Anti-Phospho-AKT (T308) Antibody [PSH08-10] HA722951

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, FC
Molecular Wt:	Predicted band size: 56 kDa
Clone number:	PSH08-10

Description: RAC(Rho family)-alpha serine/threonine-protein kinase is an enzyme that in humans is encoded by the AKT1 gene. This enzyme belongs to the AKT subfamily of serine/threonine kinases that contain SH2 (Src homology 2-like) protein domains. It is commonly referred to as PKB, or by both names as "Akt/PKB". The serine-threonine protein kinase AKT1 is catalytically inactive in serum-starved primary and immortalized fibroblasts. AKT1 and the related AKT2 are activated by platelet-derived growth factor. The activation is rapid and specific, and it is abrogated by mutations in the pleckstrin homology domain of AKT1. It was shown that the activation occurs through phosphatidylinositol 3-kinase. In the developing nervous system AKT is a critical mediator of growth factor-induced neuronal survival. Survival factors can suppress apoptosis in a transcription-independent manner by activating the serine/threonine kinase AKT1, which then phosphorylates and inactivates components of the apoptotic machinery. Mice lacking Akt1 display a 25% reduction in body mass, indicating that Akt1 is critical for transmitting growth-promoting signals, most likely via the IGF1 receptor. Mice lacking Akt1 are also resistant to cancer: They experience considerable delay in tumor growth initiated by the large T antigen or the Neu oncogene. A single-nucleotide polymorphism in this gene causes Proteus syndrome.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Thr308 of Human AKT1.

Positive control: Jurkat treated with 100nM Calyculin A for 30 minutes cell lysate, HeLa treated with 100ng/mL Calyculin A for 30 minutes cell lysate, 293T treated with 100nM Calyculin A for 15 minutes cell lysate, Jurkat cells treated with 100nM Calyculin A for 30 minutes.

Subcellular location: Cytoplasm, Nucleus, Cell membrane.

Database links: SwissProt: P31749 Human

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:100
FC	1:1,000

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

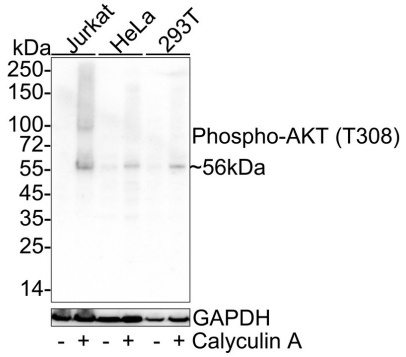

Fig1: Western blot analysis of Phospho-AKT (T308) on different lysates with Rabbit anti-Phospho-AKT (T308) antibody (HA722951) at 1/2,000 dilution.

Lane 1: Jurkat cell lysate
Lane 2: Jurkat treated with 100nM Calyculin A for 30 minutes cell lysate
Lane 3: HeLa cell lysate
Lane 4: HeLa treated with 100ng/mL Calyculin A for 30 minutes cell lysate
Lane 5: 293T cell lysate
Lane 6: 293T treated with 100nM Calyculin A for 15 minutes cell lysate

Lysates/proteins at 30 µg/Lane.

Predicted band size: 56 kDa
Observed band size: 56 kDa

Exposure time: 3 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA722951) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

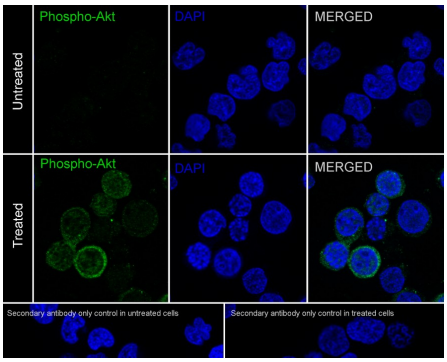

Fig2: Immunocytochemistry analysis of Jurkat cells treated with or without 100nM Calyculin A for 30 minutes labeling Phospho-AKT (T308) with Rabbit anti-Phospho-AKT (T308) antibody (HA722951) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospho-AKT (T308) antibody (HA722951) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

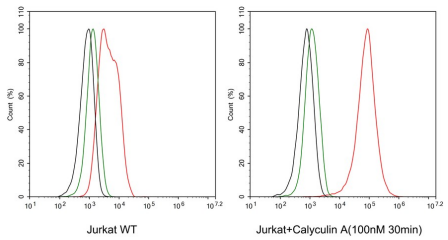

Fig3: Flow cytometric analysis of Jurkat cells treated with or without 100nM Calyculin A for 30 minutes labeling Phospho-AKT (T308).

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722951, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Huang L et al. PRMT5 activates AKT via methylation to promote tumor metastasis. Nat Commun. 2022 Jul
2. Liang XX et al. Phosphorylation of Akt at Thr308 regulates p-eNOS Ser1177 during physiological conditions. FEBS Open Bio. 2021 Jul