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Datasheet for KCAI51

AKT1 Western Chemiluminescent Blotting Kit

Overview

Description:	AKT1 Western Blot Kit - KCAI51
Item No.:	KCAI51
Size:	1 Kit
Applications:	ELISA, IF, WB
Reactivity:	Human
Host Species:	Mouse

Product Details

Background:	Rockland Immunochemicals' Chemiluminescent Western Blot Kit for AKT1 combines all of the necessary reagents with a rapid proven protocol and the extremely high signal detection of our luminol chemiluminescent substrate for the detection of native and/or recombinant human AKT1 protein. AKT1 Western Blotting kit allows for the detection of endogenous protein levels of human AKT1 present in cell lysates provided by the user. As a positive control, this kit includes a MDA-MB468 whole cell lysate proven to contain AKT1. After protein separation by SDS-PAGE and transfer, the membrane is probed with Rockland's optimized Anti-AKT1 antibody. Detection of the membrane bound antibody-antigen complex is achieved by the addition of a secondary antibody conjugated to the enzyme horseradish peroxidase. The enzyme reacts with a specialized formulation of luminol, an extremely sensitive, non-radioactive substrate that emits light and allows visualization using X-ray film or other imaging methods, including highly sensitive CCD cameras and imaging systems. AKT1 Western Blotting is ideal for investigators involved in Cell Signaling, Cancer, Neuroscience and Signal Transduction research.
Synonyms:	AKT 1 western blotting, 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, Chemiluminescent WB, Western Blotting Kit, Chemiluminescent Kit, Peroxidase Kit, Immunoblotting Kit
Host Species:	Mouse
Clonality:	Monoclonal
Clone ID:	14E5.A2.B2.H9
Format:	IgG2a
Detection Kit Type:	Chemiluminescent Western Blot Kit

Target Details

Gene Name:	AKT1
Reactivity:	Human
Purity/Specificity:	The kit is designed to detect both unphosphorylated and phosphorylated forms of the protein. Cross reactivity with AKT1 from other species has not been determined, however, the sequence of the immunogen shows 100% identity to human, mouse, and rat, therefore, cross reactivity is expected. Cross-reactivity with AKT2 and AKT3 has not been determined. This kit contains sufficient substrate for up to 5 mini blots at 7.5 x 8 cm2 (1,800 cm2) and is stable for at least 1 year when stored as indicated.
Relevant Links:	• UniProtKB - P31749 • AKT Chemiluminescent WBlot Kit Protocol

Application Details

Tested Applications:	ELISA, IF, WB
Application Note:	Use Rockland Immunochemicals' Anti-AKT1 Chemiluminescent Kit for Western Blotting for detection of human AKT1 native and recombinant proteins by western blot. Expect a band approximately 56 kDa in size corresponding to AKT1 protein. This kit is useful for both western blotting and dot blotting methods. Please read the entire product insert prior to use.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
WB:	User Optimized

Formulation

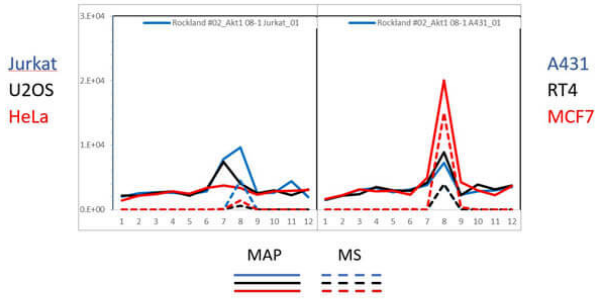
Physical State:	n/a
Preservative:	Wash buffers MUST NOT contain SODIUM AZIDE or other inhibitors of peroxidase activity!
Stabilizer:	None

Shipping & Handling

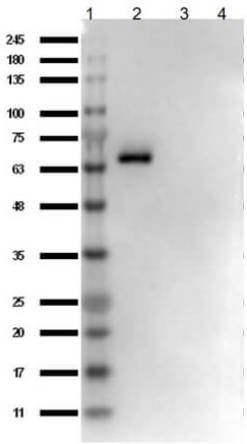
Shipping Condition:	Dry Ice
Storage Condition:	See kit insert for complete instructions.

Expiration: See kit insert for complete instructions.

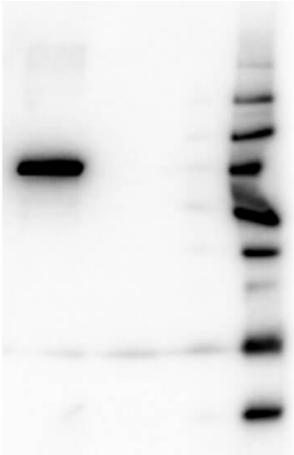
Images



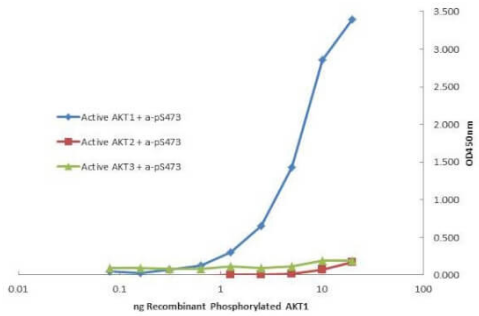
PAGE-MAP
PAGE-MAP (microsphere affinity proteomics) of Mouse Anti-AKT1 Antibody. (Catalog Number: 200-301-I51, Lot Number: 29014). Antibody array western blot binding of gelfree size separated fractions of multiple lysates (solid lines) and shotgun mass spectroscopy identification (dashed lines) of the 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.



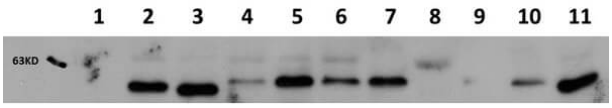
Western Blot
Western Blot of Mouse Anti-AKT1 Antibody. Lane 1: Opal Prestained Molecular Weight Protein (p/n MB-210-0500). Lane 2: AKT1 protein (p/n 009-001-P21). Lane 3: AKT2 protein (p/n 009-001-P22). Lane 4: AKT3 protein (p/n 009-001-P23). Load: 50ng. Blocking: BlockOut Buffer (p/n MB-073) for 30 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: 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.



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 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.

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