Anti-AMPK alpha 1 Antibody [SU03-48]

ET1608-40

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, FC, IP, IHC-Fr, IHC-P
Molecular Wt:	Predicted band size: 64 kDa
Clone number:	SU03-48

Description: 5'-AMP-activated protein kinase catalytic subunit alpha-1 is an enzyme that in humans is encoded by the PRKAA1 gene. The protein encoded by this gene belongs to the serine/threonine protein kinase family. It is the catalytic subunit of the 5'-prime-AMP-activated protein kinase (AMPK). AMPK is a cellular energy sensor conserved in all eukaryotic cells. The kinase activity of AMPK is activated by the stimuli that increase the cellular AMP/ATP ratio. AMPK regulates the activities of a number of key metabolic enzymes through phosphorylation. It protects cells from stresses that cause ATP depletion by switching off ATP-consuming biosynthetic pathways.

Immunogen: Synthetic peptide within Human AMPK alpha aa 501-550 / 559.

Positive control: HeLa cell lysate, MCF7 cell lysate, K-562 cell lysate, 293T cell lysate, HT-29 cell lysate, L-929 cell lysate, C6 cell lysate, HeLa, L-929, human lung cancer tissue, mouse hippocampus tissue, mouse cerebral cortex tissue.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: Q13131 Human | Q5EG47 Mouse | P54645 Rat

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:100-1:250
IF-Tissue	1:50-1:200
FC	1:1,000
IP	Use at an assay dependent concentration.
IHC-Fr	1:100
IHC-P	1:1,000

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

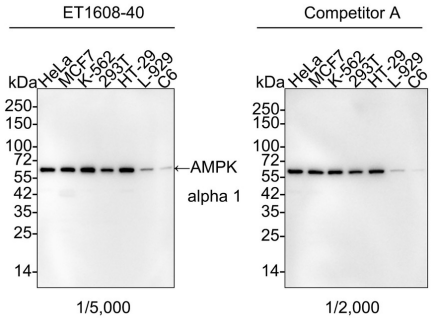

Fig1: Western blot analysis of AMPK alpha 1 on different lysates with Rabbit anti-AMPK alpha 1 antibody (ET1608-40) at 1/5,000 dilution and competitor's antibody at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: MCF7 cell lysate
Lane 3: K-562 cell lysate
Lane 4: 293T cell lysate
Lane 5: HT-29 cell lysate
Lane 6: L-929 cell lysate
Lane 7: C6 cell lysate

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 1 minute 10 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 (ET1608-40) at 1/5,000 dilution and competitor's antibody at 1/2,000 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.

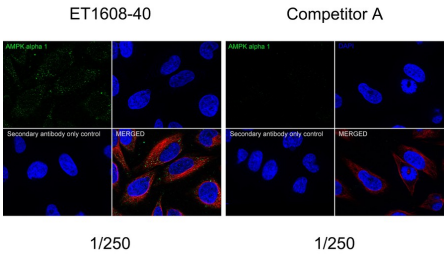

Fig2: Immunocytochemistry analysis of HeLa cells labeling AMPK alpha 1 with Rabbit anti-AMPK alpha 1 antibody (ET1608-40) at 1/250 dilution and competitor's antibody at 1/250 dilution.

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-AMPK alpha 1 antibody (ET1608-40) at 1/250 dilution and competitor's antibody at 1/250 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.

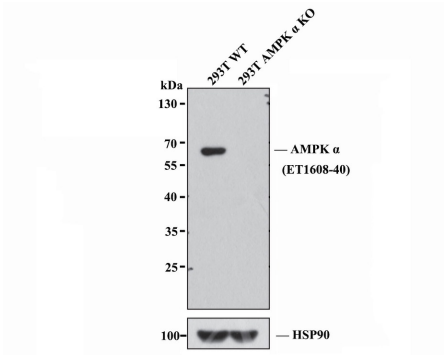

Fig3: Western blot analysis of AMPK α with anti-AMPK alpha 1 antibody [SU03-48] (ET1608-40) at 1/5,000 dilution.
Lane 1: Wild-type 293T whole cell lysate (20 µg).
Lane 2: AMPK α knockout 293T whole cell lysate (20 µg).

