Anti-Phospho-AKT (S473) Antibody [PSH04-44] - BSA and Azide free

HA750953

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, FC
Molecular Wt:	Predicted band size: 56 kDa
Clone number:	PSH04-44

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.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser473 of human Akt1.

Positive control: MCF7 treated with 50ng/mL Calyculin A for 45 minutes cell lysate, SH-SY5Y treated with 100ng/mL PDGF for 5 minutes cell lysate, HEK-293 cell lysate, NIH/3T3 treated with 100ng/mL PDGF for 5 minutes cell lysate, C6 treated with 100ng/mL PDGF for 5 minutes cell lysate, mouse spleen tissue, rat spleen tissue, human breast cancer tissue.

Subcellular location: Cytoplasm, Nucleus, Cell membrane.

Database links: SwissProt: P31749 Human | P31751 Human | Q9Y243 Human | P31750 Mouse | Q60823 Mouse | Q9WUA6 Mouse | P47196 Rat | P47197 Rat | Q63484 Rat

Recommended Dilutions:

WB	1:2,000-1:5,000
IHC-P	1:200
FC	1:10,000

Storage Buffer: PBS (pH7.4).

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

Purity: Protein A affinity purified.

Images

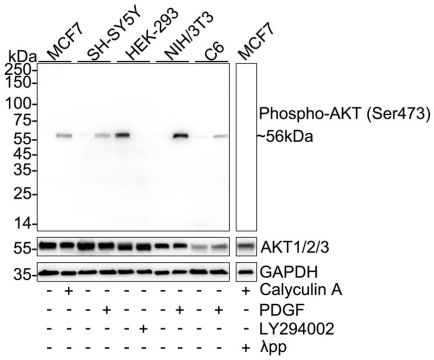

Fig1: Western blot analysis of Phospho-AKT (S473) on different lysates with Rabbit anti-Phospho-AKT (S473) antibody (HA750953) at 1/2,000 dilution and pan AKT antibody (HA721870) at 1/2,000 dilution.

Lane 1: MCF7 cell lysate
Lane 2: MCF7 treated with 50ng/mL Calyculin A for 45 minutes cell lysate
Lane 3: SH-SY5Y cell lysate
Lane 4: SH-SY5Y treated with 100ng/mL PDGF for 5 minutes cell lysate
Lane 5: HEK-293 cell lysate
Lane 6: HEK-293 treated with 50μM LY294002 for 6 hours cell lysate
Lane 7: NIH/3T3 cell lysate
Lane 8: NIH/3T3 treated with 100ng/mL PDGF for 5 minutes cell lysate
Lane 9: C6 cell lysate
Lane 10: C6 treated with 100ng/mL PDGF for 5 minutes cell lysate
Lane 11: MCF7 treated with 50ng/mL Calyculin A for 45 minutes cell lysate, then the membrane treated with λpp for 1 hour

Lysates/proteins at 20 μg/Lane.

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

Exposure time: 20 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% BSA for 1 hour at room temperature. The primary antibody (HA750953) at 1/2,000 dilution and pan AKT antibody (HA721870) at 1/2,000 dilution were used in 5% BSA at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

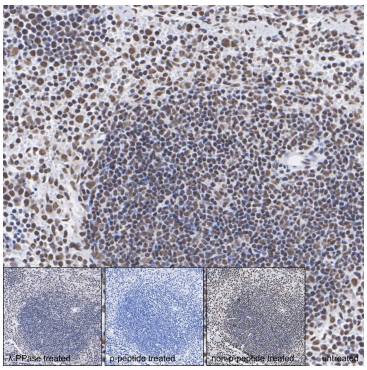

Fig2: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue untreated / treated with λpp / phospho-peptide / non-phospho-peptide with Rabbit anti-Phospho-AKT (S473) antibody (HA750953) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750953) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

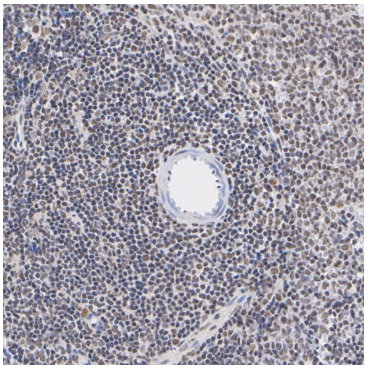

Fig3: Immunohistochemical analysis of paraffin-embedded rat spleen tissue with Rabbit anti-Phospho-AKT (S473) antibody (HA750953) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750953) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

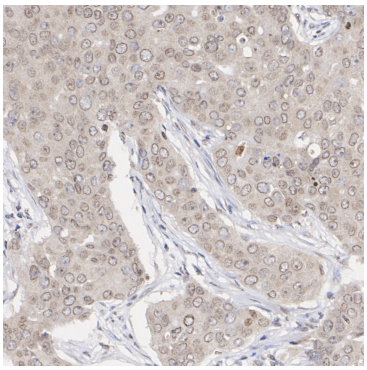

Fig4: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue with Rabbit anti-Phospho-AKT (S473) antibody (HA750953) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750953) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

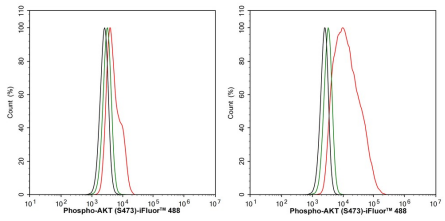

Fig5: Flow cytometric analysis of NIH/3T3 cells untreated (left) / treated with 100ng/mL PDGF for 1 hour (right) labeling Phospho-AKT (S473).

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750953, 0.1μg/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).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Lee DS et al. P2 × 7 Receptor Inhibits Astroglial Autophagy via Regulating FAK- and PHLPP1/2-Mediated AKT-S473 Phosphorylation Following Kainic Acid-Induced Seizures. Int J Mol Sci. 2020 Sep
2. Cai Q et al. MAPK6-AKT signaling promotes tumor growth and resistance to mTOR kinase blockade. Sci Adv. 2021 Nov