

Datasheet for 200-301-E75**AKT3 Antibody****Overview**

Description:	Anti-AKT3 (MOUSE) Monoclonal Antibody - 200-301-E75
Item No.:	200-301-E75
Size:	100 µg
Applications:	ELISA, IHC, WB
Reactivity:	Human, Mouse
Host Species:	Mouse

Product Details

Background:	AKT3 Antibody detects AKT3 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-AKT3 Antibody is ideal for investigators involved in Cell Signaling, Neuroscience and Signal Transduction research.
Synonyms:	Mouse anti-AKT3 antibody, AKT-3, PKB antibody, PKB gamma antibody, PKBGAMMA antibody, PRKBG antibody, Protein kinase Akt 3 antibody, Protein kinase B gamma antibody, RAC-gamma serine/threonine-protein kinase, RAC-PK-gamma
Host Species:	Mouse
Clonality:	Monoclonal
Clone ID:	25F6.F6.D8
Format:	IgG1

Target Details

Gene Name:	AKT3
-------------------	------

Reactivity:	Human, Mouse
Immunogen Type:	Conjugated Peptide
Immunogen:	Anti-AKT3 Antibody was produced in mice by repeated immunizations with a synthetic peptide corresponding to internal residues of human AKT3 protein.
Purity/Specificity:	Anti-AKT3 antibody is directed against human AKT3. The antibody detects both unphosphorylated and phosphorylated forms of the protein. Anti-AKT3 antibody was purified from ascites by Protein A chromatography. Cross reactivity with AKT3 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 AKT has not been determined.
Relevant Links:	• GenelD - 10000 • UniProtKB - Q9Y243 • NCBI - NP_001193658.1

Application Details

Tested Applications:	ELISA, IHC, WB
Application Note:	Anti-AKT3 Antibody is tested in ELISA, immunohistochemistry, and western blotting. Expect a band approximately 56 kDa in size corresponding to AKT3 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:	1:500-1:2000

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.

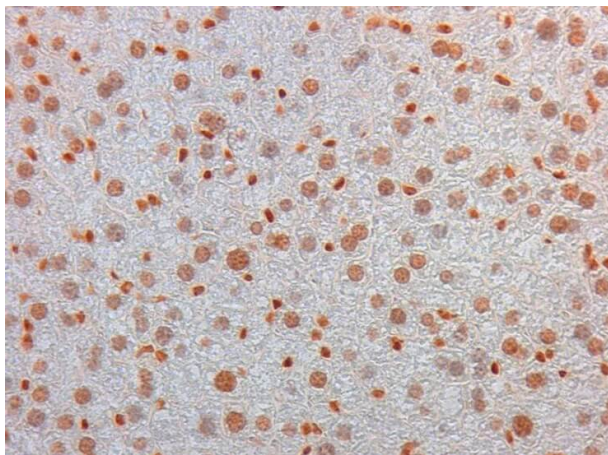
Expiration: Expiration date is one (1) year from date of receipt.

Images



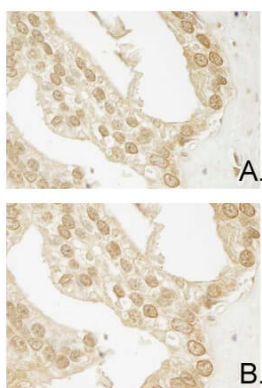
Western Blot

Western blot comparative study using Anti-AKT3 Antibody. Wild type mouse embryonic fibroblast (MEF) cells and cell lines created from Rictor knockout mice were treated with rapamycin (Rapa) and two other drugs (Drug x and Drug Y). Untreated cell lysates (-) were similarly processed along with cells from AKT3 null mice. Rockland's anti-AKT3 antibody (p/n 200-301-E75) (bottom panel) detects AKT3 (blue arrowhead) in wt MEFs. While rapamycin appears to have little affect on AKT3 levels in cells from Rictor knockouts, the two other drugs used in this study show a pronounced effect. By comparison, a competitor's anti-AKT3 antibody (top panel) appears to incorrectly recognize a 63 kDa band (red arrowhead) in MEFs and AKT3 null cells. Data provided by Dr. Yuefeng Tang, Dave Guertin Lab. U. Mass.



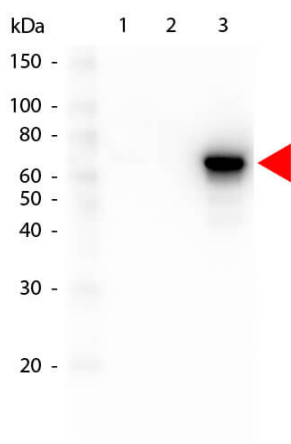
Immunohistochemistry

Immunohistochemistry of Mouse Monoclonal anti AKT3 Antibody in Mouse Embryonic Kidney. Tissue: Mouse Liver. Fixation: FFPE buffered formalin 10% conc. Ag Retrieval: Heat, Citrate pH 6.2. Pressure Cooker. Primary antibody: anti-AKT3 at 2ug/ml for 1.5 hour @ room Temp. Secondary Ab: MOUSE ON MOUSE HRP POLYMER 45" RT.



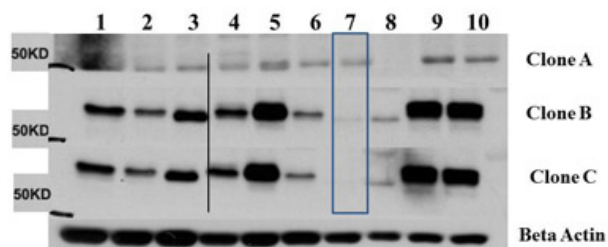
Immunohistochemistry

Immunohistochemistry of Mouse Anti-AKT3 antibody. Tissue: human prostate carcinoma. A) AKT-3 antibody produced using CELLline, B) AKT-3 antibody produced using roller bottle. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: AKT-3 antibody at 10 µg/mL for 1 h at RT. Secondary antibody: Peroxidase mouse secondary antibody at 1:10,000 for 1 h at RT. Localization: AKT3 is nuclear and occasionally cytoplasmic. Staining: AKT3 as precipitated brown signal with hematoxylin purple nuclear counterstain.



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. Lane 1: GST Tagged recombinant AKT1. Lane 2: GST Tagged recombinant AKT2. Lane 3: GST Tagged recombinant AKT3. The membrane was blocked with Blocking Buffer (p/n MB-070) for 1 h at room temperature. Mouse anti-AKT1 (p/n 200-301-E75) 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.



Western Blot

Western Blot of Mouse Anti-AKT3 antibody. Lane 1: C2C12.

Lane 2: MEF#1. Lane 3: MEF#2. Lane 4: A549. Lane 5: Calu-1. Lane 6: PC3. Lane 7: HepG2. Lane 8: Jurkat. Lane 9: SKOV3. Lane 10: 293T. Load: 35 µg per lane. Primary antibody: AKT-3 antibody at 1:1000 for overnight at 4°C. Secondary antibody: Anti mouse secondary antibody at 1:20,000 for 1 h at RT. Block: 5% BLOTTO overnight at 4°C. Predicted/Observed size: 56 kDa for AKT3.

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.