

Certificate of Analysis

Product: Anti-AKT pS473 Monoclonal Antibody

Code: 200-301-268

Lot # 22649

Size: 100 µg

Physical State: Liquid (sterile filtered)

Antibody Concentration: 1.0 mg/ml (by UV absorbance at 280 nm)

Isotype: IgG₁ Kappa

Clone: 17B6.B11.A12.C3.G7.G12

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 or below prior to opening. Dilute only prior to immediate use. For extended storage, aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. 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.

Application Note(s): This monoclonal antibody is suitable for ELISA, immunohistochemistry, immunoprecipitation and western blotting. Expect a band approximately 56 kDa in size corresponding to phosphorylated AKT protein by western blotting in the appropriate cell lysate or extract. This phospho-specific monoclonal antibody reacts with human and mouse AKT pS473 and shows minimal reactivity by ELISA against the non-phosphorylated form of the immunizing peptide. Specific conditions for reactivity should be optimized by the end user. For immunohistochemistry use formalin-fixed paraffin-embedded sections. No pre-treatment of sample is required.

Recommended Dilutions:

ELISA	1:20,000
WESTERN BLOT	1:500 - 1:3,000
IMMUNOHISTOCHEMISTRY	20 µg/ml
OTHER APPLICATIONS	User Optimized

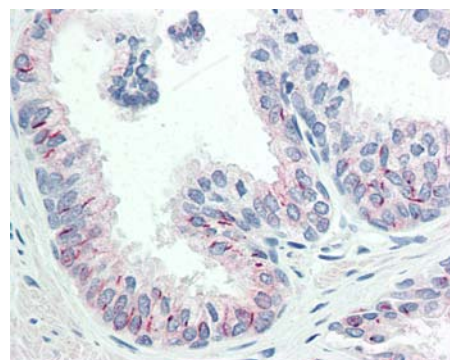
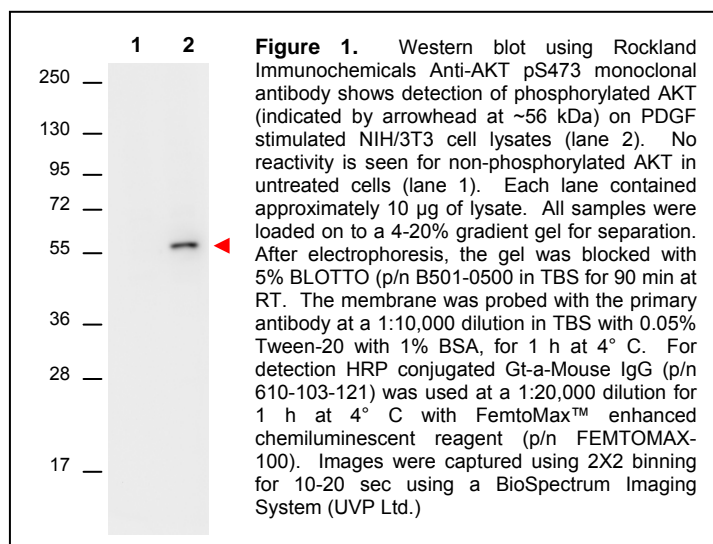


Figure 2. Immunohistochemistry using Rockland Immunochemicals Anti-AKT pS473 monoclonal antibody shows detection of phosphorylated AKT pS473 in human prostate tissue. The antibody was used at 20 µg/mL. The staining is much stronger than the weak basal level of phosphorylation in normal prostate tissue. Tissue was formalin fixed and paraffin embedded. No pre-treatment of sample was required. Signal was developed using Dako's Techmate streptavidin-biotin reagents. *Personal communication, Glenna Burner, Lifespan Biosciences, Seattle, WA.*

Purity and Specificity: This product was purified from concentrated tissue culture supernate by Protein A chromatography. This antibody is specific for human and mouse AKT protein phosphorylated at S473. A BLAST analysis was used to suggest cross-reactivity with AKT pS473 from human, mouse, rat and chimpanzee sources based on 100% homology with the immunizing sequence. Cross-reactivity with AKT from other sources has not been determined. Cross-reactivity with AKT2 and AKT3 has not been determined.

