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Datasheet for 200-301-268

AKT phospho S473 Antibody

Overview

Description:	Anti-AKT pS473 (MOUSE) Monoclonal Antibody - 200-301-268
Item No.:	200-301-268
Size:	100 µg
Applications:	ELISA, IF, IHC, Multiplex, WB, FC
Reactivity:	Human, Mouse, Rat, Monkey
Host Species:	Mouse

Product Details

Background:	AKT phospho S473 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, phospho AKT, 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, Rat, Monkey
PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide
Immunogen:	Anti-AKT pS473 (MOUSE) Monoclonal Antibody was produced by repeated immunizations with a synthetic phospho-peptide corresponding to residues surrounding S473 of human AKT1 protein, followed by hybridoma development.
Purity/Specificity:	Anti-AKT pS473 Monoclonal Antibody was purified from concentrated tissue culture supernate by Protein A chromatography. This phospho specific monoclonal antibody is specific for phosphorylated human and mouse AKT protein 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:	ELISA, IF, IHC, Multiplex, WB
Suggested Applications:	FC (Based on references)
Application Note:	Phospho AKT antibody is tested in ELISA, immunofluorescence, immunohistochemistry, flow cytometry, 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.
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
IP:	User Optimized
WB:	1:500-1:3,000

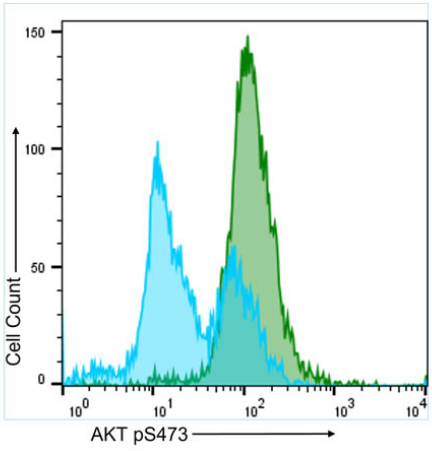
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	0.996 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

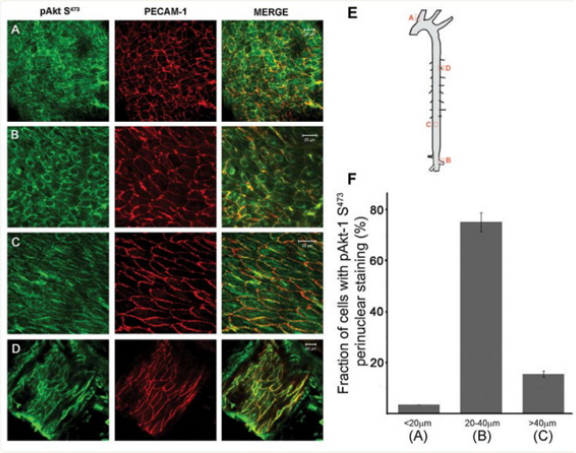
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store Anti-AKT pS473 (MOUSE) Monoclonal Antibody 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.

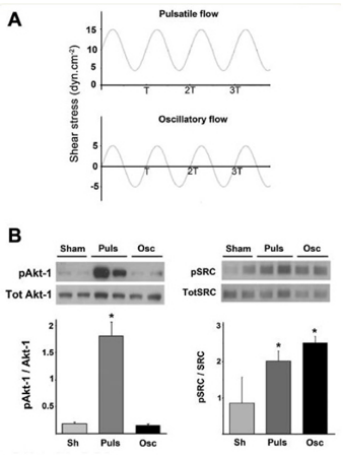
Images



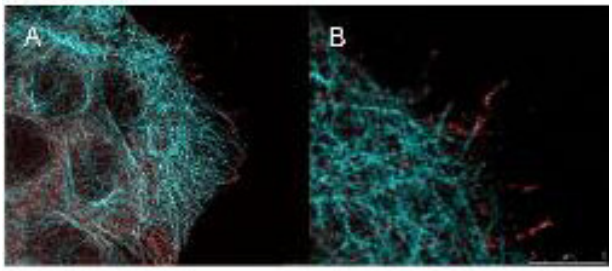
Flow Cytometry
Flow Cytometry results of Anti-AKT pS473 (MOUSE) Monoclonal Antibody. The green histogram represents the A431 cells that were stimulated for 15 minutes with 100 ng/mL EGF. The blue histogram shows the untreated A431 cell population, which is bimodal. Both populations were stained with a 1:50 dilution of the Anti-AKT pS473 (MOUSE) Monoclonal Antibody (p/n 200-301-268) for 30 mins at 4°C. The secondary antibody, Anti-MOUSE IgG (H&L) (GOAT) Antibody DyLight™ 488 Conjugated (p/n 610-141-002) was used at a 1:200 dilution for 30 mins at 4°C.



