

Certificate of Analysis

Product: Affinity Purified Anti-AKT phospho specific pS473 RABBIT

Code: 600-401-268

Lot # 18346

Size: 100 µg

Physical State: Liquid (sterile filtered)

Antibody 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

Stabilizer: None

Preservative: 0.01% (w/v) Sodium Azide

Storage Conditions: Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. Undiluted product is stable for several weeks at 4° C. Dilute only prior to immediate use. Expiration date is one (1) year from date of opening.

Background Information: 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.

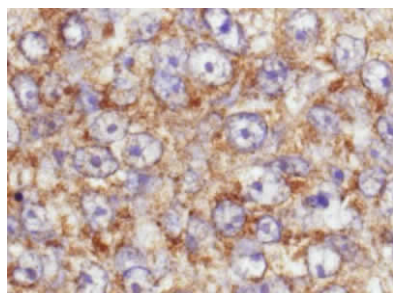


Figure 2. Immunohistochemistry. This image is a higher magnification of the breast tumor image above.

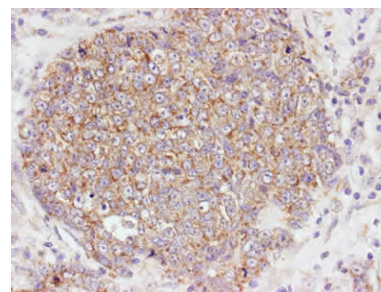


Figure 1. Immunohistochemistry. Rabbit anti-AKT pS473 was used at a 1:100 dilution to detect AKT by immunohistochemistry in human breast tumor tissue. The staining is much stronger than the weak basal level of phosphorylation in normal breast. ROCKLAND's Rabbit anti-AKT pS473 antibody was used with no pretreatment of tissue. Signal was developed using Dako's Techmate streptavidin-biotin reagents. Tissue was formalin-fixed and paraffin embedded.

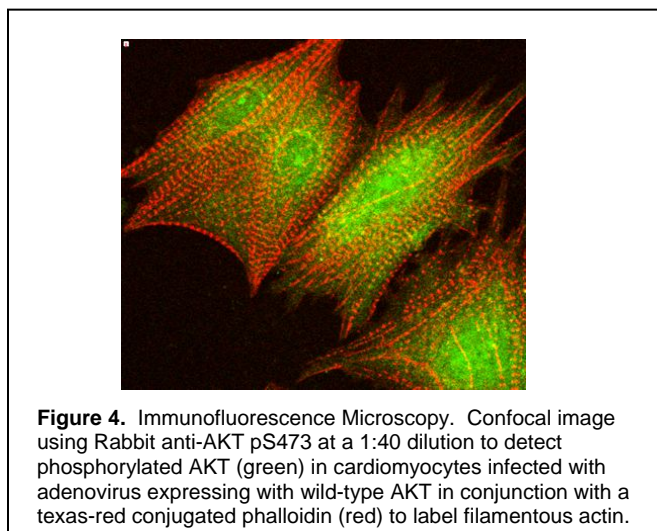
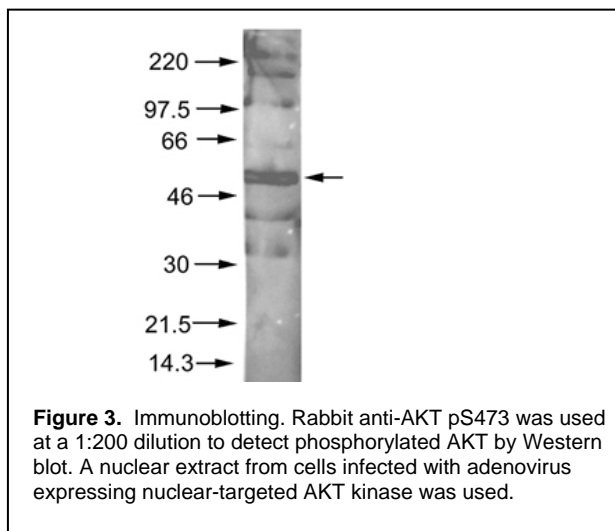
Recommended Dilutions:

ELISA	1:15,000 - 1:60,000
WESTERN BLOT	1:200 - 1:1000
IMMUNOHISTOCHEMISTRY	1:100 - 1:500
OTHER APPLICATIONS	User Optimized

Application Note(s): This antibody is phospho specific for pS473 and is suitable for western blotting, immunohistochemistry (formalin-fixed paraffin-embedded sections), immunofluorescence and ELISA. By immunoblot a single band of the expected apparent molecular weight is observed. For immunohistochemistry no pre-treatment of sample is required.

