Datasheet for 200-301-I51

AKT1 Antibody

Overview

Description:	Anti-AKT1 (MOUSE) Monoclonal Antibody - 200-301-I51
Item No.:	200-301-I51
Size:	100 µg
Applications:	ELISA, FC, WB, IP
Reactivity:	Human, Mouse
Host Species:	Mouse

Product Details

Background:	AKT1 Antibody detects AKT1 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-AKT1 Antibody is ideal for investigators involved in Cell Signaling, Neuroscience and Signal Transduction research.
Synonyms:	mouse anti-AKT1 antibody, AKT-1, PKB antibody, PKB gamma antibody, PKBGAMMA antibody, PRKBG antibody, Protein kinase Akt 1 antibody, Protein kinase B gamma antibody, RAC-gamma serine/threonine-protein kinase, RAC-PK-gamma
Host Species:	Mouse
Clonality:	Monoclonal
Clone ID:	14E5.A2.B2.H9
Format:	IgG2a

Target Details

Gene Name:	AKT1

Reactivity:	Human, Mouse
Immunogen Type:	Conjugated Peptide
Immunogen:	Anti-AKT1 Antibody was produced by repeated immunizations with a synthetic peptide corresponding to internal residues of human AKT1 protein.
Purity/Specificity:	Anti-AKT1 antibody is directed against human AKT1. The antibody detects both unphosphorylated and phosphorylated forms of the protein. Anti-AKT1 antibody was purified from ascites by Protein A chromatography. Cross reactivity with AKT1 from other species has not been determined, however, the sequence of the immunogen shows 85% identity to mouse and 92% identity with rat, therefore, cross reactivity is expected.
Relevant Links:	• UniProtKB - P31749

Application Details

Tested Applications:	ELISA, FC, WB
Suggested Applications:	IP (Based on references)
Application Note:	Anti-AKT1 Antibody has been tested in ELISA, flow cytometry, and western blotting. This antibody is suitable for immunoprecipitation and immunohistochemistry. Expect a band approximately 56 kDa in size corresponding to AKT1 protein by western blotting in the appropriate cell lysate or extract. This monoclonal antibody reacts with human AKT. Specific conditions for reactivity should be optimized by the end user. For immunohistochemistry we recommend the use of fresh frozen tissues. Attempts at staining paraffin-embedded formalin fixed tissues were negative. 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:	1:2,000 - 1:10,000
FC:	User Optimized
IHC:	20 µg/mL
WB:	1:1000

Formulation

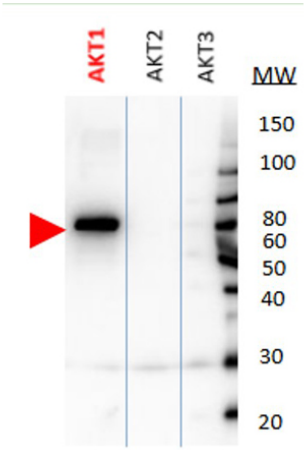
Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/ml by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide

Stabilizer:	None

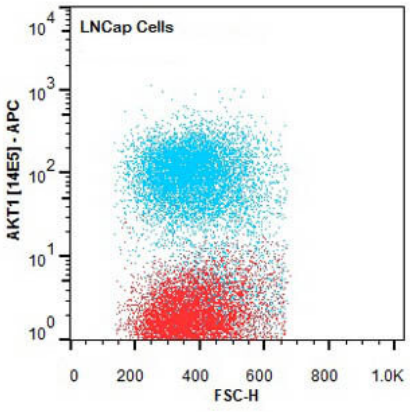
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial 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.

