Product Datasheet

AKT Antibody (orb750475)

Catalog Number	orb750475
Description	AKT1 antibody
Species/Host	Rabbit
Reactivity	Gallus, Human, Mouse, Rat
Conjugation	Unconjugated
Tested Applications	ELISA, IF, IHC, WB
Immunogen	AKT Antibody was produced from whole rabbit serum prepared by repeated immunizations with a synthetic peptide R-P-H-F-P-Q-F-S-Y-S-A-S-G-T-A corresponding to the C-terminus (460-480) of human AKT proteins conjugated to KLH using maleimide. A residue of cysteine was added to the amino terminal end to facilitate coupling. A BLAST analysis was used to suggest reactivity with this protein from rat, mouse, and chicken based on 100% homology for the immunogen sequence.
Preservatives	0.1% (w/v) Sodium Azide
Form/Appearance	Liquid (sterile filtered)
Concentration	75 mg/ml
Storage	Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.
Note	For research use only

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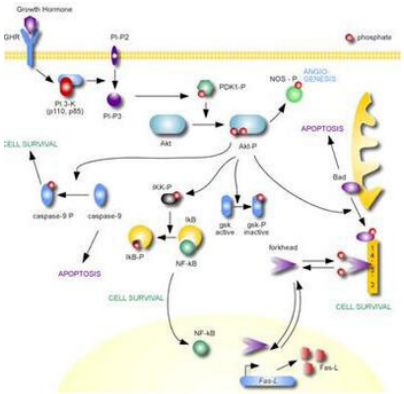
Application notes	Anti-AKT Antibody has been tested in Western Blot, Immunohistochemistry (Formalin-fixed paraffin-embedded sections), and Immunofluorescence (paraformaldehyde-fixed primary cardiomyocyte cultures). Expect a band at ~55.7kDa in 3T3 whole cell lysate or other appropriate cell lysates or tissues in western blot. Although not tested, this antibody would be useful in flow cytometry. Researchers should determine optimal titers for applications that are not stated below.
Isotype	Antiserum
Clonality	Polyclonal
Antibody Type	Primary Antibody
Purity	This product was prepared from monospecific antiserum by a delipidation and defibrination. Pan Anti-AKT Antibody reacts with the AKT from human tissues. Based on sequence we expect this antibody to react as well with rat, mouse, and chicken AKT.
Uniprot ID	**P31749**
NCBI	**62241011**
Dilution Range	ELISA: 1:2,000 - 1:10,000, IHC: 1:500 - 1:2,000, IF: 1:100 - 1:1,000, WB: 1:500 - 1:2,000
Expiration Date	12 months from date of receipt.



AKT Metabolic Pathway

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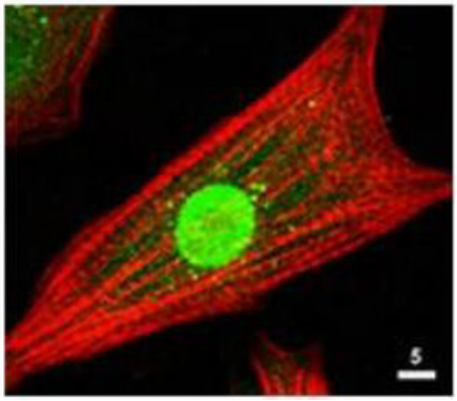
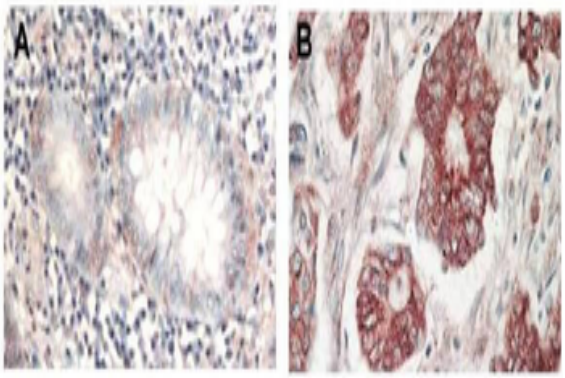
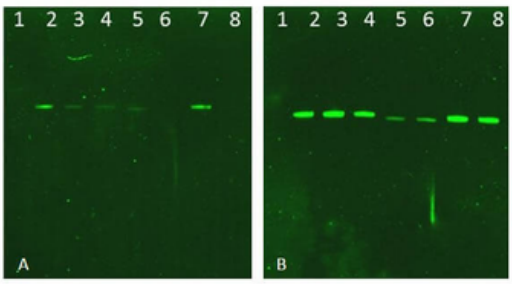


Immunofluorescence Microscopy of Rabbit Anti-AKT Antibody. Tissue: neonatal rat cardiomyocytes. Fixation: 0.5% PFA. Antigen retrieval: not required. Primary antibody: AKT antibody at 1:80 dilution for 1 h at RT. Secondary antibody: Texas-red™ conjugated rabbit secondary antibody at 1:10000 for 45 min at RT. Localization: AKT is nuclear. Staining: Anti-AKT staining appears green. Actin filaments are labeled red using a Texas-red™ conjugated phalloidin.



