Product Datasheet

AKT phospho S473 Antibody (orb344403)

Catalog Number	orb344403
Category	Antibodies
Description	Akt (phospho-S473) antibody
Clonality	Monoclonal
Species/Host	Mouse
Isotype	IgG1
Conjugation	Unconjugated
Reactivity	Human, Monkey, Mouse, Rat
Form/Appearance	Liquid (sterile filtered)
Concentration	1.0 mg/ml
Buffer/Preservatives	Preservative: 0.01% (w/v) Sodium Azide. Stabilizer: None; Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Purity	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.
Immunogen	Anti-AKT pS473 (MOUSE) Monoclonal Antibody was produced by repeated immunizations with a synthetic peptide corresponding to residues surrounding S473 of human AKT1 protein, followed by hybridoma development.

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UniProt ID	**P31749**
Tested applications	ELISA, IF, IHC, Multiplex Assay, WB
Dilution range	ELISA: 1:20,000, IHC: 20 µg/mL, IF: 1:500-1:3,000, WB: 1:500-1:3,000
Application notes	Phospho AKT antibody is tested in ELISA, immunofluorescence, immunohistochemistry, 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.
Antibody Type	Primary Antibody
Clone Number	17F6.B11
Storage	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.
Dry Ice Shipping	**Please note: This product requires shipment on dry ice. A dry ice surcharge will apply.**
Note	For research use only
NCBI	**62241011**
Expiration Date	12 months from date of receipt.

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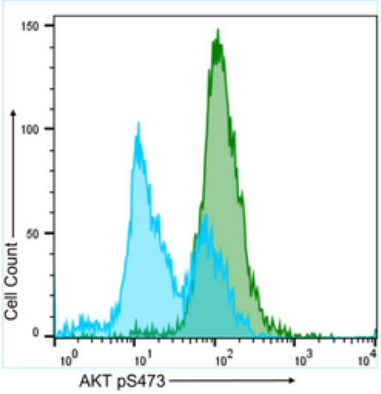
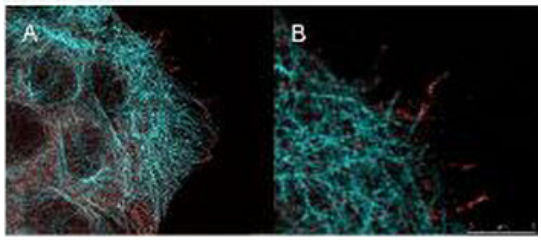
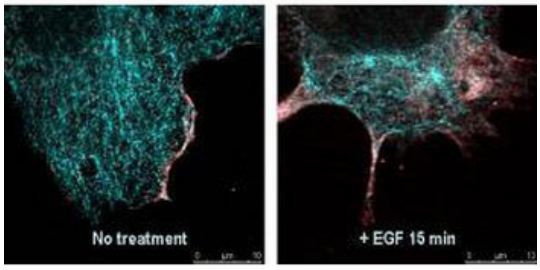


Flow Cytometry results of Anti-AKT pS473 (MOUSE) Monoclonal Antibody. The green histogram represents the A431 cells that were stimulated for 15 minutes with 100 ng/mL EGF. The blue histogram shows the untreated A431 cell population, which is bimodal. Both populations were stained with a 1:50 dilution of the Anti-AKT pS473 (MOUSE) Monoclonal Antibody (p/n orb344403) for 30 mins at 4°C. The secondary antibody, Anti-MOUSE IgG (H&L) (GOAT) Antibody DyLight™ 488 Conjugated was used at a 1:200 dilution for 30 mins at 4°C.



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 orb345510) 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 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 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 1:10000 for 45 min at RT. Localization: AKT pS473 is nuclear and occasionally cytoplasmic. Staining: AKT pS473 as red signal with tubulin (cyan).

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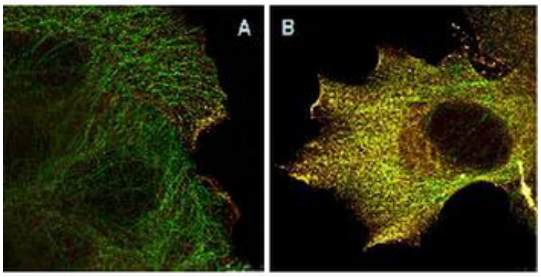
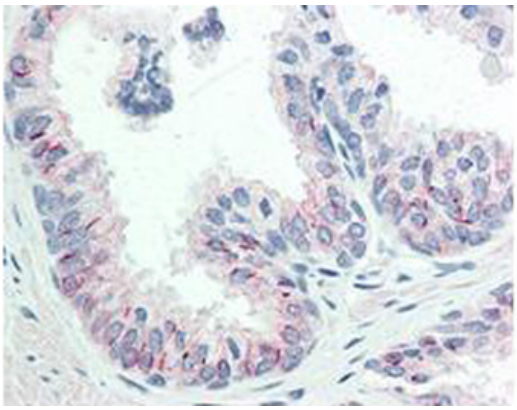


