



Product Specification Sheet

AKT1 antibody

Cat # AKT1-A

Rabbit Anti-Human AKT1 antibody

SIZE: 100 µg

Cat # AKT1-C

Recombinant Human AKT1 protein control for Western blotting

SIZE: 100 µl

AKT1 is one of 3 closely related serine/threonine-protein kinases (AKT1, AKT2 and AKT3) called the AKT kinase, and which regulate many processes including metabolism, proliferation, cell survival, growth and angiogenesis. This is mediated through serine and/or threonine phosphorylation of a range of downstream substrates. Putative human homolog of the proto-oncogene v-akt of the acutely transforming retrovirus AKT8 have been cloned. These protein-serine/threonine kinase proteins have a catalytic domain closely related to both PKA and PKC and have been designated RAC (related to A and C kinases), pkb (Protein kinase B) or Akt.

RAC protein kinase family members feature pleckstrin homology (PH) domain at the amino terminus and a protein-serine/threonine kinase catalytic domain at the carboxy terminus. The Amino terminal domain (referred to as AH-Akt Homology domain) spans from 1-148 amino acids and contains the PH domain, a region found in diverse group of signaling proteins. The PH domain (amino acids 1-106) has been implicated in interactions with other proteins such as G-protein b subunits, as well as phosphoinositides. The kinase domain is located between residues 148 to 411. These enzymes are activated by diverse ligands such as PGDF, EGF and basic FG in NIH 3T3, Rat-1 or Swiss-3T3 cells.

AKT1 (RAC-PK-a or PKB-a) is the human homolog of v-akt and is identical to RAC gene. The protein has been observed to show different migratory patterns on a western blot according to the state of phosphorylation of the protein. Phosphatase treatment has been shown to result in inactivation of the protein.

Source of Antigen and Antibodies

Uniprot: P31749

Host: Rabbit

Clonality: Polyclonal

Immunogen: Full length recombinant Human AKT1

Purification: Ammonium sulfate followed by protein affinity purification

Species Reactivity: Human

Cross reactivity: AKT1 is highly conserved across species. Reactivity is expected for species containing >80% or more homology with human AKT1

Subcellular Location: Nucleus, Cytoplasm, Membrane

Recommended Secondary Antibody: Goat anti-Rabbit IgG-HRP (ADI cat#20320)

Negative Control: Non-immune Rabbit IgG (ADI cat# 20009-1)

Form & Storage of Antibodies

Affinity pure IgG Solution

Concentration: 0.5 mg/ml Volume: 200 µl

Supplied in PBS, pH 7.4 + 0.1% BSA

The antibody can be made available carrier free or conjugated to HRP, Biotin, or FITC on request

Lyophilized powder

Lyophilized from a formulation containing PBS, pH 7.4 +3% Trehalose. Reconstitute in 200 µl PBS, 0.05% tween-20, 0.1% BSA, and a preservative to 0.5 mg/ml.

Storage:

Short-term: 4°C for 1 month

Long-term: at -20°C or below in suitable aliquots after reconstitution for 1 year. Do not expose to multiple freeze/thaw cycles or store working, diluted solutions. Glycerol may be added to a final concentration of 50% and antibodies can be stored un-aliquoted at -20°C.

Recommended Usage

ELISA: Assay dependent concentration. Typically, between 0.1-2.0 µg/ml for capture/detection antibodies. Request the carrier free or conjugated antibody.

Western Blotting: 0.5-1.0 µg/ml

Observed band size: 60 kDa

IHC-P: 1-10 µg/ml.

The above concentrations are a *suggestion*, user's must optimize their assay based on their own conditions. The antibody may work in other applications such as Immunocytochemistry or IP. These methods have not been tested by ADI.

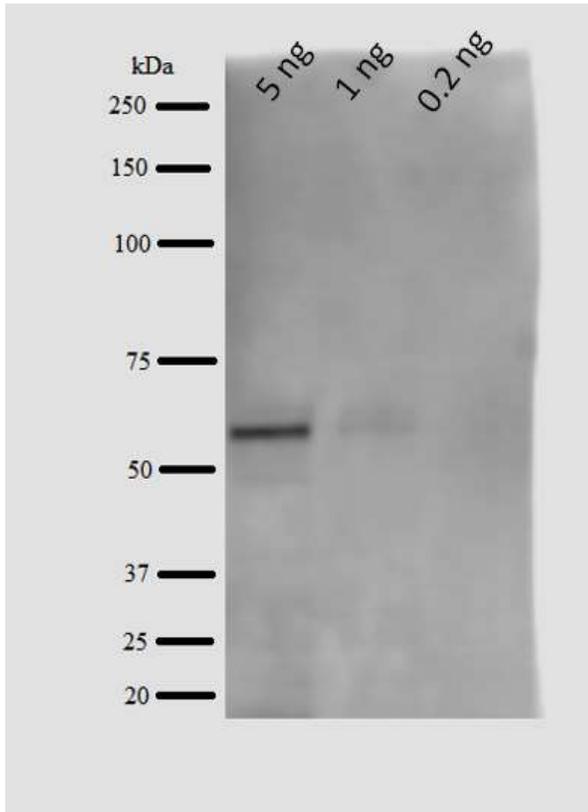
****This product is for In vitro research use only.***

Related materials available from ADI

Catalog#	Description
BCL11-A	Rabbit Anti-Human BCL-2 antibody
BCL21-A	Rabbit Anti-Mouse BCL-2 antibody
BCL2-C	Recombinant BCL-2 control for Western blotting
HP5311-A	Rabbit anti-Human P53 antibody
HP5311-C	Recombinant Human P53 protein control for Western blotting
CASP3-A	Rabbit anti-Mouse Caspase 3 antibody

AKT1-A

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Western blotting: 5, 1, and 0.2 ng of recombinant AKT1 (**AKT1-C**) was heated for 5 minutes at 95°C then electrophoretically separated on a 4-20% SDS-PAGE gel. The gel was run at 100V for ~1 hour and 30 minutes then transferred to a 0.2 µm nitrocellulose membrane using the 'Mixed MW' settings on a Transblot Turbo (Biorad). The blot was blocked for 1 hour at room temperature with 1% Casein. **AKT1-A** was diluted with TBST+0.1% BSA to 1 µg/ml and incubated overnight at 4°C. The blot was washed with TBS-T 3 times for 5 minutes each. Goat anti-rabbit IgG HRP (**ADI cat#20320**) was diluted in TBST+0.1% BSA at a 1:1,000 dilution (500 ng/ml) then incubated for 1 hour at room temperature. The blot was washed 3 times with TBS-T for 5 minutes each. The blot was then incubated with regular ECL substrate for 1 minutes and imaged on a CCD imaging system (C-DiGit, LI-COR).

AKT1-C: Contains a recombinant *E.coli* expressed full length human AKT1 protein at a concentration of 1 ng/µl in Laemmli buffer (62.5 mM Tris-HCL, pH 6.8, 2% SDS, 10% glycerol, 5% BME, and 0.002% bromphenol blue). Heat for 5 minutes at 95°C then load 1-5 µl. Store at -20°C in suitable size aliquots, do not expose to multiple freeze/thaw cycles. **Note:** Due to the addition of tags, the protein appears slightly larger than native protein.