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Datasheet for 600-401-269

AKT phospho T308 Antibody

Overview

Description:	Anti-AKT pT308 (RABBIT) Antibody - 600-401-269
Item No.:	600-401-269
Size:	100 µg
Applications:	Dot Blot, ELISA, IHC, WB
Reactivity:	Human, Mouse, Rat
Host Species:	Rabbit

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 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.
Synonyms:	rabbit anti-AKT pT308 Antibody, AKT1 phospho T308, 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:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	AKT1
Reactivity:	Human, Mouse, Rat
PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide

Immunogen:	Anti-AKT pT308 polyclonal antibody was produced by repeated immunizations with a phosphorylated synthetic peptide corresponding to residues surrounding threonine 308 of human AKT1 protein.
Purity/Specificity:	AKT phospho T308 Antibody was prepared from monospecific antiserum by immunoaffinity chromatography using phospho peptide coupled to agarose beads followed by solid phase adsorption(s) against non-phospho peptide and non-specific peptide to remove any unwanted reactivities. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-Rabbit Serum. This antibody is specific for phosphorylated human AKT. Minimal reactivity occurs against non-phosphorylated AKT. Reactivity against AKT from other species may occur but has not yet been tested.
Relevant Links:	• UniProtKB - P31749 • NCBI - 62241011 • GeneID - 207

Application Details

Tested Applications:	Dot Blot, ELISA, IHC, WB
Application Note:	AKT antibody is phospho specific for pT308 and is tested for western blotting, Immunohistochemistry, dot blot, and ELISA. This antibody is suitable for flow cytometry.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:15,000
FC:	User Optimized
IHC:	1:200
WB:	1:1000

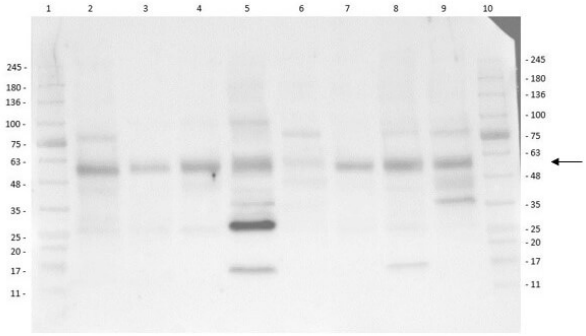
Formulation

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

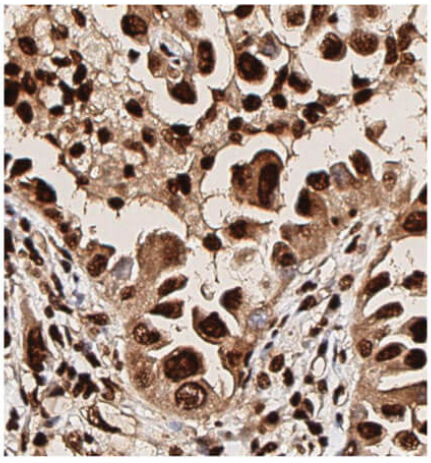
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store AKT phospho T308 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.

