Anti-AKT1/2/3 Antibody [ST48-09] - BSA and Azide free

HA750179

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Monkey
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC, IHC-Fr
Molecular Wt:	Predicted band size: 56 kDa
Clone number:	ST48-09

Description: The serine/threonine kinase Akt family contains several members, including Akt1 (also designated PKB or RacPK), Akt2 (also designated PKBβ or RacPK-β) and Akt 3 (also designated PKBγ or thyoma viral proto-oncogene 3), which exhibit sequence homology with the protein kinase A and C families and are encoded by the c-Akt proto-oncogene. All members of the Akt family have a pleckstrin homology domain. Akt1 and Akt2 are activated by PDGF stimulation. This activation is dependent on PDGFR-β tyrosine residues 740 and 751, which bind the subunit of the phosphatidylinositol 3-kinase (PI 3-kinase) complex. Activation of Akt1 by insulin or insulin-growth factor-1(IGF-1) results in phosphorylation of both Thr 308 and Ser 473. Phosphorylation of both residues is important to generate a high level of Akt1 activity, and the phosphorylation of Thr 308 is not dependent on phosphorylation of Ser 473 in vivo. Thus, Akt proteins become phosphorylated and activated in insulin/IGF-1-stimulated cells by an upstream kinase(s). The activation of Akt1 and Akt2 is inhibited by the PI kinase inhibitor wortmannin, suggesting that the protein signals downstream of the PI kinases.

Immunogen:	Recombinant protein within human AKT3 aa 300-479.
Positive control:	MCF7 cell lysate, U-2 OS cell lysate, Jurkat cell lysate, C6 cell lysate, mouse heart tissue lysate, mouse testis tissue lysate, rat heart tissue lysate, rat testis tissue lysate, MCF7, RAW264.7, C6, human brain tissue, human lung tissue, mouse brain tissue, mouse lung tissue, rat brain tissue, rat lung tissue, mouse hippocampus tissue, mouse cerebral cortex tissue.
Subcellular location:	Cell membrane, Cytoplasm, Membrane, Nucleus.
Database links:	SwissProt: P31749 Human | P31751 Human | Q9Y243 Human | P31750 Mouse | Q60823 Mouse | Q9WUA6 Mouse | P47196 Rat | P47197 Rat | Q63484 Rat

Recommended Dilutions:

WB	1:5,000-1:10,000
IF-Cell	1:100-1:200
IF-Tissue	1:500-1:1,000
IHC-P	1:2,000
FC	1:1,000
IP	1-2μg/sample
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

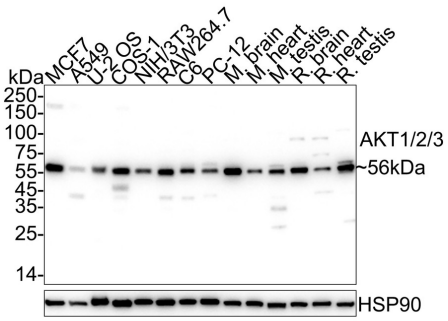

Fig1: Western blot analysis of AKT1/2/3 on different lysates with Rabbit anti-AKT1/2/3 antibody (HA750179) at 1/5,000 dilution.

Lane 1: MCF7 cell lysate (20 µg/Lane)
Lane 2: A549 cell lysate (20 µg/Lane)
Lane 3: U-2 OS cell lysate (20 µg/Lane)
Lane 4: COS-1 cell lysate (20 µg/Lane)
Lane 5: NIH/3T3 cell lysate (20 µg/Lane)
Lane 6: RAW264.7 cell lysate (20 µg/Lane)
Lane 7: C6 cell lysate (20 µg/Lane)
Lane 8: PC-12 cell lysate (20 µg/Lane)
Lane 9: Mouse brain tissue lysate (20 µg/Lane)
Lane 10: Mouse heart tissue lysate (20 µg/Lane)
Lane 11: Mouse testis tissue lysate (20 µg/Lane)
Lane 12: Rat brain tissue lysate (20 µg/Lane)
Lane 13: Rat heart tissue lysate (20 µg/Lane)
Lane 14: Rat testis tissue lysate (20 µg/Lane)

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

Exposure time: 24 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 (HA750179) 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.

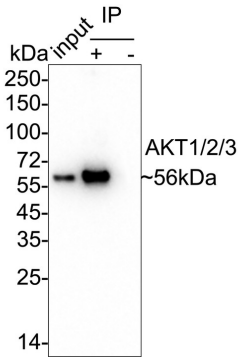

Fig2: AKT1/2/3 was immunoprecipitated from 0.2 mg MCF7 cell lysate with HA750179 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using HA750179 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: MCF7 cell lysate (input)
Lane 2: HA750179 IP in MCF7 cell lysate
Lane 3: Rabbit IgG instead of HA750179 in MCF7 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 23 seconds; ECL: K1801

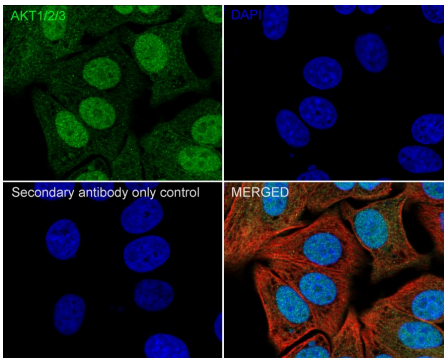

Fig3: Immunocytochemistry analysis of MCF7 cells labeling AKT1/2/3 with Rabbit anti-AKT1/2/3 antibody (HA750179) at 1/100 dilution.

