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Anti-Akt phospho S473 Monoclonal Antibody

Catalog Number: MP00024

About AKT1

AKT phospho S473 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 pS473 (MOUSE) Monoclonal Antibody is ideal for investigators involved in Cell Signaling, Cancer, Neuroscience, Signal Transduction research.

Overview

Product Name	Anti-Akt phospho S473 Monoclonal Antibody
Reactive Species	Human, Monkey, Mouse, Rat
Description	Boster Bio Anti-Akt phospho S473 Monoclonal Antibody (Catalog # MP00024). Tested in ELISA, IF, IHC, WB applications. This antibody reacts with Human, Mouse, Rat, Monkey.
Application	ELISA, IF, IHC, WB
Clonality	Monoclonal Clone: 17F6.B11
Formulation	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2, 0.01% (w/v) Sodium Azide
Storage Instructions	Store Anti-AKT pS473 (MOUSE) Monoclonal 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 date is one (1) year from date of opening. (Ship on dry ice.)
Host	Mouse
Uniprot ID	P31749

Technical Details

Immunogen	Anti-AKT pS473 (MOUSE) Monoclonal Antibody was produced by repeated immunizations with a synthetic peptide corresponding to residues surrounding S473 of human AKT1 protein, followed by hybridoma development.
Predicted Reactive Species	Hepatitis Virus
Cross Reactivity	No cross reactivity with other proteins.

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Isotype	IgG1 kappa
Form	Liquid (sterile filtered)
Concentration	1.0 mg/ml by UV absorbance at 280 nm
Purification	Anti-AKT pS473 Monoclonal Antibody was purified from concentrated tissue culture supernate by Protein A chromatography. This phospho specific monoclonal antibody is specific for phosphorylated human and mouse AKT protein at S473. A BLAST analysis was used to suggest cross-reactivity with AKT pS473 from human, mouse, rat and chimpanzee 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 has not been determined.
Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this kit. If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples. Some PubMed article(s) citing the expression level of this target are as follows: Boster Bio's internal QC testing used: ELISA: 1:20,000 Flow Cytometry: User optimized IHC: 20 µg/mL IF Microscopy: 1:500-1:3,000 IP: User optimized WB: 1:500-1:3,000

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Anti-Akt phospho S473 Monoclonal Antibody (MP00024) Images

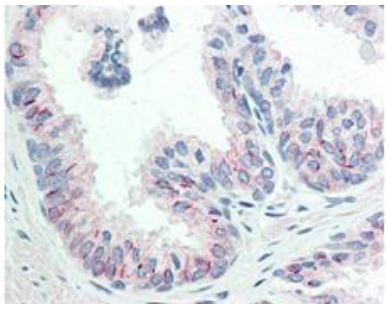
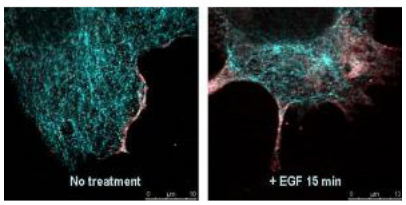
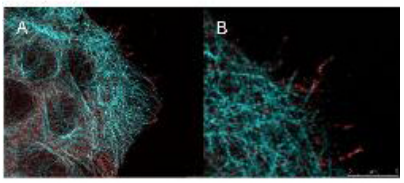


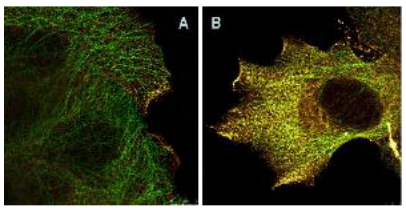
Figure 1. IHC analysis of AKT1 using anti-AKT1 antibody (MP00024).
AKT1 was detected in paraffin-embedded section. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-AKT1 Antibody (MP00024) overnight at 4°C. Biotinylated goat anti Mouse IgG antibody was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.