Immunofluorescence Microscopy of Mouse Anti-AKTpS473 antibody using STED nanoscopy to evaluate AKT activation and migration. Tissue: A431 cells. Antigen retrieval: Panel A: serum starved, unstimulated cells. Panel B: serum starved, EGF stimulated for 15 mins. A massive increase in AKT-pS473 activation, 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 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 1:10000 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.

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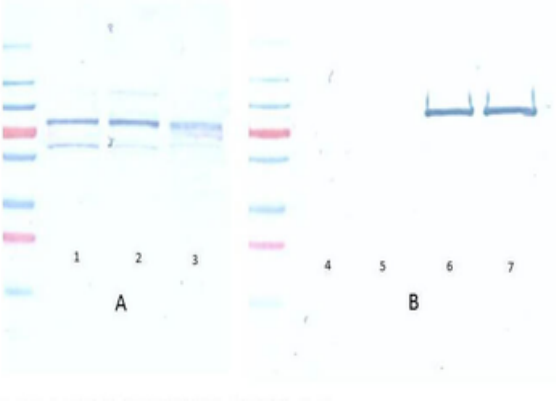
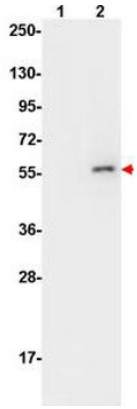
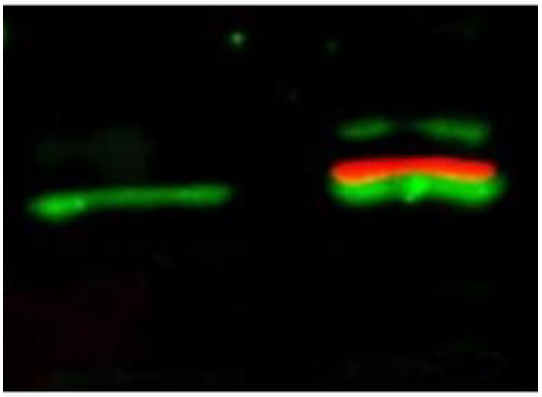


Western Blot of Mouse Anti-Akt pS473 antibody. A: Lane 1) PDGF stimulated NIH 3T3 cells (p/n orb348723) [10 µl]. Lane 2) NIH 3T3 cells (p/n orb348714) [10 µl]. Lane 3) Hela whole cell lysate (p/n orb348668) [10 µl] (weak signal). B: Lane 4) GST negative control protein (p/n orb345956) [100 ng]. Lane 5) GST negative control protein (p/n orb345956) [25 ng]. Lane 6) AKT 1 recombinant protein (p/n orb346473) [100 ng]. Lane 7) AKT 1 recombinant protein (p/n orb346473) [25 ng]. Block: 5% BSA overnight at 4°C. Primary antibody: Biorbyt monoclonal anti-AKT antibody (orb344404) used at 1:1000 for overnight at 4°C. Secondary antibody: HRP Conjugated goat anti-mouse (p/n orb347506) 1:25K for 45 min at RT. Detection: (orb348656) for 20 minutes, rinsed with deionized water, dried and scanned on conventional flatbed scanner.



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 1:10000 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 orb347385) was used at a 1:20000 dilution for 1 h at 4°C with FemtoMax™ enhanced chemiluminescent reagent. Other band(s): none.



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 1:10000 for 45 min at RT. Block: 5% BLOTTO overnight at 4°C.

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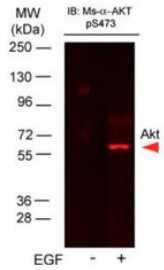

Western Blot of Mouse Anti-AKTpS473 antibody. Lane 1: A431 cells. Lane 2: A431 cells stimulated for 15 min with EGF. Load: 35 µg per lane. Primary antibody: AKTpS473 antibody at 1:400 for overnight at 4°C. Secondary antibody: DyLight™649 Conjugated Anti-AKT pS473 Monoclonal Antibody at 1:10000 for 45 min at RT. Block: Blocking Buffer for Fluorescent Western Blotting p/n orb348637 overnight at 4°C. Predicted/Observed size: 56kDa. Other band(s): none.

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