Anti-AKT1/2/3 Antibody [JE75-09]

HA721870

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Monkey
Applications:	WB, IF-Cell, FC, IP
Molecular Wt:	Predicted band size: 56 kDa
Clone number:	JE75-09

Description: Akt, also referred to as PKB or Rac, plays a critical role in controlling cell survival and apoptosis. This protein kinase is activated by insulin and various growth and survival factors to function in a wortmannin-sensitive pathway involving PI3 kinase. Akt is activated by phospholipid binding and activation loop phosphorylation at Thr308 by PDK1 and by phosphorylation within the carboxy terminus at Ser473. The previously elusive PDK2 responsible for phosphorylation of Akt at Ser473 has been identified as mammalian target of rapamycin (mTOR) in a rapamycin-insensitive complex with rictor and Sin1 . Akt promotes cell survival by inhibiting apoptosis through phosphorylation and inactivation of several targets, including Bad, forkhead transcription factors, c-Raf, and caspase-9. PTEN phosphatase is a major negative regulator of the PI3K/Akt signaling pathway. LY294002 is a specific PI3 kinase inhibitor. Another essential Akt function is the regulation of glycogen synthesis through phosphorylation and inactivation of GSK-3α and β. Akt may also play a role in insulin stimulation of glucose transport. In addition to its role in survival and glycogen synthesis, Akt is involved in cell cycle regulation by preventing GSK-3β-mediated phosphorylation and degradation of cyclin D1 and by negatively regulating the cyclin-dependent kinase inhibitors p27 Kip1 and p21 Waf1/Cip1. Akt also plays a critical role in cell growth by directly phosphorylating mTOR in a rapamycin-sensitive complex containing raptor. More importantly, Akt phosphorylates and inactivates tuberin (TSC2), an inhibitor of mTOR within the mTOR-raptor complex.

Immunogen: Recombinant protein within Human AKT1/2/3 aa 231-480 / 480.

Positive control: HeLa cell lysate, MCF7 cell lysate, A549 cell lysate, U-2 OS cell lysate, NIH/3T3 cell lysate, MCF7, COS-1 cell lysate, RAW264.7 cell lysate, C6 cell lysate, PC-12 cell lysate, Mouse brain tissue lysate, Mouse heart tissue lysate, Mouse testis tissue lysate, Rat brain tissue lysate, Rat heart tissue lysate, Rat testis tissue lysate, RAW264.7, C6.

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
IF-Cell	1:100
FC	1:1,000
IP	1-2μg/sample

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

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

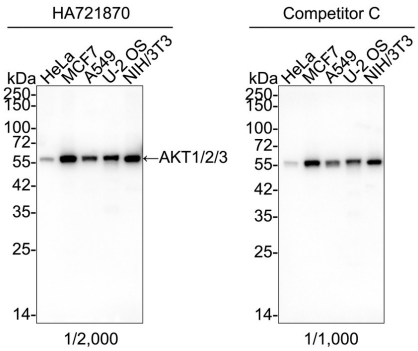

Fig1: Western blot analysis of AKT1/2/3 on different lysates with Rabbit anti-AKT1/2/3 antibody (HA721870) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: MCF7 cell lysate
Lane 3: A549 cell lysate
Lane 4: U-2 OS cell lysate
Lane 5: NIH/3T3 cell lysate

Lysates/proteins at 15 µg/Lane.

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

Exposure time: 30 seconds; ECL: K1802;

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 (HA721870) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution were 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.

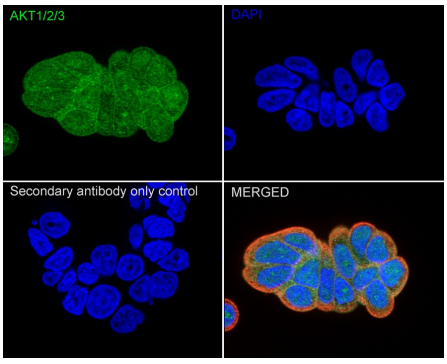

Fig2: Immunocytochemistry analysis of MCF7 cells labeling AKT1/2/3 with Rabbit anti-AKT1/2/3 antibody (HA721870) at 1/100 dilution.

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 (HA721870) 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.

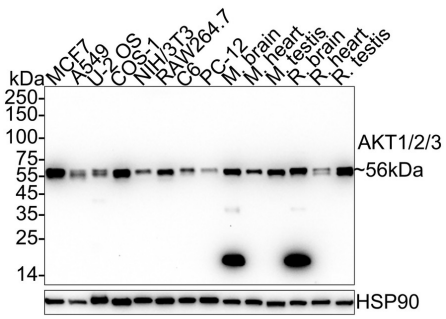

Fig3: Western blot analysis of AKT1/2/3 on different lysates with Rabbit anti-AKT1/2/3 antibody (HA721870) at 1/2,000 dilution.

