Anti-Phospho-AKT (S473) Antibody [SY28-05] - BSA and Azide free

HA750132

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Dog
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IHC-Fr
Molecular Wt:	Predicted band size: 56 kDa
Clone number:	SY28-05

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 100nM Calyculin A for 30 minutes cell lysate, NIH/3T3 treated with 100ng/mL PDGF for 1 hour cell lysate, C6 treated with 100nM Calyculin A for 30 minutes cell lysate, HEK-293 cell lysate, HeLa cells treated with 100nM Calyculin A for 30 minutes, NIH/3T3 cells treated with 100ng/mL PDGF for 1 hour, C6 cells treated with 100nM Calyculin A for 30 minutes, human breast cancer tissue, mouse lung tissue, rat brain tissue, mouse hippocampus tissue, mouse cerebral cortex tissue, mouse brain tissue.

Subcellular location: Cytoplasm, Nucleus, Cell membrane.

Database links: SwissProt: P31749 Human | P31751 Human | Q9Y243 Human | P31750 Mouse | P47196 Rat

Recommended Dilutions:

WB	1:5,000-1:10,000
IF-Cell	1:100-1:1,000
IHC-P	1:200-1:1,000
IF-Tissue	1:200-1:500
IHC-Fr	1:100

Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

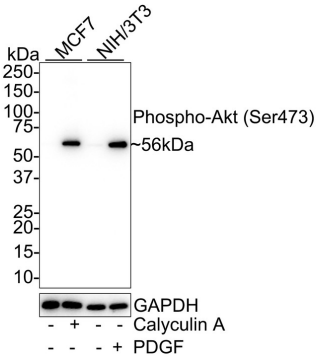

Fig1: Western blot analysis of Phospho-AKT (S473) on different lysates with Rabbit anti-Phospho-AKT (S473) antibody (HA750132) at 1/5,000 dilution.

Lane 1: MCF7 cell lysate
Lane 2: MCF7 treated with 100nM Calyculin A for 30 minutes cell lysate
Lane 3: NIH/3T3 cell lysate
Lane 4: NIH/3T3 treated with 100ng/mL PDGF for 1 hour cell lysate

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 53 seconds; ECL: K1801;
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 (HA750132) at 1/5,000 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.

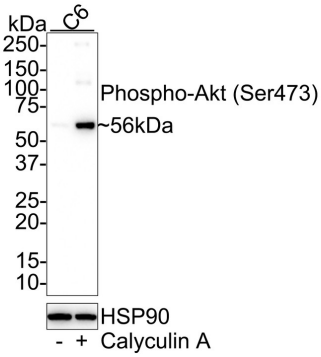

Fig2: Western blot analysis of Phospho-AKT (S473) on different lysates with Rabbit anti-Phospho-AKT (S473) antibody (HA750132) at 1/5,000 dilution.

Lane 1: C6 cell lysate
Lane 2: C6 treated with 100nM Calyculin A for 30 minutes cell lysate

Lysates/proteins at 15 µg/Lane.

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

Exposure time: 3 minutes; ECL: K1801;
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 (HA750132) at 1/5,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

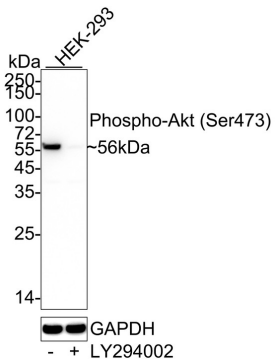

Fig3: Western blot analysis of Phospho-AKT (S473) on different lysates with Rabbit anti-Phospho-AKT (S473) antibody (HA750132) at 1/5,000 dilution.

Lane 1: HEK-293 cell lysate
Lane 2: HEK-293 treated with 50μM LY294002 for 6 hours cell lysate

Lysates/proteins at 20 μg/Lane.

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

Exposure time: 50 seconds; ECL: K1801;
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 (HA750132) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/200,000 dilution was used for 1 hour at room temperature.

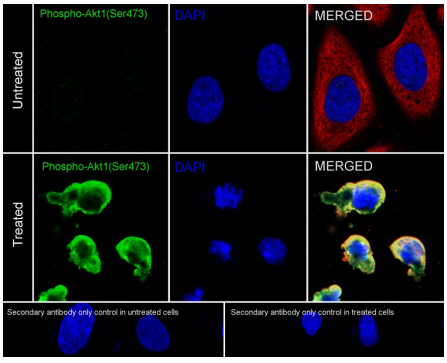

Fig4: Immunocytochemistry analysis of HeLa cells treated with or without 100nM Calyculin A for 30 minutes labeling Phospho-AKT (S473) with Rabbit anti-Phospho-AKT (S473) antibody (HA750132) at 1/200 dilution.

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 (S473) antibody (HA750132) at 1/200 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.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

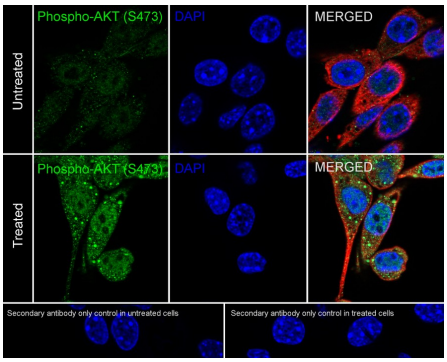

Fig5: Immunocytochemistry analysis of NIH/3T3 cells treated with or without 100ng/mL PDGF for 1 hour labeling Phospho-AKT (S473) with Rabbit anti-Phospho-AKT (S473) antibody (HA750132) at 1/100 dilution.