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-AKT1/2/3 antibody (HA750179) 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.

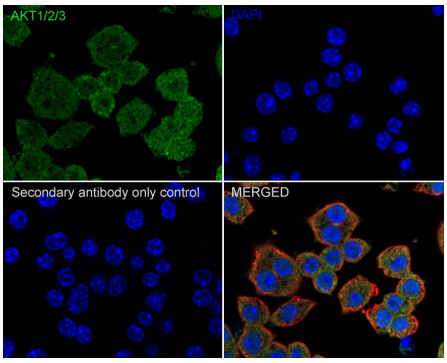

Fig4: Immunocytochemistry analysis of RAW264.7 cells labeling AKT1/2/3 with Rabbit anti-AKT1/2/3 antibody (HA750179) at 1/100 dilution.

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-AKT1/2/3 antibody (HA750179) 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.

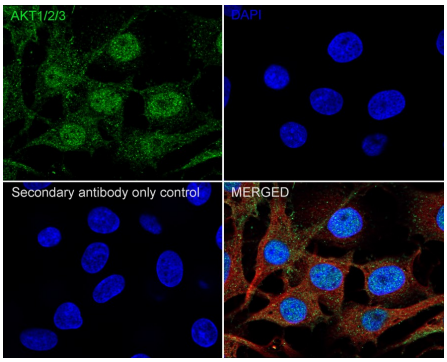

Fig5: Immunocytochemistry analysis of C6 cells labeling AKT1/2/3 with Rabbit anti-AKT1/2/3 antibody (HA750179) at 1/100 dilution.

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-AKT1/2/3 antibody (HA750179) 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.

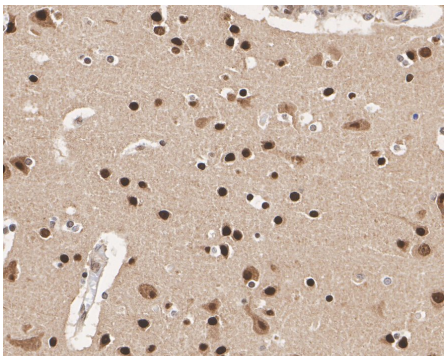

Fig6: Immunohistochemical analysis of paraffin-embedded human brain tissue with Rabbit anti-AKT1/2/3 antibody (HA750179) at 1/2,000 dilution.

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 (HA750179) at 1/2,000 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.

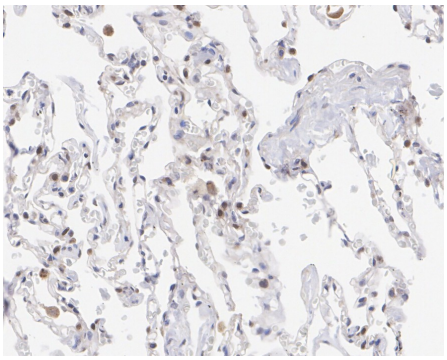

Fig7: Immunohistochemical analysis of paraffin-embedded human lung tissue with Rabbit anti-AKT1/2/3 antibody (HA750179) at 1/2,000 dilution.

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 (HA750179) at 1/2,000 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.

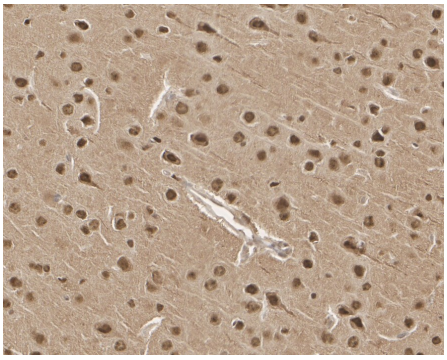

Fig8: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-AKT1/2/3 antibody (HA750179) at 1/2,000 dilution.

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 (HA750179) at 1/2,000 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.

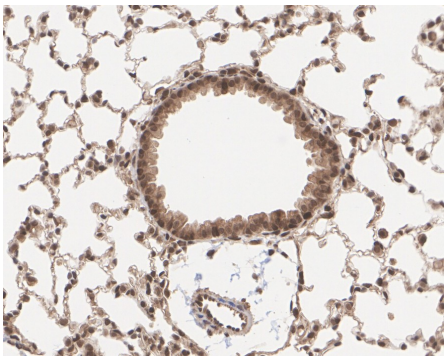

Fig9: Immunohistochemical analysis of paraffin-embedded mouse lung tissue with Rabbit anti-AKT1/2/3 antibody (HA750179) at 1/2,000 dilution.

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 (HA750179) at 1/2,000 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.

