ROCKLAND

Order online now!

www.rockland.com
tech@rockland.com
+1 484.791.3823

Datasheet for 200-301-B19

AKT phospho S473 Antibody

Overview

Description:	Anti-AKT pS473 (MOUSE) Monoclonal Antibody - 200-301-B19
Item No.:	200-301-B19
Size:	1 mg
Applications:	IF, IHC, WB
Reactivity:	Human, Mouse
Host Species:	Mouse

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. Anti-AKT pS473 (MOUSE) Monoclonal Antibody is ideal for investigators involved in Cell Signaling, Cancer, Neuroscience, Signal Transduction research.
Synonyms:	mouse anti-AKT pS473 Antibody, 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:	Mouse
Clonality:	Monoclonal
Clone ID:	17F6.B11
Format:	IgG1

Target Details

Gene Name:	AKT1

Reactivity:	Human, Mouse
PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide
Immunogen:	This monoclonal antibody was produced by repeated immunizations with a synthetic phospho-peptide corresponding to residues surrounding S473 of human AKT1 protein.
Purity/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.
Relevant Links:	• UniProtKB - P31749 • NCBI - 62241011 • GeneID - 207

Application Details

Tested Applications:	IF, IHC, WB
Application Note:	This monoclonal antibody is tested in ELISA, immunohistochemistry, immunofluorescent microscopy, 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. Cell Signaling, Cancer, Neuroscience, Signal Transduction research.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:20,000
FC:	User Optimized
IF:	1:500 - 1:3,000
IHC:	20 µg/ml
WB:	1:500 - 1:3,000

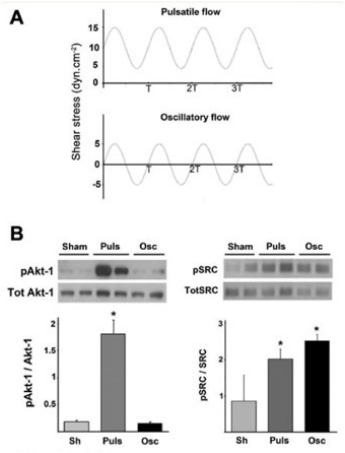
Formulation

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

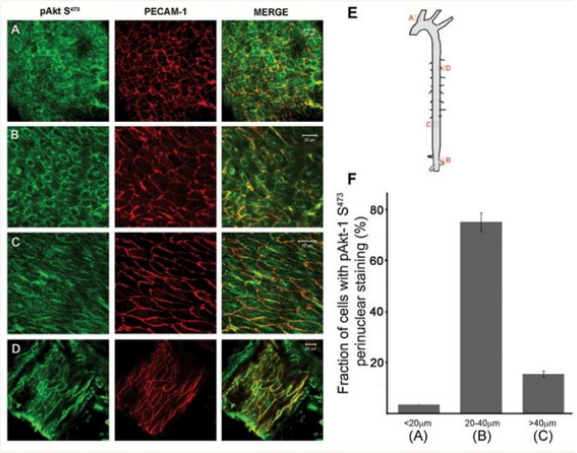
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C 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:	Expiration date is one (1) year from date of receipt.

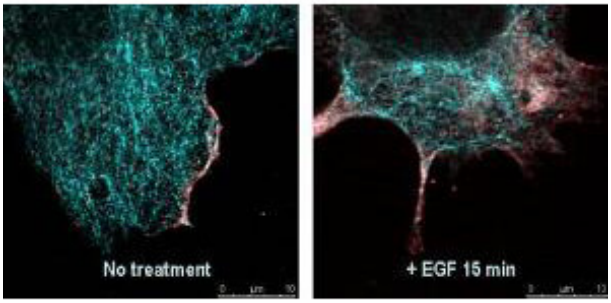
Images



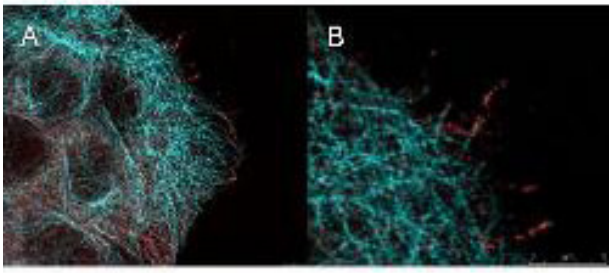
Western Blot
A forward flow component is required for flow-induced Akt-1 activation. A: Pulsatile (Puls) and oscillatory (Osc) flow were applied at similar frequency and amplitude of flow (10 dyn cm−2, T = 1 s). B: Akt-1 (S473) and Src (Y416) phosphorylation levels respectively in non-adapted HUVEC monolayers after 5 min of pulsatile or oscillatory flow or in sham-mounted slides (Sh). Ratio pAkt-1/Akt-1: Puls: 1.82 ±0.25, Osc: 0.15 ±0.01, P= 0.032. Representative blots with duplicates are shown above. Fig 4. PMID: 23913776