Immunohistochemistry of Rabbit Anti-AKT antibody. Tissue: (A) normal colon tissue, (B) colon tumor tissue. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: AKT antibody at 1:1000 dilution for 1 h at RT. Secondary antibody: Peroxidase rabbit secondary antibody at 1:10000 for 45 min at RT. Localization: AKT is nuclear. Staining: AKT as precipitated red signal with hematoxylin purple nuclear counterstain.



Western Blot of Rabbit AKT Antibodies. Lane 1: NIR MW protein ladder. Lane 2: AKT1, recombinant: orb346473. Lane 3: AKT1, phosphatase-treated: orb346472. Lane 4: AKT1, mutant T308A/S473A: orb346474. Lane 5: AKT2, recombinant: orb346475. Lane 6: AKT2, phosphatase-treated: orb346470. Lane 7: AKT3, recombinant: orb346476. Lane 8: AKT3, phosphatase-treated: orb346471. Load: 50 ng per lane. Blot A: orb345379 Anti-Akt pT308 used at 1:2270, Blot B: orb750474 Anti-Akt used 1:1000.

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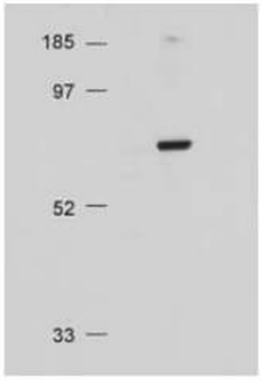
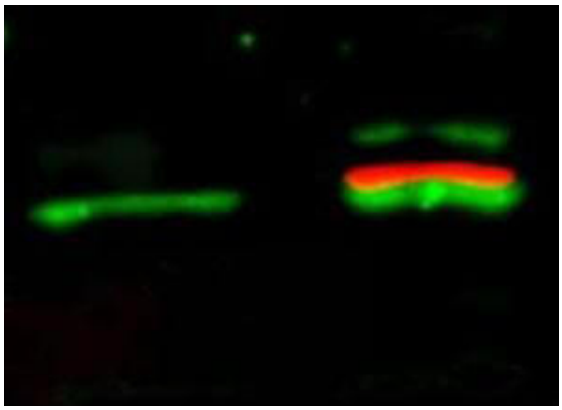


Western Blot of Rabbit Anti-AKT antibody. Lane 1: Molecular Weight. Lane 2: NIH/3T3 whole cell lysate. Load: 20 µg lysate per lane. Primary antibody: Anti-AKT antibody at 1:500 for overnight at 4°C. Secondary antibody: HRP conjugated GT-a-Rabbit IgG (orb347654) at 1:10000 preceded color development using Pierce Chemical's SuperSignal™ substrate. Block: MOPS buffer overnight at 4°C. Predicted/Observed size: 56 kDa, 56 kDa for AKT. Other band(s): none.



Western Blot of simultaneous detection of unphosphorylated and phosphorylated Rabbit Anti-AKT 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: 35 µg per lane. Primary antibody: rabbit anti-Akt (pan) and mouse anti-Akt pS473 specific antibodies at 1:1000 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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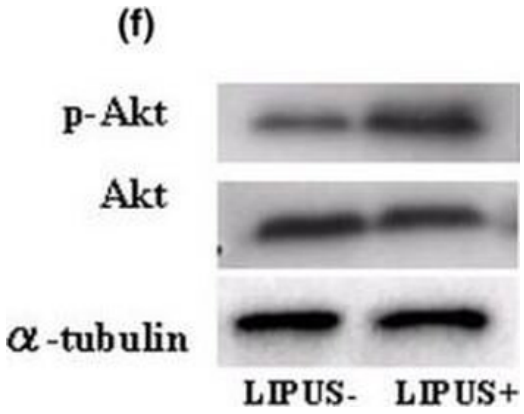

Western blotting analysis. (a) Type-II collagen. (b) Type-IX collagen. (c) Focal adhesion kinase (FAK) and phosphorylated FAK (p-FAK). (d) Paxillin and phosphorylated Paxillin (p-Paxillin). (e) Mitogen-activated protein kinase (MAPK) and phosphorylated MAPK (p-MAPK). There are no evident differences in the expression levels of total MAPK and p-MAPK between the two groups. (f) Akt and phosphorylated Akt (p-Akt). There were no differences found in the intensity the total Akt expression between the two groups, but p-Akt was found at higher levels in the LIPUS group (US+) in comparison with the control group (US-). (g) Cyclin B1 and cyclin D1. (h) Changes of proliferating cell nuclear antigen (PCNA) using MEK1 inhibitor (PD98059) and phosphatidylinositol 3-OH kinase (PI3K) inhibitor (LY294002). Chondrocytes were pretreated with MEK1 inhibitor (PD98059, 250 μm/ml) and PI3K inhibitor (LY294002, 250 μm/ml) for 12 hours and 24 hours followed by stimulation with LIPUS for 20 minutes. Each sample was harvested 2 hours after LIPUS stimulation and the influence of these inhibitors was judged in western blotting analysis of the expression of PCNA.

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