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Datasheet for 200-302-E75

AKT3 FITC Antibody

Overview

Description:	Anti-AKT3 (MOUSE) Fluorescein Conjugated Monoclonal Antibody - 200-302-E75
Item No.:	200-302-E75
Size:	50 µg
Applications:	Dot Blot, ELISA
Reactivity:	Human, Mouse, Rat
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-AKT3 (MOUSE) PE conjugated Monoclonal Antibody is ideal for investigators involved in Cell Signaling, Cancer, Neuroscience, Signal Transduction research.
Synonyms:	Mouse anti-AKT3 antibody FITC conjugation, fluorescein conjugated Mouse anti-AKT 3 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
Conjugate:	Fluorescein (FITC)
Clonality:	Monoclonal
Clone ID:	25F6.F6.D8
Format:	IgG1
F/P Ratio:	2.7

Target Details

Gene Name:	AKT3
Reactivity:	Human, Mouse, Rat
Immunogen Type:	Conjugated Peptide
Immunogen:	Anti-AKT3 Antibody was prepared from tissue culture supernatant by Protein A affinity chromatography using 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 tissue culture 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:	• UniProtKB - Q9Y243 • NCBI - NP_001193658.1 • GeneID - 10000

Application Details

Tested Applications:	Dot Blot, ELISA
Application Note:	Anti-AKT3 FITC Antibody has been tested by ELISA and dot blot and is suitable for Flow Cytometry, 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:	User Optimized
FC:	User Optimized
IF:	User Optimized
IHC:	User Optimized
WB:	User Optimized

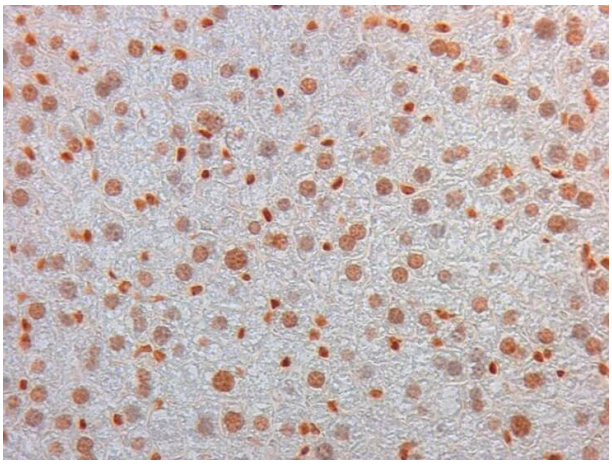
Formulation

Physical State:	Lyophilized
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.5 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	10 mg/mL Bovine Serum Albumin (BSA) - Immunoglobulin and Protease free
Reconstitution Volume:	50µL
Reconstitution Buffer:	Restore with deionized water (or equivalent)

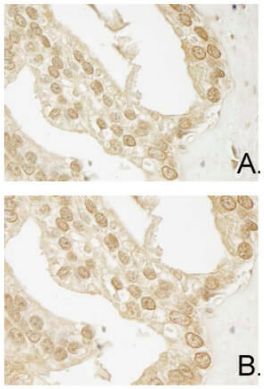
Shipping & Handling

Shipping Condition:	Wet Ice
Storage Condition:	Store vial at 4° C prior to restoration. Restore with deionized water (or equivalent). For extended storage aliquot contents and freeze at -20° C or below. 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.

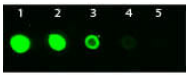
Images



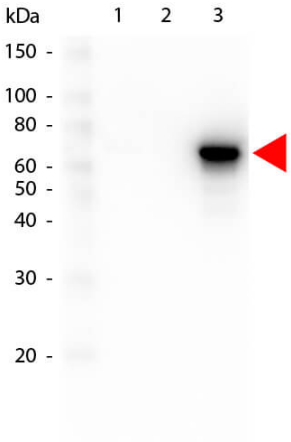
Immunohistochemistry
Immunohistochemistry of Mouse Monoclonal anti AKT3 Antibody in Mouse Embryonic Kidney. Tissue: Mouse Liver. Fixation: FFPE buffered formalin 10% conc. Ag Retrieval: Heat, Citrate pH 6.2. Pressure Cooker. Primary antibody: anti-AKT3 at 2ug/ml for 1.5 hour @ room Temp. Secondary Ab: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



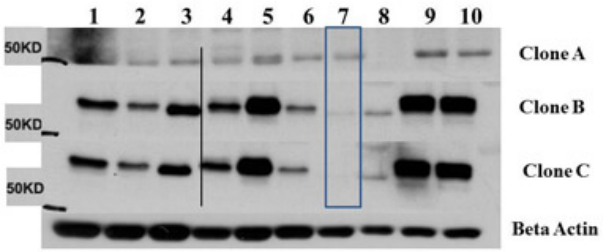
Immunohistochemistry
Immunohistochemistry of Mouse Anti-AKT3 antibody. Tissue: human prostate carcinoma. A) AKT-3 antibody produced using CELLine, B) AKT-3 antibody produced using roller bottle. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: AKT-3 antibody 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.



Dot Blot
Dot Blot of Mouse anti-AKT3 Monoclonal Antibody Fluorescein Conjugated. Antigen: His-tagged AKT3. Load: Lane 1 - 100 ng Lane 2 - 33.3 ng Lane 3 - 11.1 ng Lane 4 - 3.70 ng Lane 5 - 1.23 ng. Primary antibody: n/a. Secondary antibody: Mouse anti-AKT3 Monoclonal Antibody Fluorescein Conjugated at 1:1,000 for 60 min at RT. Block: MB-070 for 1 HR at RT.

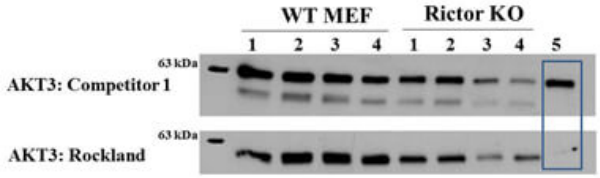


Western Blot
Western Blot of AKT isoform specific antibodies: 50 ng of recombinant GST-AKT1, -AKT2 and -AKT3 proteins were separated by SDS PAGE (4-20% gel) and transferred onto 0.2 µm nitrocellulose. Lane 1: GST Tagged recombinant AKT1. Lane 2: GST Tagged recombinant AKT2. Lane 3: GST Tagged recombinant 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.



Western Blot

Western Blot of Mouse anti-AKT3 antibody. Lane 1: Control. Lane 2: Rapa. Lane 3: T50. Lane 4: T250. Lane 5: Control. Lane 6: Rapa. Lane 7: T50. Lane 8: T250. Lane 9: AKT3 null. 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

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