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Datasheet for 100-401-401

AKT Antibody

Overview

Description:	Anti-AKT (RABBIT) Antibody - 100-401-401
Item No.:	100-401-401
Size:	200 µL
Applications:	IF, IHC, WB
Reactivity:	Human, Mouse, Rat, Chicken
Host Species:	Rabbit

Product Details

Background:	AKT Antibody detects AKT which 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 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 Antibody is ideal for investigators involved in Cell Signaling, Neuroscience, Signal Transduction, VEGF Signaling, and apoptosis research.
Synonyms:	rabbit anti-AKT antibody, RAC-PK-alpha, Protein kinase B, PKB, C-AKT, RAC-alpha serine/threonine-protein kinase, Proto-oncogene c-Akt, AKT1, AKT Serine/Threonine Kinase 1, AKT 1 Antibody, AKT-1 Antibody
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	Antiserum

Target Details

Gene Name:	AKT1

Reactivity:	Human, Mouse, Rat, Chicken
Immunogen Type:	Conjugated Peptide
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.
Purity/Specificity:	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.
Relevant Links:	• UniProtKB - P31749 • NCBI - 62241011 • GeneID - 207

Application Details

Tested Applications:	IF, IHC, WB
Application Note:	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.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:2,000 - 1:10,000
FC:	User Optimized
IF:	1:100 - 1:1,000
IHC:	1:500 - 1:2,000
WB:	1:500 - 1:2,000

Formulation

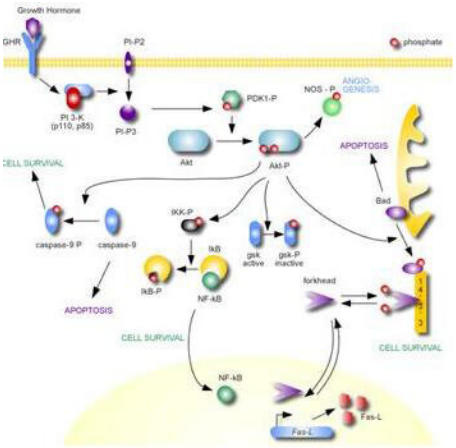
Physical State:	Liquid (sterile filtered)
Concentration:	85 mg/mL by Refractometry

Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

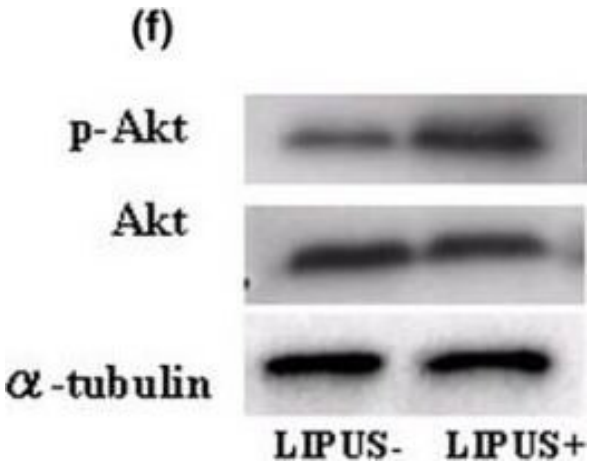
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store Anti-Akt 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.

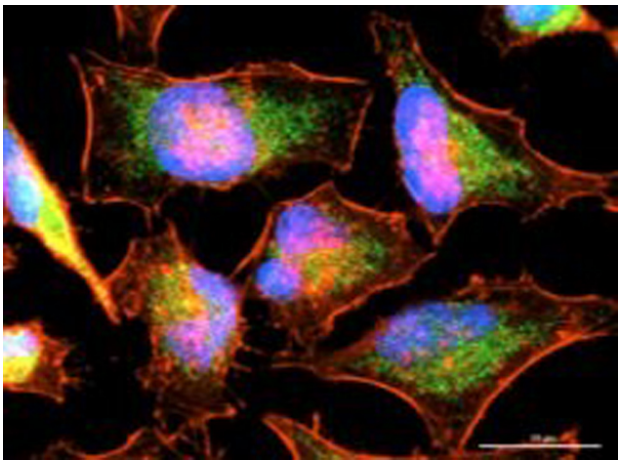
Images



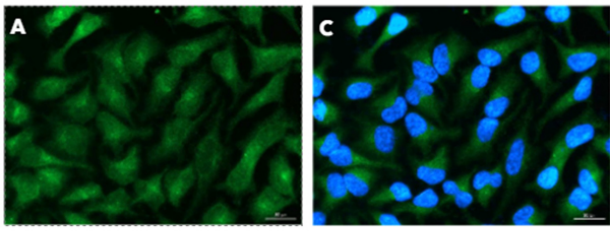
Pathway
AKT Metabolic Pathway



Western Blot
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. Figure provided by CiteAb. Source: Arthritis Res Ther, PMID: 18616830.



Immunofluorescence Microscopy
Immunofluorescence of Rabbit Anti-AKT Antibody. Cell line: HeLa. Primary Antibody: AKT (p/n 100-401-401) at 1:500 overnight at 2-8°C. Secondary Antibody: Goat Anti-Rabbit IgG (H&L) Antibody DyLight™ 488 Conjugated Pre-Adsorbed (p/n 611-141-122) at 1:1000 at room temp for 1hr. Fixative: 4% PFA. Permeabilization: 1X PBS, 0.3% Triton X-100. Nuclear stain: Hoechst 33342. Counterstain: Phalloidin at 1:400. Expected Localization: Cytoplasmic/slightly nuclear. Magnification: 40X with deconvolution and digital zoom.



