

Datasheet for K-I51

AKT1 Antibody Combo Pack**Overview**

Description:	AKT1 Antibody Combo Pack - K-I51
Item No.:	K-I51
Size:	1 Pair
Applications:	ELISA, FC, WB
Reactivity:	Human
Host Species:	Mouse

Product Details

Background:	This AKT1 Antibody Combo Pack contains: Mouse Anti-AKT1 and Rabbit Anti-Mouse IgG 1, 2a, 2b, 3 HRP Antibody. 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:	AKT 1 antibody pair, 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, primary and secondary antibody pair, Primary +Secondary pair, matched antibody pair
Host Species:	Mouse
Clonality:	Monoclonal
Clone ID:	14E5.A2.B2.H9
Format:	IgG2a
Detection Kit Type:	Combo Pack

Target Details

Gene Name:	AKT1
Reactivity:	Human
Immunogen Type:	Conjugated Peptide
Immunogen:	Anti-AKT1 Antibody was produced by repeated immunizations in mice with a synthetic peptide corresponding to internal residues of human AKT1 protein. Anti-Mouse IgG HRP secondary antibody was produced by repeated immunizations in rabbit with Mouse IgG whole molecule.
Purity/Specificity:	This AKT1 primary and secondary antibody pair has been carefully chosen to give best results. 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. HRP secondary antibody was prepared from monospecific antiserum by immunoaffinity chromatography using Mouse IgG coupled to agarose beads followed by solid phase adsorption (s) to remove any unwanted reactivities. This product shows balanced reactivity to Mouse IgG1, IgG2a, IgG2b and IgG3 proteins and is suitable to screen IgG class hybridoma clones. Minimal cross reactivity is observed against other Mouse immunoglobulin classes or light chain proteins. This antibody pair is sufficient for up to 5 mini blots at 7.5 x 8 cm² (1,800 cm²).
Relevant Links:	• UniProtKB - P31749 • NCBI - NP_001014431.1 • GeneID - 207

Application Details

Tested Applications:	ELISA, FC, WB
Application Note:	Anti-AKT1 Antibody with the matched secondary are suitable for ELISA, immunohistochemistry, and western blotting. Expect a band approximately 56 kDa in size corresponding to AKT1 protein by western blotting in the appropriate cell lysate or extract. This primary 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

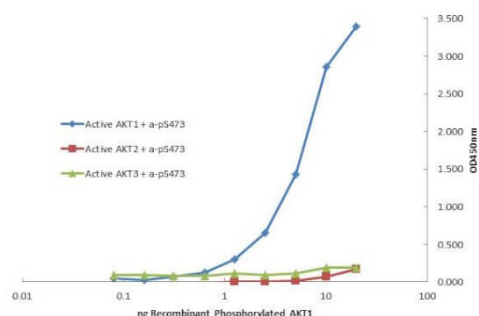
Formulation

Physical State:	Liquid and Lyophilized
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
Reconstitution Volume:	1.0 mL
Reconstitution Buffer:	Restore with deionized water (or equivalent)

Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Primary antibody: the vial contains a relatively low volume of reagent (25 µL). Store vial at -20° C or below prior to opening. 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. Secondary antibody: Store secondary antibody at 4° C prior to restoration. For extended storage aliquot antibody and freeze at -20° C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. Anti-Mouse IgG HRP secondary antibody 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

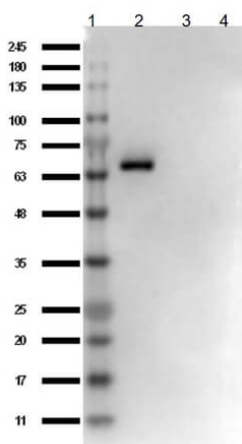
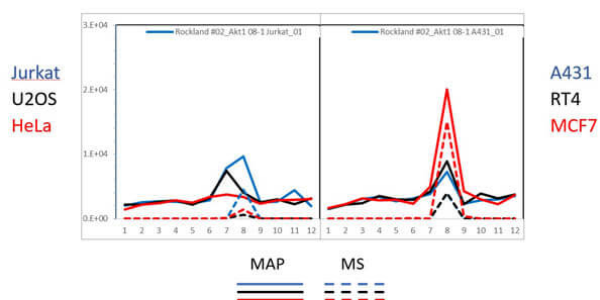


ELISA

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.

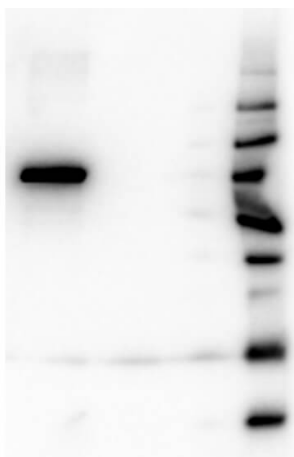
PAGE-MAP

PAGE-MAP (microsphere affinity proteomics) of Mouse Anti-AKT1 Antibody. (Catalog Number: 200-301-151, 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.



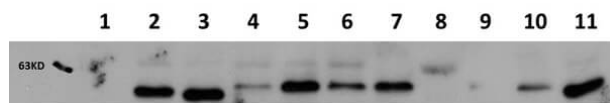
Western Blot

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: GST Tagged recombinant AKT1. Lane 2: GST Tagged recombinant AKT2. Lane 3: GST Tagged recombinant AKT3. Load: 25 ng 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: 78 kDa for AKT1. Other band(s): none.



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.

Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.