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary Anti-AMPK alpha 1 antibody (ET1608-40, 1/5,000) and Anti-HSP90 antibody (ET1605-56, 1/10,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG H&L (HRP) Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

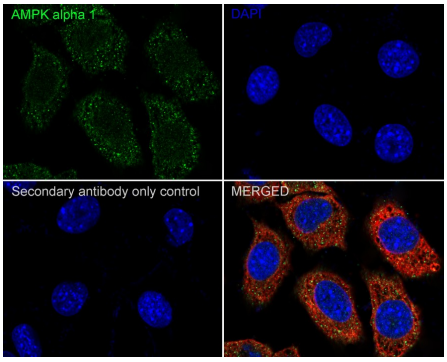

Fig4: Immunocytochemistry analysis of L-929 cells labeling AMPK alpha 1 with Rabbit anti-AMPK alpha 1 antibody (ET1608-40) at 1/100 dilution.

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-AMPK alpha 1 antibody (ET1608-40) 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.

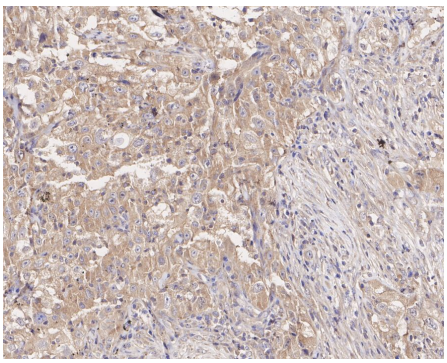

Fig5: Immunohistochemical analysis of paraffin-embedded human lung cancer tissue with Rabbit anti-AMPK alpha 1 antibody (ET1608-40) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) 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 (ET1608-40) 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.

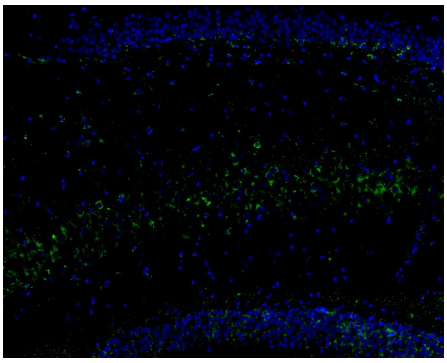

Fig6: Immunofluorescence analysis of frozen mouse hippocampus tissue labeling AMPK alpha 1 with Rabbit anti-AMPK alpha 1 antibody (ET1608-40).

The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ET1608-40, 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.

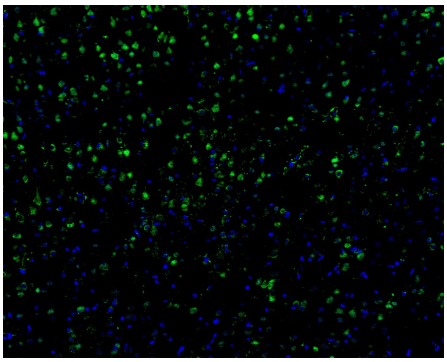

Fig7: Immunofluorescence analysis of frozen mouse cerebral cortex tissue labeling AMPK alpha 1 with Rabbit anti-AMPK alpha 1 antibody (ET1608-40).

The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ET1608-40, 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.

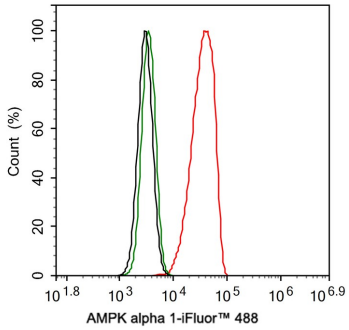

Fig8: Flow cytometric analysis of HeLa cells labeling AMPK alpha 1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1608-40, 1ug/ml) (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).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

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. Lieberthal W et al. Susceptibility to ATP depletion of primary proximal tubular cell cultures derived from mice lacking either the a1 or the a2 isoform of the catalytic domain of AMPK. BMC Nephrol 14:251 (2013).