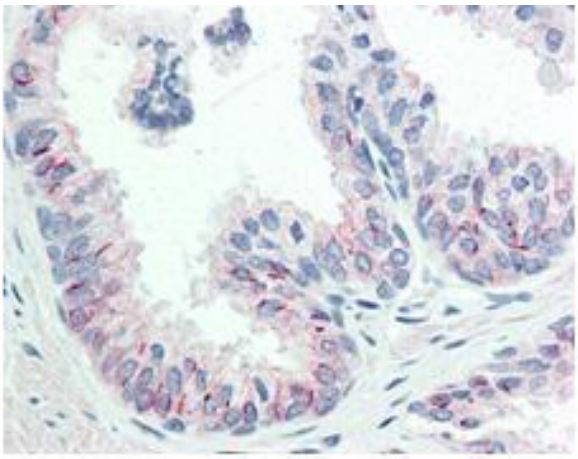
Immunofluorescence Microscopy
Cell morphology and spatiolocalization of phosphorylated Akt-1 in the mouse aorta. En face immunostaining of pAkt-1S473 (green) and PECAM-1 (red) in the brachiocephalic area (A), the renal artery bifurcation (B), the distal part of the descending aorta (C), and the adjacent intercostals artery (D). Scale bar is 20 µm. E: Sites along the aorta where samples were derived. F: Bar graph illustrating the fraction of EC expressing perinuclear pAkt-1S473 staining in regards to cell diameter from areas shown in a, b, and c. Fig 1. PMID: 23913776



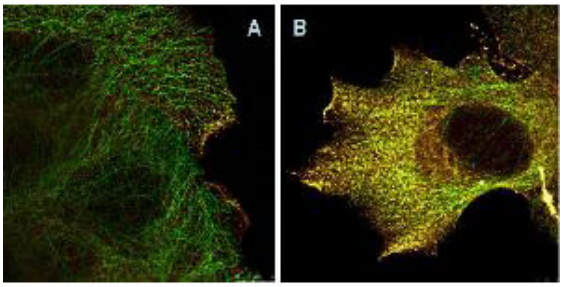
Immunofluorescence Microscopy
Immunofluorescence confocal microscopy of Mouse Anti-AKT pS473 antibody. Tissue: EGF treated A431 cells. Fixation: 0.5% PFA. Antigen retrieval: EGF 15 min. Primary antibody: AKT pS473 antibody at 10 µg/mL for 1 h at RT. Secondary antibody: DyLight 488™ Goat anti-Rabbit IgG, MAb anti-AKT pS473, atto-647N anti-Mouse IgG (Active Motif). at 1:10,000 for 45 min at RT. Localization: AKT pS473 is nuclear and occasionally cytoplasmic. Staining: AKT pS473 as red signal with tubulin (cyan).



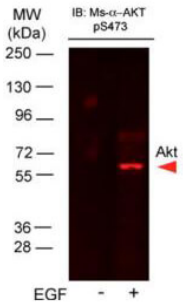
Immunofluorescence Microscopy
High resolution STED immunofluorescence nanoscopy of Mouse anti-AKT pS473 antibody. Tissue: A431 cells. The merge images (A) and at high magnification (B) show phosphorylated AKT colocalized with the distal microtubules. Fixation: 4% paraformaldehyde for 5 min and after washes blocked with 10% NGS/0.2% Triton X-100 for 30 min. Antigen retrieval: serum deprivation for 12 h. Primary antibody: AKT pS473 antibody at 10 µg/mL and α-tubulin (cyan) (p/n 600-401-880) at 1.4 µg/mL for 1 h at RT. Secondary antibody: Atto 647N anti-Mouse IgG (ATTO TEC GmbH), and DyLight™488 anti-Rabbit IgG (p/n 611-141-122) were used at 1.0 µg/mL for 1h at RT for indirect detection. Localization: AKT pS473 is in the cytoplasm and also organized at the periphery of the cell. Staining: AKT pS473 as red signal with bis-benzimide (blue) nuclear counterstain.