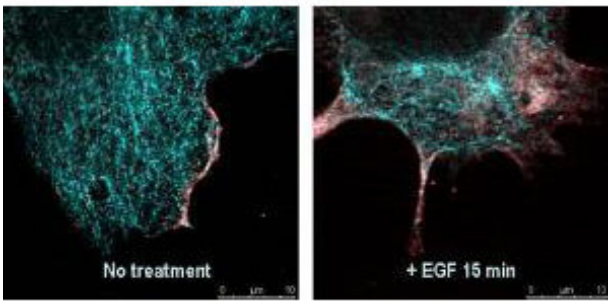
Immunofluorescence Microscopy
Cell morphology and spatiolocalization of phosphorylated Akt-1 in the mouse aorta. En face immunostaining of pAkt-1S473 (green) and PECAM-1 (red) in the brachiocephalic area (A), the renal artery bifurcation (B), the distal part of the descending aorta (C), and the adjacent 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



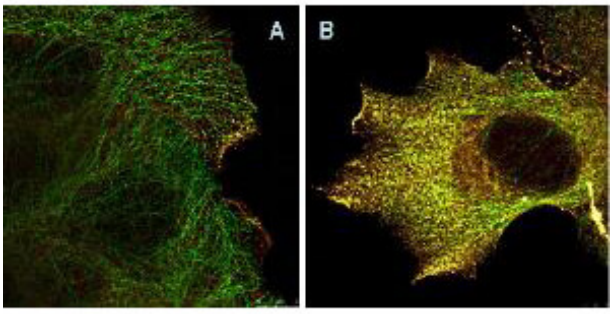
Western Blot
A forward flow component is required for flow-induced Akt-1 activation. A: Pulsatile (Puls) and oscillatory (Osc) flow were applied at similar frequency and amplitude of flow (10 dyn cm−2, T = 1 s). B: Akt-1 (S473) and Src (Y416) phosphorylation levels respectively in non-adapted HUVEC monolayers 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



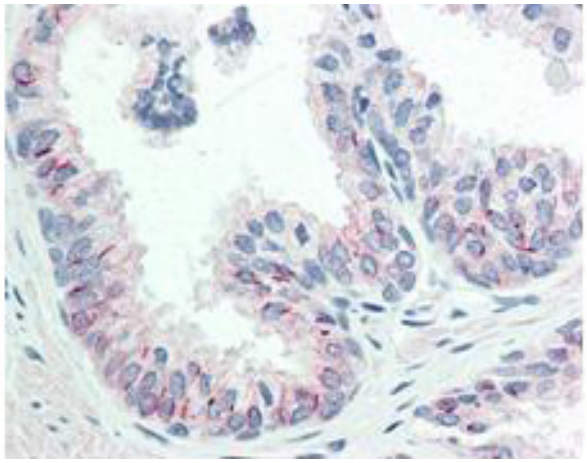
Immunofluorescence Microscopy
High resolution STED immunofluorescence nanoscopy of Mouse anti-AKT pS473 antibody. Tissue: A431 cells. The merge images (A) and at high magnification (B) show phosphorylated AKT colocalized with the distal microtubules. Fixation: 4% paraformaldehyde for 5 min and after 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.



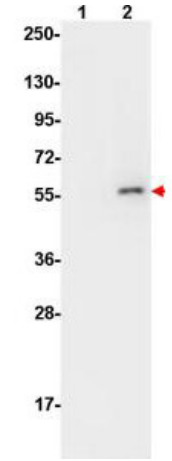
Immunofluorescence Microscopy
Immunofluorescence confocal microscopy of Mouse Anti-AKT pS473 antibody. Tissue: EGF treated A431 cells. Fixation: 0.5% PFA. Antigen retrieval: EGF 15 min. Primary antibody: AKT pS473 antibody at 10 µg/mL for 1 h at RT. Secondary antibody: DyLight 488™ Goat anti-Rabbit IgG, 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).