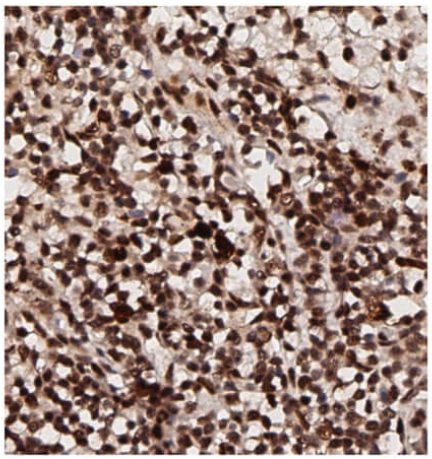
Images



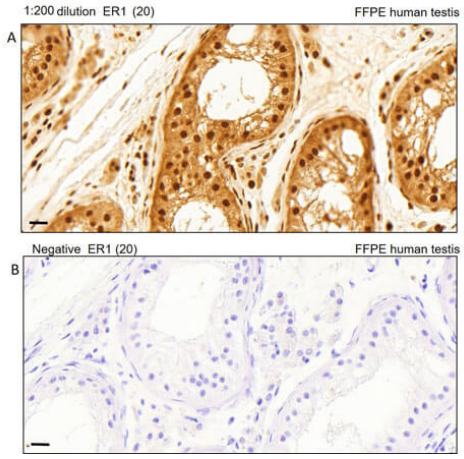
Western Blot
Multi-Lysate Western Blot of Rabbit Anti-AKT pT308 Antibody. Lane 1: Opal Pre-stained Molecular Weight Marker (p/n MB-210-0500). Lane 2: Human Spleen Lysate. Lane 3: Hu Small Intestine Lysate. Lane 4: Hu Placenta Lysate. Lane 5: Hu Skeletal Muscle Lysate. Lane 6: Hu Brain Cerebellum Lysate. Lane 7: Hu Lung Lysate. Lane 8: Hu Tonsil Lysate. Lane 9: Hu Thymus Lysate. Lane 10: Opal Pre-stained Molecular Weight Marker (p/n Mb-210-0500). Primary Antibody: Anti-AKT pT308 at 1:1000 overnight at 2-8°C. Secondary Antibody: Goat Anti-Rabbit IgG HRP (p/n 611-103-122) at 1:40,000 at RT for 60mins. Block: BlockOut Buffer (p/n MB-073). Predicted MW: ~55kDa. Observed MW: ~28, ~58kDa. Notes: Ubiquitous.



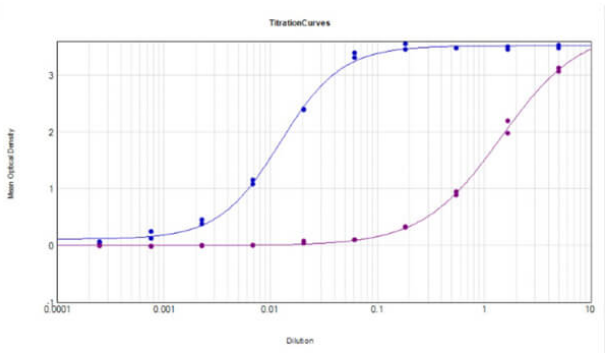
Immunohistochemistry
Immunohistochemistry of Rabbit Anti-AKT pT308 Antibody. Tissue: human lung tissue. Antigen retrieval: HIER using citrate buffer for 20 minutes. Fixative: None. Primary Antibody: Anti-AKT phosphoT308 at 1:200 for 30 minutes at RT. Secondary Antibody: Anti-Rabbit Poly-HRP-IgG Ready to Use for 8 minutes at RT. Counterstain: Hematoxylin. Substrate: DAB. Analysis: Strong staining in nucleus. May be suitable with more dilutions.



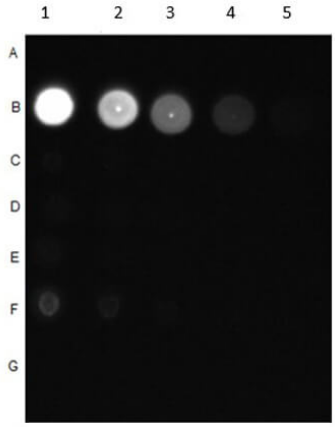
Immunohistochemistry
Immunohistochemistry of Rabbit Anti-AKT pT308 Antibody. Tissue: human breast tissue (lymph nodes). Antigen retrieval: HIER using citrate buffer for 20 minutes. Fixative: None. Primary Antibody: Anti-AKT phosphoT308 at 1:200 for 30 minutes at RT. Secondary Antibody: Anti-Rabbit Poly-HRP-IgG Ready to Use for 8 minutes at RT. Counterstain: Hematoxylin. Substrate: DAB. Analysis: Strong staining in nucleus. May be suitable with more dilutions.



