

Datasheet for 200-301-269S**AKT phospho T308 Antibody****Overview**

Description:	Anti-AKT pT308 (MOUSE) Monoclonal Antibody - 200-301-269S
Item No.:	200-301-269S
Size:	25 µL
Applications:	ELISA, IHC, WB, IF
Reactivity:	Human, Mouse
Host Species:	Mouse

Product Details

Background:	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 AKT1, 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 pT308 monoclonal antibody is ideal for investigators involved in Cancer, Cell Signaling, Neuroscience, Signal Transduction research.
Synonyms:	mouse anti-AKT pT308 Antibody, RAC-PK-alpha, Protein kinase B, PKB, C-AKT, RAC-alpha serine/threonine-protein kinase, Proto-oncogene c-Akt, AKT1, AKT 1, AKT-1
Host Species:	Mouse
Clonality:	Monoclonal
Clone ID:	18F3.H11
Format:	IgG1

Target Details

Gene Name:	AKT1
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Reactivity:	Human, Mouse
PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide
Immunogen:	Anti-AKT pT308 monoclonal antibody was produced by repeated immunizations with a synthetic phospho-peptide corresponding to residues surrounding T308 of human AKT1 protein.
Purity/Specificity:	Anti-AKT Phospho pT308 antibody was purified from concentrated tissue culture supernate by Protein A chromatography. This antibody is specific for human and mouse AKT protein phosphorylated at T308. A BLAST analysis was used to suggest cross-reactivity with AKT pT308 from most vertebrate species sources based on 100% homology with the immunizing sequence. Cross-reactivity with AKT from other sources has not been determined. Cross-reactivity with AKT2 and AKT3 will likely occur. Cancer, Cell Signaling, Neuroscience, Signal Transduction research.
Relevant Links:	• UniProtKB - P31749 • NCBI - 62241011 • GeneID - 207

Application Details

Tested Applications:	ELISA, IHC, WB
Suggested Applications:	IF (Based on references)
Application Note:	Anti-AKT pT308 antibody is tested for ELISA, immunohistochemistry, immunoprecipitation and western blotting. Expect a band approximately 56 kDa in size corresponding to phosphorylated AKT protein by western blotting in the appropriate cell lysate or extract. This phospho-specific monoclonal antibody reacts with human and mouse AKT pT308 and shows minimal reactivity by ELISA against the non-phosphorylated form of the immunizing peptide. Specific conditions for reactivity should be optimized by the end user. Use formalin-fixed paraffin-embedded sections for immunohistochemistry. 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:	1:20,000
FC:	User Optimized
IF:	1:25
IHC:	20 µg/ml
IP:	User Optimized
WB:	1:500 - 1:3,000

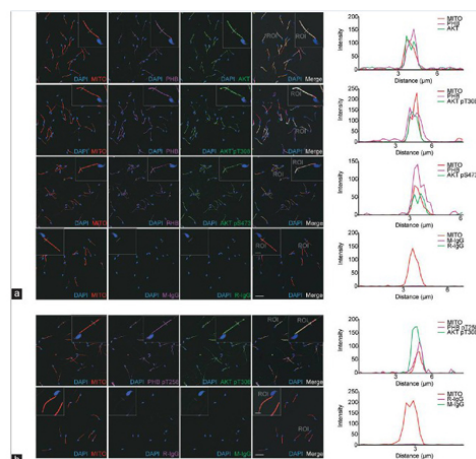
Formulation

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

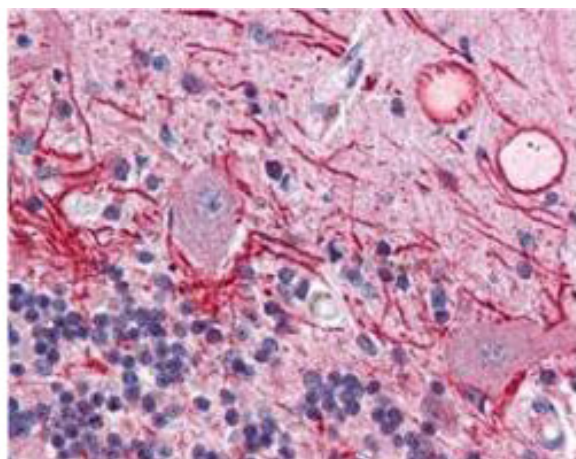
Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge 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 three (3) months from date of receipt.

Images



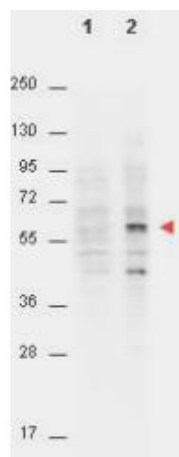
Immunofluorescence Microscopy

PHB and phospho-PHB (pT258) colocalize with AKT and phospho-AKT in the mitochondrial sheath of mouse mature sperm. Colocalization of (a) PHB (magenta) or (b) phospho-PHB (pT258; magenta) with AKT (green) or phospho-AKT (pT308, pS473; green), and the mitochondrial sheath (red) of mouse cauda epididymal sperm, was determined using a mitochondrial membrane potential probe (MITO) and confocal fluorescence microscopy. The inserts showed the image amplified for "Line Scan" colocalization analysis. The plot profiles of MITO (red), PHB (magenta) or phospho-PHB (pT258; magenta), AKT (green) or phospho-AKT (pT308, pS473; green) colocalization along the region of interest (ROI) were constructed and analyzed using LAS AF Lite software, as shown in the last column on the right. The negative controls (the last rows of a and b) were run with rabbit (R-IgG) and mouse (M-IgG) IgG isotypes, instead of specific primary antibodies. Scale bars = 20 µm. For amplified image (insert), scale bars = 5 µm. PHB: prohibitin; AKT: protein kinase B; DAPI: 4',6-diamidino-2-phenylindole. Fig 1. PMID: 32859869



Immunohistochemistry

Immunohistochemistry of Mouse anti-AKT pT308 antibody. Tissue: human brain cerebellum tissue (40X). Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: AKT pT308 antibody at 20 µg/mL for 1 h at RT. Secondary antibody: Peroxidase rabbit secondary antibody at 1:10,000 for 45 min at RT. Localization: staining of Purkinje neurons and cell processes in the cerebellum, cytosolic as well as occasionally nuclear. Staining: AKT pT308 as precipitated red signal with hematoxylin purple nuclear counterstain.



Western Blot

Western Blot of Mouse anti-AKT pT308 antibody. Lane 1: non-phosphorylated AKT in untreated NIH/3T3 cells (p/n W10-000-358). Lane 2: phosphorylated AKT on PDGF stimulated NIH/3T3 cell lysates (p/n W10-001-377). Load: 15 µg per lane. Primary antibody: AKT pT308 antibody at a 1:4,000 dilution in TBS with 3% BSA, for 3 h at 4° C. Secondary antibody: Peroxidase conjugated Gt-a-Mouse IgG (Fc) (p/n 610-1303) was used at a 1:40,000 dilution for 1 h at 4° C. Block: 3% BSA (p/n BSA-30) in TBS for 30 min at RT. Predicted/Observed size: (indicated by arrowhead at ~56 kDa). Other band(s): unspecific.

References

- Li XH et al. Prohibitin (PHB) interacts with AKT in mitochondria to coordinately modulate sperm motility. *Asian J Androl.* (2020)

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.