Immunofluorescence confocal microscopy of Mouse Anti-AKT pS473 antibody.Tissue: EGF treated A431 cells.Fixation:0.5% PFA. Antigen retrieval:EGF 15 min.Primary antibody:AKT pS473 antibody at 10 µg/mL



High resolution STED immunofluorescence nanoscopy of Mouse anti-AKT pS473 antibody.Tissue:A43the cell.Staining:AKT pS473as red signal with bis-benzimide (blue) nuclear counterstain.



Immunofluorescence Microscopy of Mouse Anti-AKTpS473 antibody using STED nanoscopy to evaluate AKT activation and migration.Tissue:A43cells with EGF, a rapid activation of AKT is observed (Panel B) along with a coincident change in the tubulin organization (yellow signal), as well as an extensive cell shape-change (cell membrane folding) and accumulation of AKTpS473 at the cell periphery.

Figure 5. Western blot analysis of AKT1 using anti-AKT1 antibody (MP00024).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
After Electrophoresis, proteins were transferred to a

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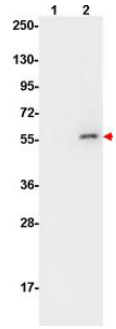

Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-AKT1 antigen affinity purified polyclonal antibody (Catalog # MP00024) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-Mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # SA1021) with Tanon 5200 system. A specific band was detected for AKT1.

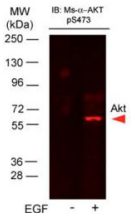

Figure 6. Western blot analysis of AKT1 using anti-AKT1 antibody (MP00024).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-AKT1 antigen affinity purified polyclonal antibody (Catalog # MP00024) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-Mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # SA1021) with Tanon 5200 system. A specific band was detected for AKT1.

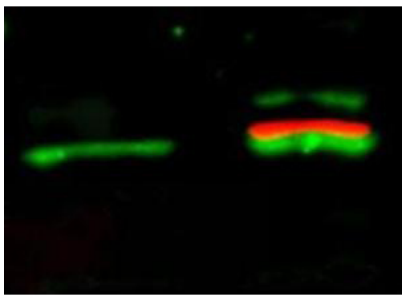

Figure 7. Western blot analysis of AKT1 using anti-AKT1 antibody (MP00024).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-AKT1 antigen affinity purified polyclonal antibody (Catalog # MP00024) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-Mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # SA1021) with Tanon 5200 system. A specific band was detected for AKT1.

Figure 8. Western blot analysis of AKT1 using anti-AKT1 antibody (MP00024).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
After Electrophoresis, proteins were transferred to a

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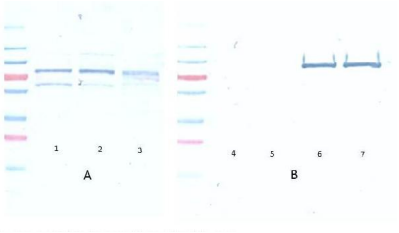

Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-AKT1 antigen affinity purified polyclonal antibody (Catalog # MP00024) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-Mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # SA1021) with Tanon 5200 system. A specific band was detected for AKT1.

15 Publications Citing This Product

1. PubMed ID: 10.3748/wjg.v25.i40.6063, Insulin-like growth factor 2 mRNA-binding protein 1 promotes cell proliferation via activation of AKT and is directly targeted by microRNA-494 in pancreatic cancer

2. PubMed ID: 29044143, Li R, Cui K, Liu K, Li H, Zhang Y, Liu X, Chen R, Li M, Wang T, Wang S, Liu J, Rao K. Sci Rep. 2017 Oct 18;7(1):13464. doi: 10.1038/s41598-017-12907-1. Metabolic syndrome in rats is associated with erectile dysfunction by impairing PI3K/Akt/eNOS a...

3. PubMed ID: 27456341, Hyperthermia induced HIF-1a expression of lung cancer through AKT and ERK signaling pathways

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