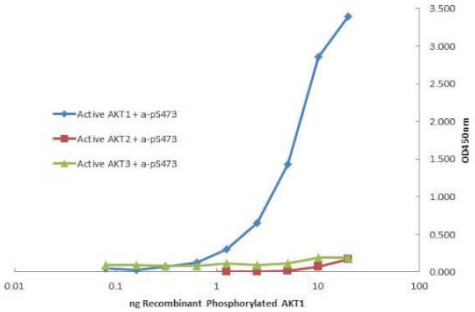
Images



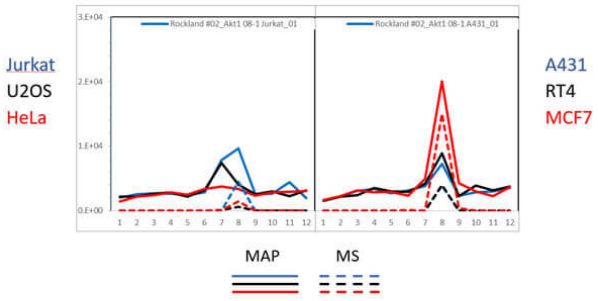
Western Blot
Western Blot of AKT isoform specific antibodies: 50 ng of recombinant GST-AKT1, -AKT2 and -AKT3 proteins were separated by SDS PAGE (4-20% gel) and transferred onto 0.2 µm nitrocellulose. The membrane was blocked with Blocking Buffer (p/n MB-070) for 1 h at room temperature. Mouse anti-AKT1 (p/n 200-301-I51) at a dilution of 1 µg/mL in Blocking Buffer and incubated for 16 h at 4°C. Rabbit anti-Mouse IgG HRP (p/n 610-403-C46) secondary antibodies were applied at 0.05 µg/mL in Blocking Buffer and incubated for 1 h at room temperature followed by detection with FemtoMax™ chemiluminescent reagent (p/n FEMTOMAX-110). Predicted/Observed size: The expected size for all recombinant GST-AKT isoforms is 85 kDa. Arrowheads indicate the position of AKT isoform.



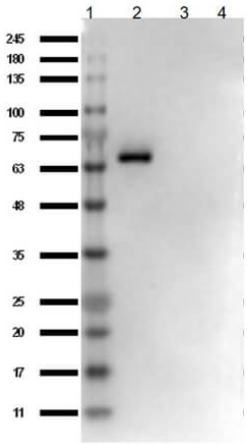
Flow Cytometry
Flow Cytometry of Mouse anti-AKT1 antibody. Cells: LNCap Cells. Stimulation: none. Primary antibody: Allophycocyanin AKT1 antibody at 1.0 µg/mL for 20 min at 4°C.



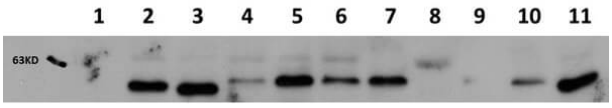
ELISA
Plate was coated with monoclonal anti AKT1 antibody (capture antibody) followed by incubation with recombinant AKT1 (p/n 009-001-P21), AKT2 (p/n 009-001-P22), AKT3 (p/n 009-001-P23) proteins. Binding was detected with biotinylated monoclonal anti-AKT pS473. The signal shows specificity of the monoclonal anti-AKT1 antibody to recombinant isoform AKT1 protein and not the isoform 2 and 3.



PAGE-MAP
PAGE-MAP (microsphere affinity proteomics) of Mouse Anti-AKT1 Antibody. (Catalog Number: 200-301-I51, Lot Number: 29014). Antibody array western blot binding of gelfree size separated fractions of multiple lysates (solid lines) and shotgun mass spectroscopy identification (dashed lines) of the target band run in parallel correlate confirming the specificity of this antibody against AKT1. Data was provided by the Lund-Johansen lab of Oslo University Hospital. For more information on PAGE-MAP/IP-MS identification of antibody specificity and its large-scale implementation for antibody validation see Sikorski et. al., (2018) Nature Methods 15, 909-912.



Western Blot
Western Blot of Mouse Anti-AKT1 Antibody. Lane 1: Opal Prestained Molecular Weight Protein (p/n MB-210-0500). Lane 2: AKT1 protein (p/n 009-001-P21). Lane 3: AKT2 protein (p/n 009-001-P22). Lane 4: AKT3 protein (p/n 009-001-P23). Load: 50ng. Blocking: BlockOut Buffer (p/n MB-073) for 30 min at RT. Primary Antibody: Anti-AKT1 at 1ug/mL o/n at 4°C. Secondary Antibody: Rabbit Anti-Mouse IgG HRP (p/n 610-403-C46, Lot 20121) at 1:40,000 in MB-073 for 30 min at RT.



Western Blot
Western Blot of Mouse Anti-AKT1 antibody. Lane 1: AKT-1 Null. Lane 2: WT. Lane 3: MEF #1. Lane 4 : A549 (p/n W09-001-GX4). Lane 5: Calu-1. Lane 6: PC-3 (p/n W09-001-GV6). Lane 7: HepG2 (p/n W09-001-GJ5). Lane 8: Jurkat (p/n W09-001-370). Lane 9: SKOV3 (p/n W09-001-GX9). Lane 10: HEK293T (p/n W09-001-GX5). Lane 11: C2C12 (p/n W10-001-GL7). Load: 20 ug per lane. Primary antibody: AKT1 antibody at 1:1,000 for overnight at 4°C. Secondary antibody: Peroxidase mouse secondary antibody at 1:40,000 for 30 min at RT. Block: MB-070 for 30 min at RT. Predicted/Observed size: 56 kDa for AKT1. Note that phosphorylated AKT migrates slower and is observed in lanes 6 and 8 where AKT is constitutively activated in PC-3 and Jurkat cells. Other band(s): none. Data provided by Dr Yuefeng Tang, Dave Guertin Lab. U. Mass.

References

- Sikorski et al. A high-throughput pipeline for validation of antibodies. *Nature Methods* (2018)

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