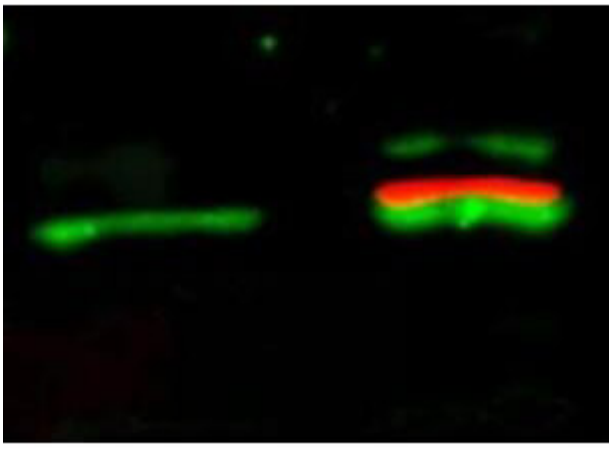
Immunohistochemistry
Immunohistochemistry of Mouse anti-AKT pS473 antibody. Tissue: human prostate tissue. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: AKT pS473 antibody at 20 µg/mL for 1 h at RT. Secondary antibody: Dako's Techmate streptavidin-biotin reagents at 1:10,000 for 45 min at RT. Localization: AKT pS473 is nuclear and occasionally cytoplasmic. Staining: AKT pS473 as precipitated red signal with hematoxylin purple nuclear counterstain.



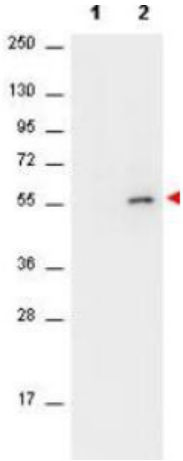
Immunofluorescence Microscopy
Immunofluorescence Microscopy of Mouse Anti-AKTpS473 antibody using STED nanoscopy to evaluate AKT activation and migration. Tissue: A431 cells. Antigen retrieval: Panel A: serum starved, unstimulated cells. Panel B: serum starved, EGF stimulated for 15 mins. A massive increase in AKT-pS473 activation, as measured by intensity signal, peaked at 15 minutes and was associated with depolymerized tubulin. Staining: Panel A shows STED data (AKT-pS473, red channel) collected simultaneously with confocal signal (a-tubulin, green channel). Upon stimulation of cells with EGF, a rapid activation of AKT is observed (Panel B) along with a coincident change in the tubulin organization (yellow signal), as well as an extensive cell shape-change (cell membrane folding) and accumulation of AKTpS473 at the cell periphery.



Western Blot
Western Blot of Mouse Anti-AKTpS473 antibody. Lane 1: A431 cell lysate (p/n W09-000-361). Lane 2: A431 cells stimulated for 15 min with EGF (p/n W09-000-362). Load: 35 µg per lane. Primary antibody: AKTpS473 antibody at 1:400 for overnight at 4°C. Secondary antibody: DyLight™649 Conjugated Anti-AKT pS473 Monoclonal Antibody (p/n 200-343-268) at 1:10,000 for 45 min at RT. Block: Blocking Buffer for Fluorescent Western Blotting (p/n MB-070) overnight at 4°C. Predicted/Observed size: 56kDa. Other band(s): none.



Western Blot
Western Blot of Mouse Anti-Akt pS473 antibody. Lane 1: unstimulated NIH/3T3 lysates (p/n W10-000-358) contain inactive unphosphorylated Akt1, green band. Lane 2: PDGF stimulated NIH/3T3 lysate (p/n W10-001-377) contains both inactive (green band) and activated phosphorylated Akt1 (red band). Load: 10 µg per lane. Primary antibody: rabbit anti-Akt (pan) (p/n 100-401-401) and mouse anti-Akt pS473 (p/n 200-301-B19) specific antibodies at 1:400 for overnight at 4°C. Secondary antibody: anti-rabbit IgG DyLight™ 549 (green) and anti-mouse IgG DyLight™ 649 conjugated (red) secondary antibodies at 1:10,000 for 45 min at RT. Block: 5% BLOTTO overnight at 4°C.



Western Blot
Western Blot of Mouse anti-AKT antibody. Lane 1: unstimulated NIH/3T3 cell lysates (p/n W10-000-358). Lane 2: PDGF stimulated NIH/3T3 cell lysates (p/n W10-001-377). Load: 10 µg per lane. Primary antibody: AKT antibody at 1:400 for overnight at 4°C. Secondary antibody: HRP conjugated Gt-a-Mouse IgG (p/n 610-103-121) was used at a 1:40,000 dilution for 1 h at 4° C with FemtoMax™ enhanced chemiluminescent reagent (p/n FEMTOMAX-100). Block: 5% BLOTTO (p/n B501-0500 in TBS for 2h at RT. Observed size: ~56 kDa for AKT. Other band(s): none.

References

- Melchior, B et al. Distinctive subcellular Akt-1 responses to shear stress in endothelial cells. *Journal of Cellular Biochemistry* (2014)

Disclaimer

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. 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. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.