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Datasheet for 200-301-269

AKT phospho T308 Antibody

Overview

Description:	Anti-AKT pT308 (MOUSE) Monoclonal Antibody - 200-301-269
Item No.:	200-301-269
Size:	100 µg
Applications:	ELISA, IHC, WB, IF
Reactivity:	Human, Mouse
Host Species:	Mouse

Product Details

Background:	AKT1 pT308 antibody detects phosphorylated Akt. AKT is involved in cellular survival pathways, by inhibiting apoptotic processes. Akt is also able to induce protein synthesis pathways, and is therefore a key signaling protein in the cellular pathways that lead to skeletal muscle hypertrophy, and general tissue growth. 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 with 3 isoforms known as AKT1, AKT2, AKT3. AKT is 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 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
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 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.
Relevant Links:	• NCBI - 62241011 • UniProtKB - P31749 • 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

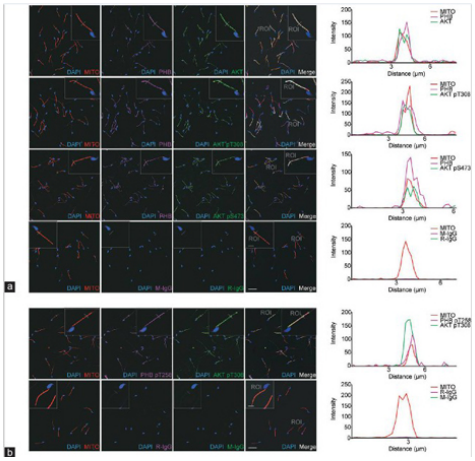
Formulation

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

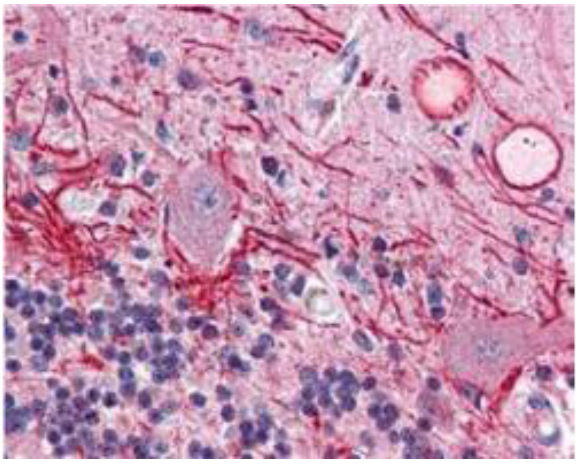
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store Anti-AKT pT308 antibody 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.

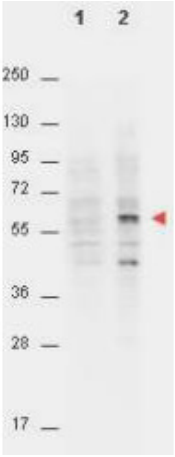
Images



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 pT308antibody 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

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