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### Certificate of Analysis

**Product:** Affinity Purified Anti-AKT phospho specific pT308 RABBIT

**Code:** 600-401-269

**Lot #** 20173

**Size:** 100 µg

**Physical State:** Liquid (sterile filtered)

**Antibody Concentration:** 0.72 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. This product is stable for several weeks at 4° C as an undiluted liquid. 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 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.

**Application Note(s):** This antibody is phospho specific for pT308 and is suitable for western blotting and ELISA.

**Recommended Dilutions:** This product was assayed against the peptide immunogen in a standard sandwich ELISA using Peroxidase conjugated anti-Rabbit IgG [H&L] (Goat) code #611-1302 and ABTS (2,2'-azino-bis-[3-ethylbenthiiazoline-6-sulfonic acid]) code # ABTS-100 as a substrate for 30 minutes at room temperature. A working dilution of 1:20,000 to 1:70,000 is suggested for this product. A single band of the expected apparent molecular weight was observed by immunoblot using a 1:500 dilution of the antibody. Optimal titers for other applications should be determined by the researcher.

**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.

**Immunogen:** This whole rabbit serum was prepared by repeated immunizations with a synthetic peptide corresponding to aa 302-311 of human AKT protein conjugated to KLH.

**Protein Sequence:** Human AKT (PKB), 480 aa, predicted MW 55.6 kDa

1	msdvaivkeg	wlhrgeyik	twrpryflk	ndgtfigyke	rpqdvqrea	plnfsvaqc
61	qlmkerprp	ntfiirclqw	ttviertfhv	etpeereewt	taiqtvadgl	kkqeeemdf
121	rsgspdsnsg	aeemevslak	pkhrvtmnef	eyklilgkgt	fgkvilvkek	atgryyamki
181	lkkevivaqd	evahtltenr	vlqnsrhpfl	talkysfqth	drlcfvmeya	nggelffhls
241	rervfsedra	rfygaeivsa	ldylhseknv	vyrdlkleni	mldkdghiki	tdfglckegi
301	<b>kdgatk<sup>1</sup>fc</b>	<b>gtpeylapev</b>	ledndygrav	dwwglgvvmy	emmcgrlpfy	nqdheklfel
361	ilmeeirfpr	tlgpeaksl	sgllkkdpkq	rlgggsedak	eimqhrffag	ivwqhvyekk
421	lspfpkpqvt	setdtryfde	eftaqmitit	ppdqddsmec	vdserrphfp	qfsysasgta

### 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.

Hedjazifar, S., Jenn Dahl, L.E., Shimokawa, H. and Baeckstrom, D. (2005) PKB mediates c-erbB2-induced epithelial beta1 integrin conformational inactivation through Rho-independent F-actin rearrangements. *Exp. Cell Res.* **307** (1), 259-275.

Saji, M., Vasko, V., Kada, F., Allbritton, E.H., Burman, K.D. and Ringel, M.D. (2005) Akt1 contains a functional leucine-rich nuclear export sequence. *Biochem. Biophys. Res. Commun.* **332** (1), 167-173.

Sutherland, B.W., Kucab, J., Wu, J., Lee, C., Cheang, M.C., Yorida, E., Turbin, D., Dedhar, S., Nelson, C., Pollak, M., Leighton Grimes, H., Miller, K., Badve, S., Huntsman, D., Blake-Gilks, C., Chen, M., Pallen, C.J. and Dunn, S.E. (2005) Akt phosphorylates the Y-box binding protein 1 at Ser102 located in the cold shock domain and affects the anchorage-independent growth of breast cancer cells. *Oncogene* **24** (26), 4281-4292.

### Related Product(s):

<a href="#">#000-000-401</a>	CONTROL PEPTIDE for Rabbit-anti-AKT (Human/Mouse/Rat specific)
<a href="#">#100-401-401</a>	Rabbit-anti-AKT (Human/Mouse/Rat specific)
<a href="#">#600-401-269</a>	Rabbit-anti-AKT pT308 (Human)
<a href="#">#600-401-268</a>	Rabbit-anti-AKT pS473 (Human)
<a href="#">#611-703-127</a>	Peroxidase Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (DONKEY) MX10
<a href="#">#611-132-122</a>	IRDye® 800 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
<a href="#">#B501-0500</a>	BLOTTO (500 g)
<a href="#">#BSA-30</a>	30% BOVINE SERUM ALBUMIN SOL'N in 0.85% sodium chloride (no preservative or stabilizer) (5)
<a href="#">#B304</a>	NORMAL GOAT SERUM (NGS) (10 ml)
<a href="#">#KIA-003</a>	<b>MaxTag™</b> Anti-RABBIT IgG Kit for Immunoblotting
<a href="#">#MB-070</a>	Blocking Buffer for Fluorescent Western Blotting

**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.

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