Order online now!

www.rockland.com
tech@rockland.com
+1 484.791.3823

Datasheet for K401

AKT Isoform Antibody Sampler Kit

Overview

Description:	AKT Isoform Antibody Sampler Kit - K401
Item No.:	K401
Size:	1 Kit
Reactivity:	Human
Host Species:	Mouse

Product Details

Background:	This primary antibody kit is designed to detect and quantify endogenous protein levels of human AKT1, AKT2 and AKT3. Each antibody is designed to recognize a specific isoform of AKT. AKT 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 Antibodies are ideal for investigators involved in Cell Signaling, Neuroscience and Signal Transduction research.
Synonyms:	AKT isoform antibody kit, AKT-1, AKT-2, AKT-3, Protein kinase Akt antibody, Protein kinase B antibody, RAC-gamma serine/threonine-protein kinase, RAC-PK-gamma, antibody sampler,
Host Species:	Mouse
Clonality:	Monoclonal
Clone ID:	various
Format:	IgG2a

Target Details

Gene Name:	AKT1, AKT2, AKT3

Reactivity:	Human
Immunogen Type:	Conjugated Peptide
Immunogen:	All Anti-AKT isoform specific Antibodies were produced by repeated immunizations with a synthetic peptide corresponding to internal residues of human AKT1, AKT2 or AKT3 protein.
Purity/Specificity:	This primary antibody kit has been carefully chosen to recognize all 3 isoforms of AKT. Anti-AKT1 antibody is directed against human AKT1. Anti-AKT2 antibody is directed against human AKT2. Anti-AKT3 antibody is directed against human AKT3. The antibodies detect both unphosphorylated and phosphorylated forms of the protein. The antibodies were purified from ascites by Protein A chromatography.
Relevant Links:	• UniProtKB - Q9Y243 • UniProtKB - P31751 • UniProtKB - P31749

Application Details

Application Note:	Anti-AKT Antibodies are suitable for ELISA, immunohistochemistry, and western blotting. Expect a band approximately 56 kDa in size corresponding to AKT proteins by western blotting in the appropriate cell lysate or extract. All 3 primary monoclonal antibodies 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. This sampler kit consists of sample sizes of anti-AKT1, anti-AKT2 and anti-AKT3 mouse monoclonal antibody and HRP conjugated anti-Mouse IgG secondary antibody (p/n 200-301-I51S; 200-301-BR8S; 200-301-J36S and 610-403-C46S, respectively).
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
Other:	User Optimized

Formulation

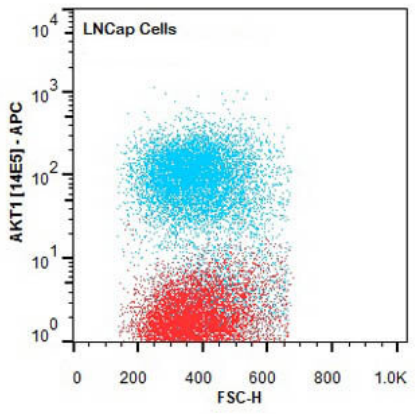
Physical State:	Liquid and Lyophilized
Concentration:	1.0 mg/ml by UV absorbance at 280 nm

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

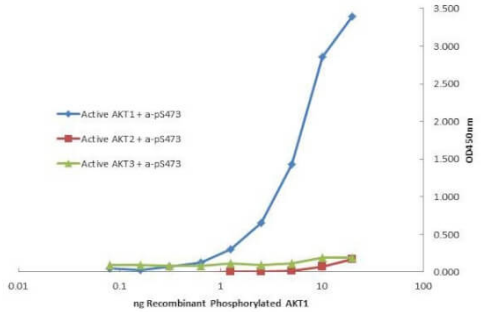
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Each 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.
Expiration:	Expiration date is one (1) year from date of receipt.

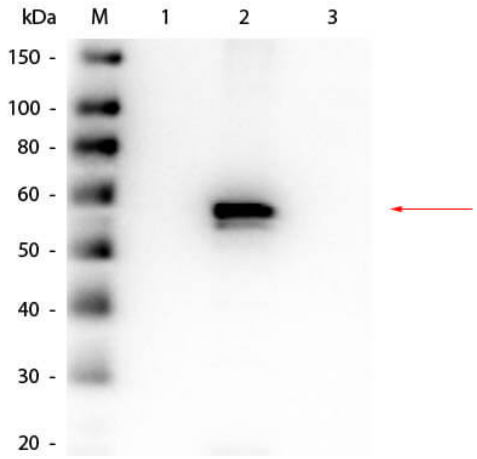
Images



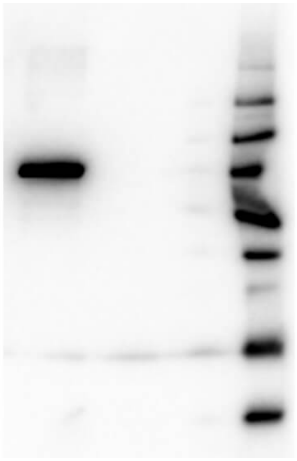
Flow Cytometry
Flow Cytometry of Mouse anti-AKT1 antibody. Cells: LNCap Cells. Stimulation: none. Primary antibody: Allophycocyanin AKT1 antibody at 1.0 µg/mL for 20 min at 4°C.



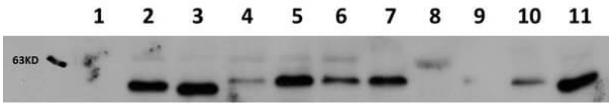
ELISA
Plate was coated with monoclonal anti AKT1 antibody (capture antibody) followed by incubation with recombinant AKT1 (p/n 009-001-P21), AKT2 (p/n 009-001-P22), AKT3 (p/n 009-001-P23) proteins. Binding was detected with biotinylated monoclonal anti-AKT pS473. The signal shows specificity of the monoclonal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



Western Blot

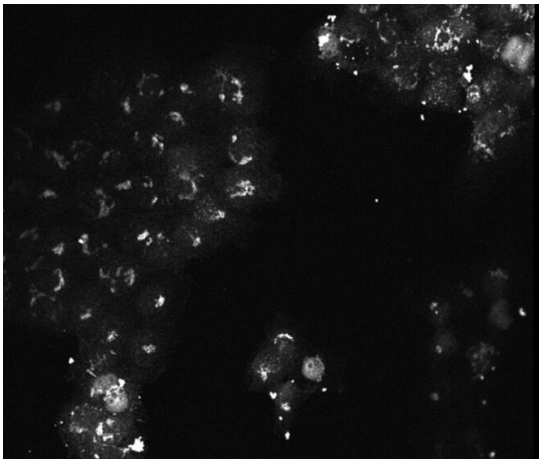


Western Blot
Western Blot of Mouse Anti-AKT1 antibody. Lane 1: GST Tagged recombinant AKT1. Lane 2: GST Tagged recombinant AKT2. Lane 3: GST Tagged recombinant AKT3. Load: 25 ng per lane. Primary antibody: AKT1 antibody at 1:1,000 for overnight at 4°C. Secondary antibody: Peroxidase mouse secondary antibody 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.



Immunofluorescence Microscopy

Immunofluorescence of Mouse monoclonal anti-AKT3 antibody. Cell Type: A431 cells. Fixation: 4% paraformaldehyde 10 min. Permeabilization: 0.5% Triton X 30 min. Primary Ab: (p/n 200-301-J36) at 1:250 for 72 hours 4°C. Secondary Ab: (p/n 610-142-121) at 1:1000 overnight 4°C.

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.