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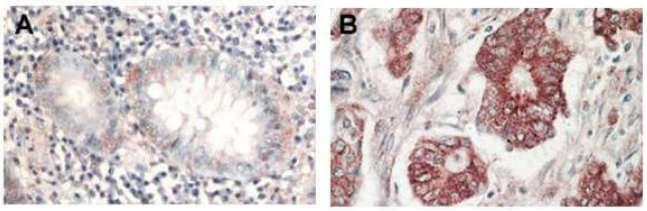
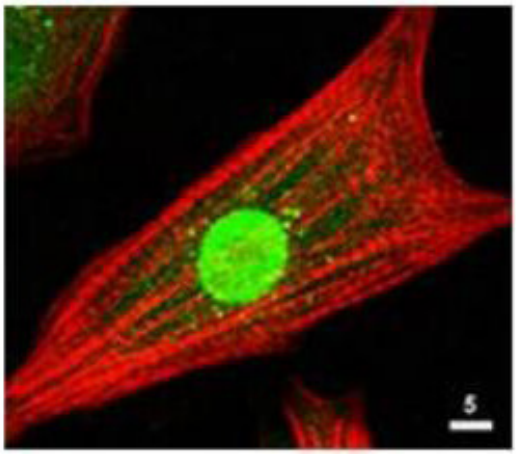
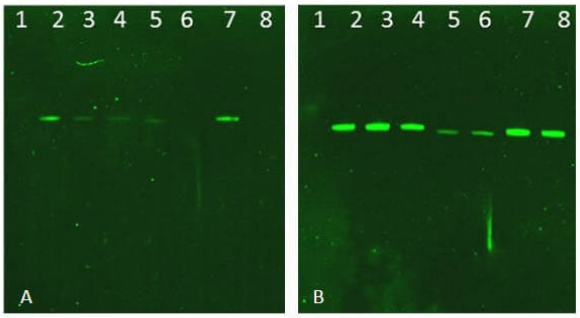


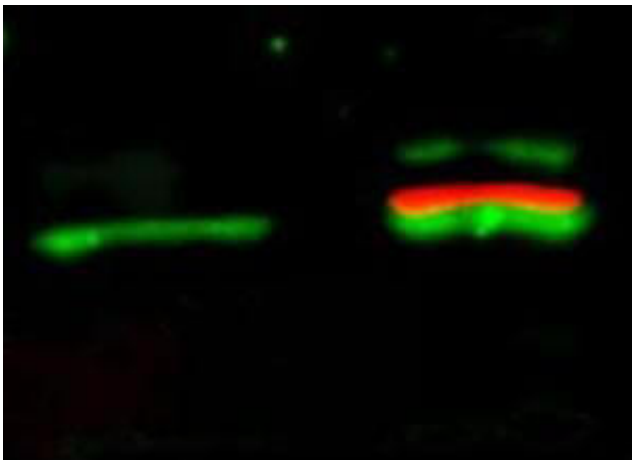
Immunohistochemistry
Immunohistochemistry of Rabbit Anti-AKT antibody. Tissue: (A) normal colon tissue, (B) colon tumor tissue. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: AKT antibody at 1:1,000 dilution for 1 h at RT. Secondary antibody: Peroxidase rabbit secondary antibody at 1:10,000 for 45 min at RT. Localization: AKT is nuclear. Staining: AKT as precipitated red signal with hematoxylin purple nuclear counterstain.



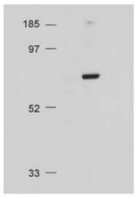
Immunofluorescence Microscopy
Immunofluorescence Microscopy of Rabbit Anti-AKT Antibody. Tissue: neonatal rat cardiomyocytes. Fixation: 0.5% PFA. Antigen retrieval: not required. Primary antibody: AKT antibody at 1:80 dilution for 1 h at RT. Secondary antibody: Texas-red™ conjugated rabbit secondary antibody at 1:10,000 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.



Western Blot
Western Blot of Rabbit AKT Antibodies. Lane 1: NIR MW protein ladder. Lane 2: AKT1, recombinant: 009-001-P21. Lane 3: AKT1, phosphatase-treated: 009-001-I51. Lane 4: AKT1, mutant T308A/S473A: 009-001-P22. Lane 5: AKT2, recombinant: 009-001-P23. Lane 6: AKT2, phosphatase-treated: 009-001-E71. Lane 7: AKT3, recombinant: 009-001-P24. Lane 8: AKT3, phosphatase-treated: 009-001-E75. Load: 50ng per lane. Blot A: 600-401-269 Anti-Akt pT308 used at 1:2270, Blot B: 100-401-401 Anti-Akt used 1:1000.



Western Blot
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 1:10,000 for 45 min at RT. Block: 5% BLOTTO overnight at 4°C.



Western Blot
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 (611-103-122) at 1:10,000 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.

References

- Sato et al. Sodium butyrate enhances the growth inhibitory effect of sunitinib in human renal cell carcinoma cells. *Oncology Letters* (2017)
- Ishigami, T. et al. Inhibition of ICAM2 induces radiosensitization in oral squamous cell carcinoma cells. *British Journal of Cancer* (2008)
- Takeuchi R et al. Low-intensity pulsed ultrasound activates the phosphatidylinositol 3 kinase/Akt pathway and stimulates the growth of chondrocytes in three-dimensional cultures: a basic science study. *Arthritis Res Ther.* (2008)

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