Immunogen: This whole rabbit serum was prepared by repeated immunizations with a synthetic peptide corresponding to the C-terminus aa 460-480 of human, mouse, rat and chicken AKT proteins conjugated to KLH.

Purity and Specificity: This product was prepared from monospecific antiserum by immunoaffinity chromatography using phospho peptide coupled to agarose beads followed by solid phase adsorption(s) against 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 AKT. Minimal reactivity occurs against non-phosphorylated AKT. Reactivity against AKT from other species may occur but has not yet been tested.

Relevant Link(s): Swiss Prot: [P31749](#)NCBI Link [AAH00479](#)**Protein Sequence:** Human AKT1 protein, 480 aa, predicted MW 55.7kDa

1	msdvaivkeg	wlhkrgeyik	twrpryflk	ndgtfigyke	rpqvdqrea	plnnfsvaqc
61	qlmkteprp	ntfiirclqw	ttvierthv	etpeereewt	taiqtvadgl	kkqeeemdf
121	rsgspsdnsg	aeemevslak	pkhrvtmnef	eylklgkgt	fgkvlvkek	atgryyamki
181	lkkevivaqd	evahtltenr	vlqnsrhpfl	talkysfqth	drlicvmeya	nggelfhls
241	rervfsedra	rfgaeivsa	ldylhseknv	vyrdklenl	mldkdghiki	tdfglckegi
301	kdgatmkfvc	gtpeylapev	ledndygrav	dwwglgvvmy	emmcgrlpfy	nqdhckfel
361	ilmeeirfpr	tigpeaksll	sgllkkdpkq	rlgggsedak	eimqhrffag	iwqhvyeck
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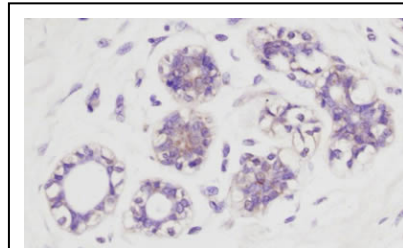


Figure 5. Immunohistochemistry. Rabbit anti-AKT pS473 was used at a 1:100 dilution to detect AKT by immunohistochemistry in normal human breast tissue. AKT is weakly phosphorylated in normal tissue in the breast. The phosphorylated AKT is clearly localized in the cytoplasm. ROCKLAND's Rabbit anti-AKT pS473 antibody was used with no pretreatment of tissue. Signal was developed using Dako's Techmate streptavidin-biotin reagents. Tissue was formalin-fixed and paraffin embedded.

Related Product(s):

#000-000-401 CONTROL PEPTIDE for Rb-anti-AKT (Hu/Ms/Rt)
 #100-401-401 Rabbit-anti-AKT (Human/Mouse/Rat specific)
 #600-401-269 Rabbit-anti-AKT pT308 (Human)
 #600-401-268 Rabbit-anti-AKT pS473 (Human)
 #611-103-122 HRP Anti-Rabbit IgG [H&L] MX10 (GOAT)
 #611-132-122 IRDye800 Anti-Rabbit IgG [H&L] MX10 (GOAT)

General References:

Lawlor, M. A. and Alessi, D.R. (2001). PKB/AKT: a key mediator of cell proliferation, survival and insulin responses. *J. Cell Science* **114**:2903-2910.

Alessi, D. R. (2001). Discovery of PDK1, one of the missing links in insulin signal transduction. *Biochem. Soc. Trans.* **29**,1 -14.

USDA Certification: All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation.

Note: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.