Anti-SIN1 Antibody [A6G10]

HA601008

Product Type:	Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 59 kDa
Clone number:	A6G10

Description: Subunit of mTORC2, which regulates cell growth and survival in response to hormonal signals. mTORC2 is activated by growth factors, but, in contrast to mTORC1, seems to be nutrient-insensitive. mTORC2 seems to function upstream of Rho GTPases to regulate the actin cytoskeleton, probably by activating one or more Rho-type guanine nucleotide exchange factors. mTORC2 promotes the serum-induced formation of stress-fibers or F-actin. mTORC2 plays a critical role in AKT1 'Ser-473' phosphorylation, which may facilitate the phosphorylation of the activation loop of AKT1 on 'Thr-308' by PDK1 which is a prerequisite for full activation. mTORC2 regulates the phosphorylation of SGK1 at 'Ser-422'. mTORC2 also modulates the phosphorylation of PRKCA on 'Ser-657'. Within mTORC2, MAPKAP1 is required for complex formation and mTORC2 kinase activity. MAPKAP1 inhibits MAP3K2 by preventing its dimerization and autophosphorylation. Inhibits HRAS and KRAS signaling. Enhances osmotic stress-induced phosphorylation of ATF2 and ATF2-mediated transcription. Involved in ciliogenesis, regulates cilia length through its interaction with CCDC28B independently of mTORC2 complex.

Immunogen: Recombinant protein within human SIN1 aa 350-522.

Positive control: Hela cell lysate, A549 cell lysate, Hela, human kidney tissue, MCF-7.

Subcellular location: Nucleus, Cell membrane, Cytoplasmic vesicle.

Database links: SwissProt: Q9BPZ7 Human

Recommended Dilutions:

WB	1:500
IF-Cell	1:100
IHC-P	1:600
FC	1:500-1:1,000

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

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

Purity: Protein G affinity purified.

Images

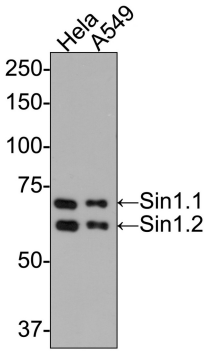

Fig1: Western blot analysis of SIN1 on different lysates with Mouse anti-SIN1 antibody (HA601008) at 1/500 dilution.

Lane 1: Hela cell lysate
Lane 2: A549 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 59 kDa
Observed band size: 60/70 kDa

Exposure time: 2 minutes;

8% 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 (HA601008) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:100,000 dilution was used for 1 hour at room temperature.

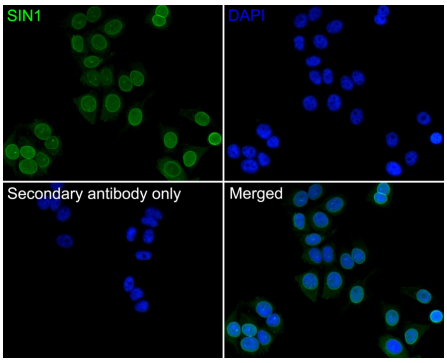

Fig2: Immunocytochemistry analysis of Hela cells labeling SIN1 with Mouse anti-SIN1 antibody (HA601008) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 30 minutes, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes, and then blocked with 2% BSA for 30 minutes at room temperature. Cells were then incubated with Mouse anti-SIN1 antibody (HA601008) at 1/100 dilution in 2% BSA overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) 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.

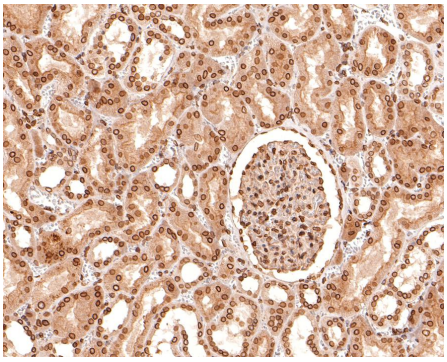

Fig3: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Mouse anti-SIN1 antibody (HA601008) at 1/600 dilution.

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 (HA601008) at 1/600 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.

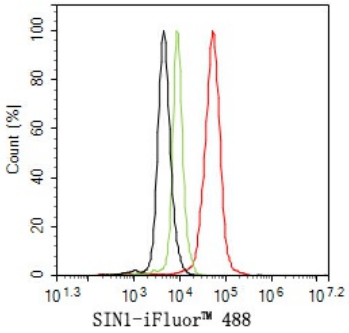

Fig4: Flow cytometric analysis of MCF-7 cells labeling SIN1.

Cells were fixed and permeabilized, and then blocked with 2% negative goat serum for 15 minutes at room temperature.Then stained with the primary antibody (HA601008, 1ug/ml) (red) compared with Mouse IgG1 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-Mouse IgG Secondary antibody (HA1125) 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. Huang Y. et. al. Sin1 promotes proliferation and invasion of prostate cancer cells by modulating mTORC2-AKT and AR signaling cascades. Life Sci. 2020 May
2. Xu H. et. al. Nitidine Chloride Inhibits SIN1 Expression in Osteosarcoma Cells. Mol Ther Oncolytics. 2019 Feb