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

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 2 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 (HA721870) at 1/2,000 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.

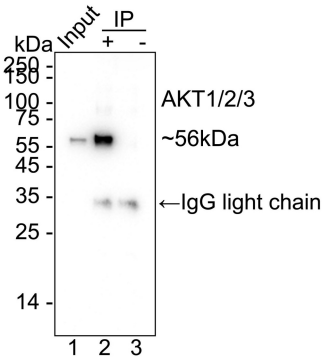

Fig4: AKT1/2/3 was immunoprecipitated from 0.2 mg MCF7 cell lysate with HA721870 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using HA721870 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: MCF7 cell lysate (input)
Lane 2: HA721870 IP in MCF7 cell lysate
Lane 3: Rabbit IgG instead of HA721870 in MCF7 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 6 seconds; ECL: K1801

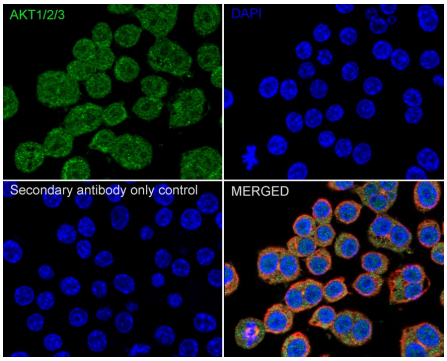

Fig5: Immunocytochemistry analysis of RAW264.7 cells labeling AKT1/2/3 with Rabbit anti-AKT1/2/3 antibody (HA721870) at 1/100 dilution.

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 (HA721870) 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.

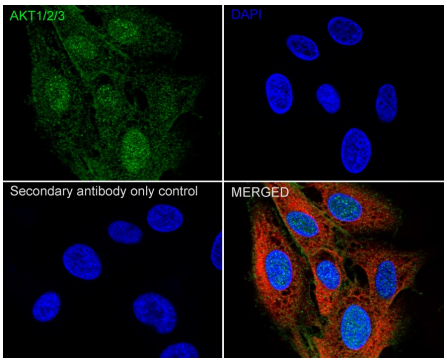

Fig6: Immunocytochemistry analysis of C6 cells labeling AKT1/2/3 with Rabbit anti-AKT1/2/3 antibody (HA721870) at 1/100 dilution.

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 (HA721870) 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.

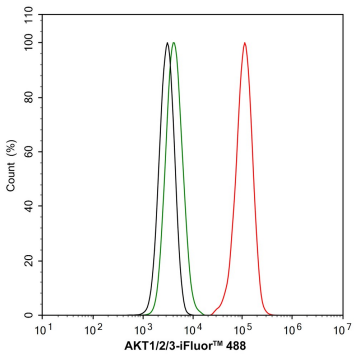

Fig7: Flow cytometric analysis of C6 cells labeling AKT1/2/3.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721870, 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).

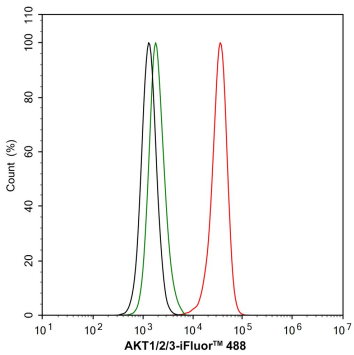

Fig8: Flow cytometric analysis of RAW264.7 cells labeling AKT1/2/3.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721870, 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).

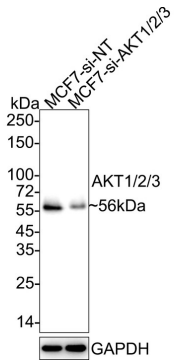

Fig9: Western blot analysis of AKT1/2/3 on different lysates with Rabbit anti-AKT1/2/3 antibody (HA721870) at 1/2,000 dilution.

Lane 1: MCF7-si NT cell lysate
Lane 2: MCF7-si AKT1/2/3 cell lysate

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 7 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 (HA721870) 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.

Background References

1. Tian X et al. Costunolide is a dual inhibitor of MEK1 and AKT1/2 that overcomes osimertinib resistance in lung cancer. Mol Cancer. 2022 Oct
2. Chen Z et al. Nuclear DEK preserves hematopoietic stem cells potential via NCoR1/HDAC3-Akt1/2-mTOR axis. J Exp Med. 2021 May