Blocking Buffer for Fluorescent Western Blotting (MB-070) for 30 min at RT. Predicted/Observed size: ~56 kDa for AKTpS473.

References

- Morisaki T et al. Combining celecoxib with sorafenib synergistically inhibits hepatocellular carcinoma cells in vitro. *Anticancer Res.* (2013)
- Henle SJ, Wang G, Liang E, Wu M, Poo MM, Henley JR. Asymmetric PI(3,4,5)P3 and Akt signaling mediates chemotaxis of axonal growth cones. *J Neurosci.* (2011)

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