Immunohistochemistry
Immunohistochemistry of Rabbit Anti-AKT pT308 Antibody. Tissue: human testis tissue. Antigen retrieval: Heat induced antigen retrieval was performed using Leica Bond Epitope Retrieval Buffer 1 (Citrate solution, pH6.0) for 20 minutes. Fixative: None. Primary Antibody: (A). Anti-AKTpT308 at 1:200 for 30 minutes at RT. (B). Negative control. Secondary Antibody: Anti-Rabbit Poly-HRP-IgG Ready to Use for 8 minutes at RT. Counterstain: Hematoxylin. Substrate: DAB. Analysis: Cells in seminiferous ducts and Leydig cells show moderate cytoplasmic staining.

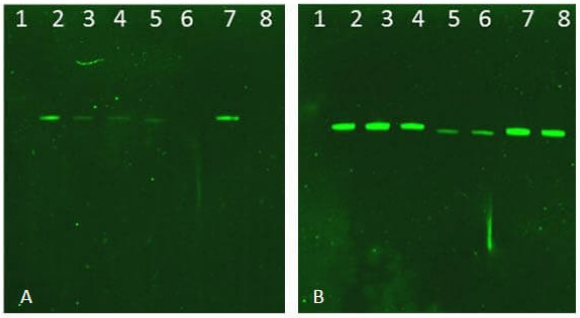


ELISA
ELISA Results of Rabbit Anti-AKT pT308 Antibody tested against BSA-conjugated non-phospho [purple] and phospho [blue] forms of immunizing peptide. Each well was coated in duplicate with either 0.1µg of conjugate. The working dilution is 1:81,300. The starting dilution of antibody was 5µg/ml and the X-axis represents the Log10 of a 3-fold dilution. This titration is a 4-parameter curve fit where the IC50 is defined as the titer of the antibody. Assay performed using HRP conjugate stabilizer (p/n MB-076), Goat Anti-Rabbit HRP conjugated (p/n 611-103-122) and TMB substrate (p/n TMBE-1000).



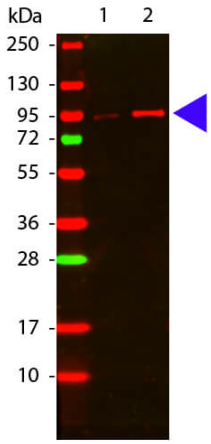
Dot Blot

Dot Blot of Rabbit Anti-AKT pT308 Antibody. Dilutions in Columns: (1) 100ng, (2) 33.33ng, (3) 11.11ng, (4) 3.7ng, (5) 1.23ng. Tested BSA Peptide Reactivity in Rows: (A) AKT1-BSA, (B) AKT1 pT308-BSA, (C) AKT1 S473-BSA, (D) AKT1 pS473-BSA, (E) CDC27 T244-BSA, (F) CDC27 pT244-BSA, (G) BSA control. Primary Antibody: Anti-AKT pT308 at 1µg/mL overnight at 2-8°C. Secondary Antibody: Goat anti-Rabbit IgG HRP (p/n 611-103-122) at 1:70,000 at RT for 30mins. Block: BlockOut Buffer (p/n MB-073).



Western Blot

Western Blot of Rabbit AKT Antibodies. Lane 1: NIR MW protein ladder. Lane 2: AKT1, recombinant: 009-001-P21. Lane 3: AKT1, phosphatase-treated: 009-001-I51. Lane 4: AKT1, mutant T308A/S473A: 009-001-P22. Lane 5: AKT2, recombinant: 009-001-P23. Lane 6: AKT2, phosphatase-treated: 009-001-E71. Lane 7: AKT3, recombinant: 009-001-P24. Lane 8: AKT3, phosphatase-treated: 009-001-E75. Load: 50ng per lane. Blot A: 600-401-269 Anti-Akt pT308 used at 1:2270, Blot B: 100-401-401 Anti-Akt used 1:1000.



Western Blot

Western Blot of Rabbit anti-Akt phospho T308 antibody. Lane 1: GST tagged AKT1 un-active recombinant protein. Lane 2: GST tagged AKT1 active recombinant protein. Load: 50 ng per lane. Primary antibody: Akt phospho T308 antibody at 1:1,000 for overnight at 4°C. Secondary antibody: DyLight™ 649 rabbit secondary antibody at 1:20,000 for 30 min at RT. Block: MB-070 for 30 min at RT. Other band(s): none.

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