Hybridoma: Produced by fusing BALB/c mouse splenocytes and mouse myeloma SP2/O cells using conventional technology.

Immunogen: This monoclonal antibody was produced by repeated immunizations with a synthetic peptide corresponding to residues surrounding S473 of human AKT1 protein.

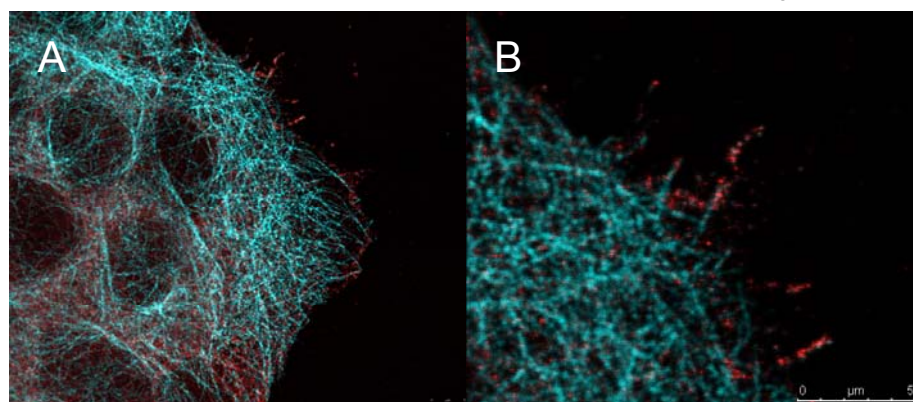


Figure 3. High resolution STED Immunofluorescence nanoscopy. Rockland Immunochemicals' Anti-AKT pS473 Monoclonal Antibody (p/n 200-301-268) was used at 10 $\mu\text{g}/\text{mL}$ for 1 h at RT to stain A431 cells after serum deprivation for 12 h. Cells were fixed in 4% paraformaldehyde for 5 min and after washes blocked with 10% NGS/0.2% Triton X-100 for 30 min. Phosphorylated AKT (red) is observed to be localized in the cytoplasm and also organized at the periphery of the cell. Sequential staining at 1.4 $\mu\text{g}/\text{mL}$ for 1 h at RT with rabbit anti- α tubulin (cyan) (p/n 600-401-880) is also shown. The merge images (A) and at high magnification (B) show phosphorylated AKT colocalized with the distal microtubules. High magnification images (D, E and F) show phosphorylated AKT appears to be colocalized to microtubules. Images were taken with a 100x objective on a Leica TCS STED (Stimulated Emission and Depletion), Leica Microsystems, Inc. Exton, PA, USA. Atto 647N anti-Mouse IgG (ATTO TEC GmbH), and DyLight™488 anti-Rabbit IgG (p/n 611-141-122) were used at 1.0 $\mu\text{g}/\text{mL}$ for 1 h at RT for indirect detection. Prolong® antifade reagent (Invitrogen) was used to mount slides. *Personal Communication, Myriam Gastard, Leica Microsystems Inc, Exton, PA (USA).*

Relevant Link(s): Swiss Prot: [P31749](#)

NCBI Link [NP_005154](#)

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.

Jones, P.F., Jakubowicz, T., Pitossi, F.J., Maurer, F. and Hemmings, B.A. (1991) Molecular cloning and identification of a serine/threonine protein kinase of the second-messenger subfamily. *Proc. Natl. Acad. Sci. U.S.A.* **88** (10), 4171-4175.

Staal, S.P. (1987) Molecular cloning of the akt oncogene and its human homologues AKT1 and AKT2: amplification of AKT1 in a primary human gastric adenocarcinoma. *Proc. Natl. Acad. Sci. U.S.A.* **84** (14), 5034-5037.

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#BSA-30	30% BOVINE SERUM ALBUMIN SOL'N in 0.85% sodium chloride (no preservative or stabilizer) (500 ml)
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#MB-070	Blocking Buffer for Fluorescent Western Blotting

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