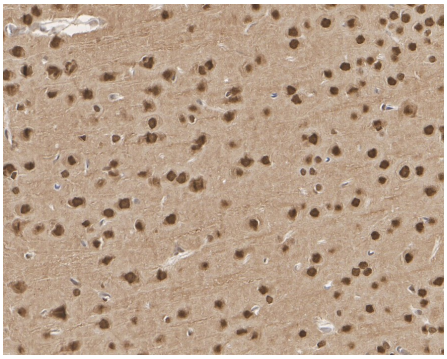

Fig10: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-AKT1/2/3 antibody (HA750179) at 1/2,000 dilution.

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 (HA750179) at 1/2,000 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.

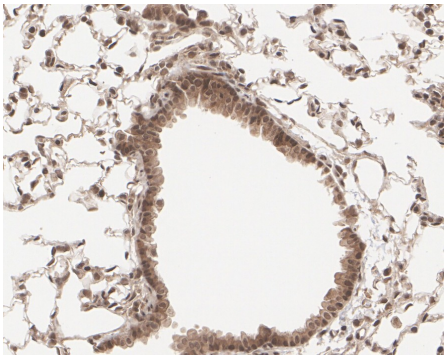

Fig11: Immunohistochemical analysis of paraffin-embedded rat lung tissue with Rabbit anti-AKT1/2/3 antibody (HA750179) at 1/2,000 dilution.

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 (HA750179) at 1/2,000 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.

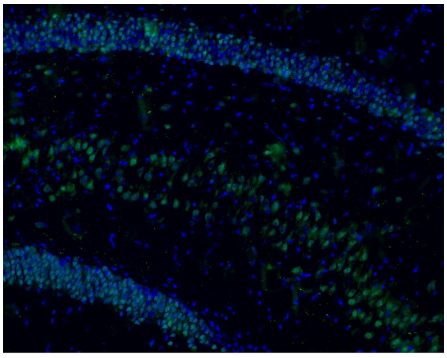

Fig12: Application: IHC-Fr

Species: Mouse

Site: Hippocampus

Sample: Frozen section

Antibody concentration: 1/100

Antigen retrieval: The section was pre-treated using 1% SDS buffer (in PBS, pH 7.4) for 5 minutes at room temperature.

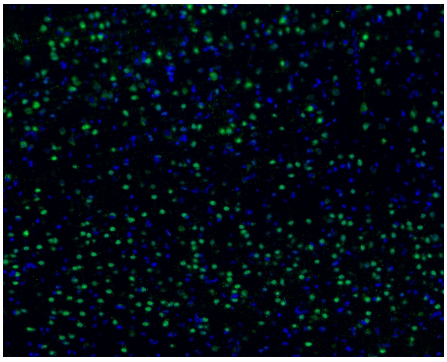

Fig13: Application: IHC-Fr

Species: Mouse

Site: Cerebral cortex

Sample: Frozen section

Antibody concentration: 1/100

Antigen retrieval: The section was pre-treated using 1% SDS buffer (in PBS, pH 7.4) for 5 minutes at room temperature.

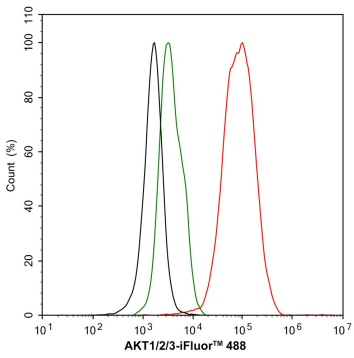

Fig14: Flow cytometric analysis of MCF7 cells labeling AKT1/2/3.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750179, 1/1,000) (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).

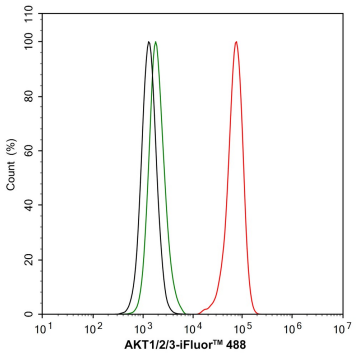

Fig15: Flow cytometric analysis of RAW264.7 cells labeling AKT1/2/3.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750179, 1/1,000) (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).

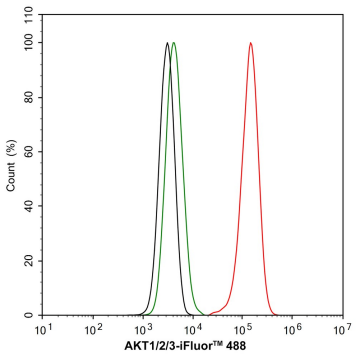

Fig16: Flow cytometric analysis of C6 cells labeling AKT1/2/3.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750179, 1/1,000) (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. Buendia, I. et al. 2015. The Melatonin-N,N-Dibenzyl(N-methyl)amine Hybrid ITH91/IQM157 Affords Neuroprotection in an in Vitro Alzheimer's Model via Hemo-oxygenase-1 Induction. ACS chemical neuroscience. 6: 288-96.
2. Siendones, E. et al. 2014. Membrane-bound CYB5R3 is a common effector of nutritional and oxidative stress response through FOXO3a and Nrf2. Antioxidants & redox signaling. 21: 1708-25.