Anti-Phospho-AKT (S473) Antibody [PSH24-43] HA724447

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IP
Molecular Wt:	Predicted band size: 56 kDa
Clone number:	PSH24-43

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: 293T (Human embryonic kidney cells) cell lysate, 293T treated with 100 nM Calyculin A for 15 minutes cell lysate, C2C12 treated with 100 nM Insulin for 30 minutes cell lysate, C6 (Rat glioma cell) cell lysate, C6 treated with 50 ng/mL Calyculin A for 45 minutes cell lysate.

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	1:1,000-1:2,000
IP	1-2μg/sample

Storage Buffer: 1*PBS (pH7.4), 0.1% BSA, 40% Glycerol, 0.2% Proclean 950.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

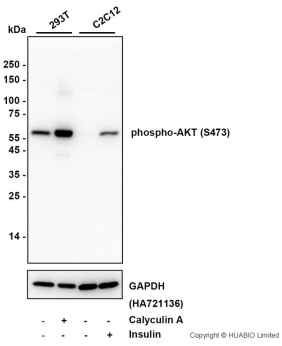

Fig1: Western blot analysis of Phospho-AKT (S473) on different lysates with Rabbit anti-Phospho-AKT (S473) antibody (HA724447) at 1/1,000 dilution.

Lane 1: 293T (Human embryonic kidney cells) cell lysate
Lane 2: 293T treated with 100 nM Calyculin A for 15 minutes cell lysate
Lane 3: C2C12 (Mouse myoblasts) cell lysate
Lane 4: C2C12 treated with 100 nM Insulin for 30 minutes cell lysate

Lysates/proteins at 15 µg/Lane.
Exposure time: 20 seconds; ECL: K1801

Blocking: 5% NFDM/TBST, 1 hour at room temperature
Primary antibody: HA724447, 1/1,000 in primary antibody dilution buffer (K1803), overnight at 4 °C
Secondary antibody: Goat anti-Rabbit IgG-HRP (HA1001), 1/50,000 in 5% NFDM/TBST, 1 hour at room temperature

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

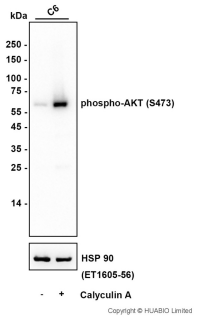

Fig2: Western blot analysis of Phospho-AKT (S473) on different lysates with Rabbit anti-Phospho-AKT (S473) antibody (HA724447) at 1/2,000 dilution.

Lane 1: C6 (Rat glioma cell) cell lysate
Lane 2: C6 treated with 50 ng/mL Calyculin A for 45 minutes cell lysate

Lysates/proteins at 10 µg/Lane.
Exposure time: 17 seconds; ECL: K1801

Blocking: 5% NFDM/TBST, 1 hour at room temperature
Primary antibody: HA724447, 1/2,000 in primary antibody dilution buffer (K1803), overnight at 4 °C
Secondary antibody: Goat anti-Rabbit IgG-HRP (HA1001), 1/50,000 in 5% NFDM/TBST, 1 hour at room temperature

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

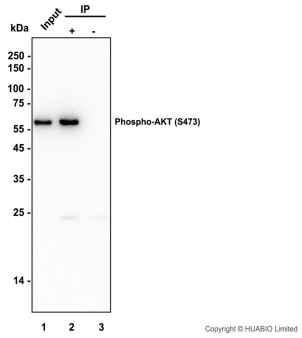

Fig3: Immunoprecipitation (IP)

Phospho-AKT (S473) was immunoprecipitated in 0.2 mg NIH/3T3 (Mouse fibroblast) treated with 100 nM Calyculin A for 30 minutes cell lysate with HA724447 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA724447 at 1/5,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: NIH/3T3 treated with Calyculin A cell lysate (input)
Lane 2: HA724447 IP in NIH/3T3 treated with Calyculin A cell lysate
Lane 3: Rabbit IgG instead of HA724447 in NIH/3T3 treated with Calyculin A cell lysate

Exposure time: 11 seconds
Blocking: 5% NFDM/TBST, 1 hour at room temperature

Primary dilution: HA724447, 1/2,000 in primary antibody dilution buffer (K1803), 2 hours at room temperature

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

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