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 (S473) antibody (HA750132) 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.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

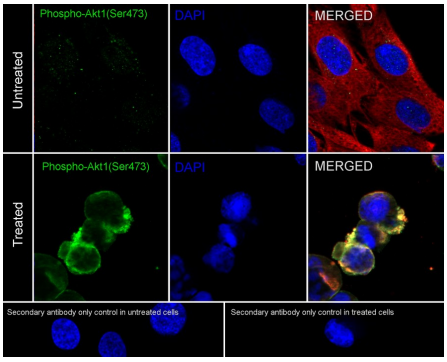

Fig6: Immunocytochemistry analysis of C6 cells treated with or without 100nM Calyculin A for 30 minutes labeling Phospho-AKT (S473) with Rabbit anti-Phospho-AKT (S473) antibody (HA750132) at 1/1,000 dilution.

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 (S473) antibody (HA750132) at 1/1,000 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.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

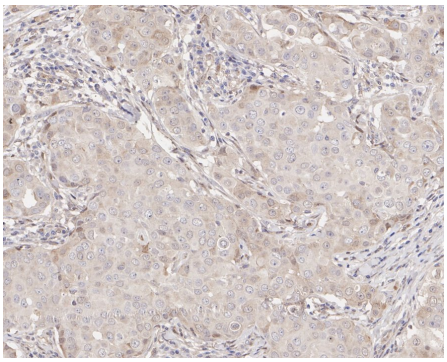

Fig7: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue with Rabbit anti-Phospho-AKT (S473) antibody (HA750132) at 1/200 dilution.

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 (HA750132) 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.

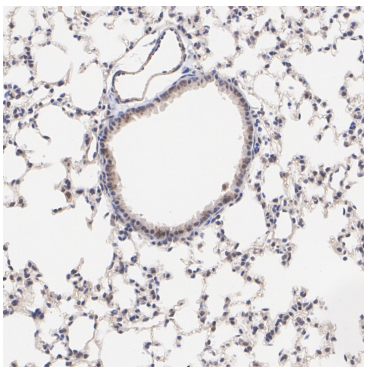

Fig8: Immunohistochemical analysis of paraffin-embedded mouse lung tissue with Rabbit anti-Phospho-AKT (S473) antibody (HA750132) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 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 (HA750132) at 1/1,000 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.

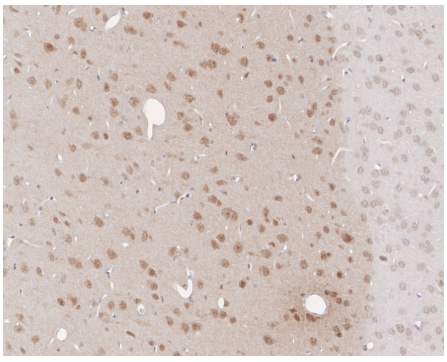

Fig9: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-Phospho-AKT (S473) antibody (HA750132) at 1/1,000 dilution.

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 (HA750132) at 1/1,000 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.

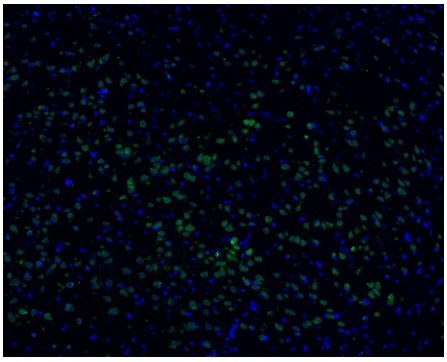

Fig10: Application: IHC-Fr

Species: Mouse

Site: Cerebral cortex

Sample: Frozen section

Antibody concentration: 1/200

Antigen retrieval: Not required

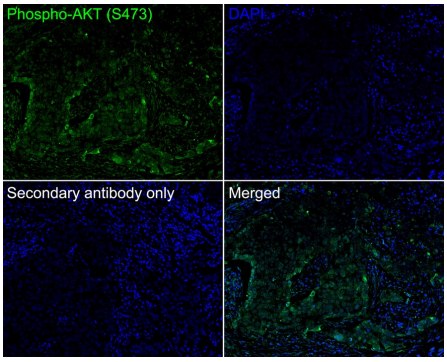

Fig11: Application: IF-tissue

Species: Human

Site: Breast cancer

Sample: Paraffin-embedded section

Antibody concentration: 1/200

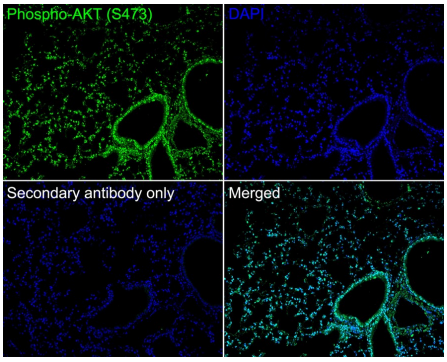

Fig12: Application: IF-tissue

Species: Mouse

Site: Lung

Sample: Paraffin-embedded section

Antibody concentration: 1/200

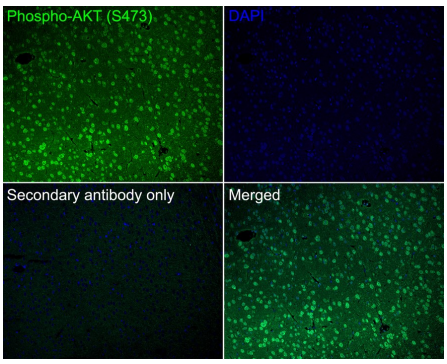

Fig13: Application: IF-tissue

Species: Mouse

Site: Cerebral cortex

Sample: Paraffin-embedded section

Antibody concentration: 1/200

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