Anti-Phospho-AMPK alpha 1 (S496) Antibody [SD0810]

ET1612-72

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IP, IHC-Fr
Molecular Wt:	Predicted band size: 64 kDa
Clone number:	SD0810

Description: AMPK (5'-AMP-activated protein kinase) is a heterotrimeric complex comprising a catalytic α subunit and regulatory β and γ subunits. It protects cells from stresses that cause ATP depletion by switching off ATP-consuming bio-synthetic pathways. AMPK is activated by high AMP and low ATP through a mechanism involving allosteric regulation, promotion of phosphorylation by an upstream protein kinase known as AMPK kinase, and inhibition of dephosphorylation. Activated AMPK can phosphorylate and regulate in vivo hydroxy-methylglutaryl-CoA reductase and acetyl-CoA carboxylase, which are key regulatory enzymes of sterol synthesis and fatty acid synthesis, respectively. AMPKα1 (5'-AMP-activated protein kinase catalytic subunit alpha-1), also known as PRKAA1, is a 559 amino acid protein that belongs to the CAMK Ser/Thr protein kinase family and protein kinase superfamily. Highly phosphorylated, AMPKα1 exists as two alternatively spliced isoforms and is encoded by a gene that maps to human chromosome 5p13.1.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser496 of Human AMPK alpha 1 aa 471-520 / 559.

Positive control: SiHa cell lysate, HUVEC cell lysate, K-562 cell lysate, mouse hippocampus tissue, mouse cerebral cortex tissue.

Subcellular location: Nucleus, Cytoplasm.

Database links: SwissProt: Q13131 Human | Q5EG47 Mouse

Recommended Dilutions:

WB	1:500-1:1,000
IP	Use at an assay dependent concentration.
IHC-Fr	1:100

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

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

Purity: Protein A affinity purified.

Images

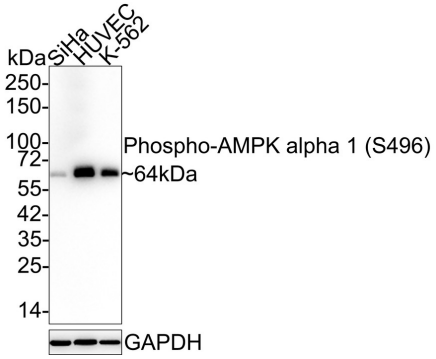

Fig1: Western blot analysis of Phospho-AMPK alpha 1 (S496) on different lysates with Rabbit anti-Phospho-AMPK alpha 1 (S496) antibody (ET1612-72) at 1/5,000 dilution.

Lane 1: SiHa cell lysate
Lane 2: HUVEC cell lysate
Lane 3: K-562 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 64 kDa
Observed band size: 64 kDa

Exposure time: 3 minutes;

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 (ET1612-72) 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.

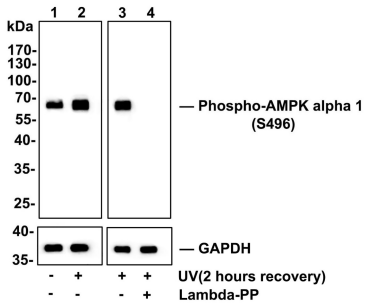

Fig2: Western blot analysis of Phospho-AMPK alpha 1(S496) on HUVEC cell lysates.

Lane 1: HUVEC cells, whole cell lysate, 10ug/lane
Lane 2/3: HUVEC cells treated with UV(2 hours recovery), whole cell lysates, 10ug/lane
Lane 4: HUVEC cells treated with UV(2 hours recovery), then treated with 2.8ug/ul lambda-PP for 30 minutes, whole cell lysates, 10ug/lane

All lanes :
Anti-Phospho-AMPK alpha 1(S496) antibody (ET1612-72) at 1:500 dilution. Anti-GAPDH antibody (ET1601-4) at 1:10,000 dilution. Goat Anti-Rabbit IgG H&L (HRP) (HA1001) at 1/200,000 dilution.

Predicted band size:64 kDa
Observed band size:64 kDa

Blocking and diluting buffer: 5% BSA.

Exposure time: 2 minutes 14 seconds

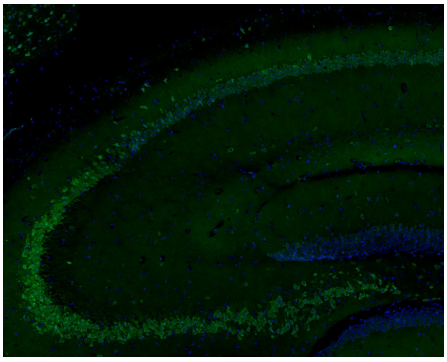

Fig3: Immunofluorescence analysis of frozen mouse hippocampus tissue labeling Phospho-AMPK alpha 1 (S496) with Rabbit anti-Phospho-AMPK alpha 1 (S496) antibody (ET1612-72).

The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ET1612-72, green) at 1/100 dilution overnight at 4℃, washed with PBS. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) was used as the secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue). Image acquisition was performed with KFBIO KF-FL-400 Scanner.

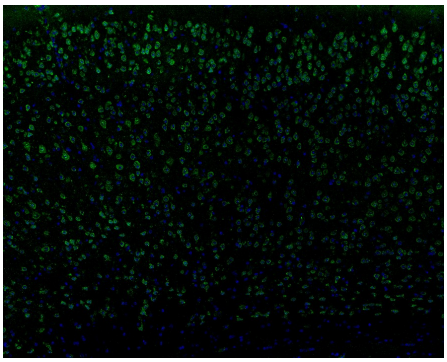

Fig4: Immunofluorescence analysis of frozen mouse cerebral cortex tissue labeling Phospho-AMPK alpha 1 (S496) with Rabbit anti-Phospho-AMPK alpha 1 (S496) antibody (ET1612-72).

The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ET1612-72, green) at 1/100 dilution overnight at 4℃, washed with PBS. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) was used as the secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue). Image acquisition was performed with KFBIO KF-FL-400 Scanner.

Background References

1. Chang TJ et al. Glucagon-like peptide-1 prevents methylglyoxal-induced apoptosis of beta cells through improving mitochondrial function and suppressing prolonged AMPK activation. Sci Rep 6:23403 (2016).
2. Wang YG & Yang TL Liraglutide reduces fatty degeneration in hepatic cells via the AMPK/SREBP1 pathway. Exp Ther Med 10:1777-1783 (2015).