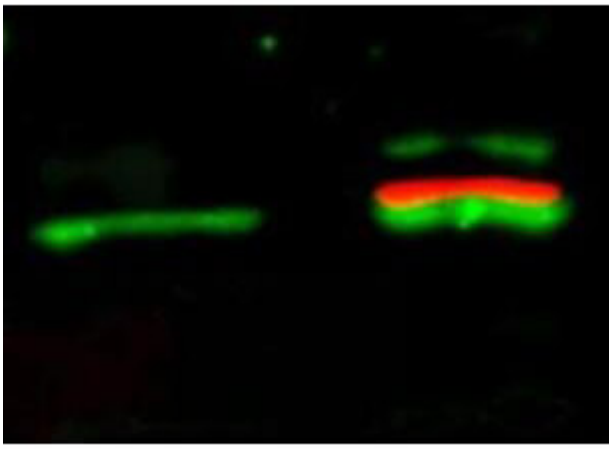
Immunofluorescence Microscopy
Immunofluorescence Microscopy of Mouse Anti-AKTpS473 antibody using STED nanoscopy to evaluate AKT activation and migration. Tissue: A431 cells. Antigen retrieval: Panel A: serum starved,unstimulated cells. Panel B: serum starved, EGF stimulated for 15 mins. A massive increase 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.



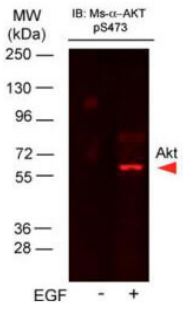
Immunohistochemistry
Immunohistochemistry of Mouse anti-AKT pS473 antibody. Tissue: human prostate tissue. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: AKT pS473 antibody at 20 µg/mL for 1 h at RT. Secondary antibody: Dako's Techmate streptavidin-biotin 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.



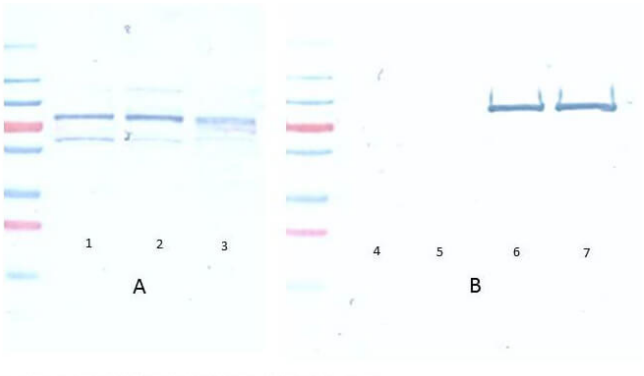
Western Blot
Western Blot of Mouse Anti-AKT pS473 antibody. Lane 1: non-phosphorylated AKT in untreated cells. Lane 2: phosphorylated AKT (indicated by arrowhead at ~56 kDa) on PDGF stimulated NIH/3T3 cell lysates. Load: 10 µg per lane. Primary antibody: AKT pS473 antibody at 1:10,000 in TBS with 0.05% Tween-20 with 1% BSA, for 1 h at 4° C. Secondary antibody: HRP conjugated Gt-a-Mouse IgG (p/n 610-103-121) was used at a 1:20,000 dilution for 1 h at 4° C with FemtoMax™ enhanced chemiluminescent reagent (p/n FEMTOMAX-100). Other band(s): none.



Western Blot
Western Blot of Mouse Anti-Akt pS473 antibody. Lane 1: unstimulated NIH/3T3 lysates contain inactive unphosphorylated Akt1, green band. Lane 2: PDGF stimulated NIH/3T3 lysate contains both inactive (green band) and activated phosphorylated Akt1 (red band). Load: 10 µg per lane. Primary antibody: rabbit anti-Akt (pan) and mouse anti-Akt pS473 specific antibodies at 1:400 for overnight at 4°C. Secondary antibody: DyLight™ 549 conjugated anti-rabbit IgG (green) and DyLight™ 649 conjugated anti-mouse IgG (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-AKTpS473 antibody. Lane 1: A431 cells. Lane 2: A431 cells stimulated for 15 min with EGF. 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. A: Lane 1) PDGF stimulated NIH 3T3 cells (p/n W10-001-377) [10µl]. Lane 2) NIH 3T3 cells (p/n W10-000-358) [10µl]. Lane 3) Hela whole cell lysate (p/n W09-000-364) [10µl] (weak signal). B: Lane 4) GST negative control protein (p/n 000-001-200) [100ng]. Lane 5) GST negative control protein (p/n 000-001-200) [25ng]. Lane 6) AKT 1 recombinant protein (p/n 009-001-P21) [100ng]. Lane 7) AKT 1 recombinant protein (p/n 009-001-P21) [25ng]. Block: 5% BSA overnight at 4°C. Primary antibody: Rockland monoclonal anti-AKT antibody (200-301-268S, lot no. 27843) used at 1:1000 for overnight at 4°C. Secondary antibody: HRP Conjugated goat anti-mouse (p/n 610-403-C46, lot 20121) 1:25K for 45 min at RT. Detection: (TMBM-100) for 20 minutes, rinsed with deionized water, dried and scanned on conventional flatbed scanner.

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