Anti-Phospho-MNK1 (T250 + T255) Antibody [PSH17-95]

HA723960

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IHC-P, IF-Cell, FC
Molecular Wt:	Predicted band size: 51 kDa
Clone number:	PSH17-95

Description: MAP kinase-interacting serine/threonine-protein kinase 1 is an enzyme that in humans is encoded by the MKNK1 gene. MKNK1 has been shown to interact with MAPK1 and Eukaryotic translation initiation factor 4 gamma. May play a role in the response to environmental stress and cytokines. Appears to regulate translation by phosphorylating EIF4E, thus increasing the affinity of this protein for the 7-methylguanosine-containing mRNA cap.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Thr250 and Thr255 of human MNK1.

Positive control: HeLa cell lysate, HeLa treated with 25μg/mL anisomycin for 30 minutes cell lysate, NIH/3T3 starved for 24 hours cell lysate, NIH/3T3 starved for 24 hours then treated with 10% FBS for 30 minutes cell lysate, human lung cancer tissue, NIH/3T3, HeLa.

Subcellular location: Cytoplasm; Nucleus.

Database links: SwissProt: Q9BUB5 Human | O08605 Mouse

Recommended Dilutions:

WB	1:5,000
IHC-P	1:200
IF-Cell	1:100
FC	1:1,000

Storage Buffer: 1*PBS (pH7.4), 0.1% BSA, 40% Glycerol, 0.2% Proclean 950.

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

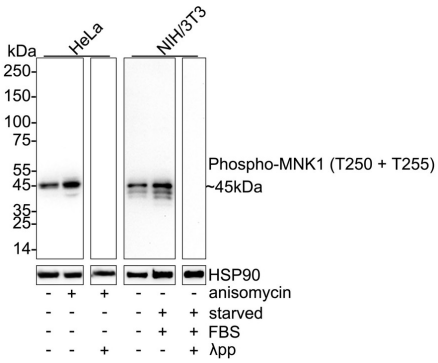

Fig1: Western blot analysis of Phospho-MNK1 (T250 + T255) on different lysates with Rabbit anti-Phospho-MNK1 (T250 + T255) antibody (HA723960) at 1/5,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 25µg/mL anisomycin for 30 minutes cell lysate
Lane 3: HeLa treated with 25µg/mL anisomycin for 30 minutes cell lysate, then the membrane treated with λpp for 1 hour
Lane 4: NIH/3T3 starved for 24 hours cell lysate
Lane 5: NIH/3T3 starved for 24 hours then treated with 10% FBS for 30 minutes cell lysate
Lane 6: NIH/3T3 starved for 24 hours then treated with 10% FBS for 30 minutes cell lysate, then the membrane treated with λpp for 1 hour

Lysates/proteins at 20 µg/Lane.

Predicted band size: 51 kDa
Observed band size: 45 kDa

Exposure time: 1 minute 16 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 (HA723960) at 1/5,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

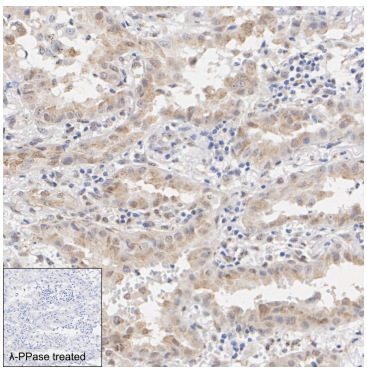

Fig2: Immunohistochemical analysis of paraffin-embedded human lung cancer tissue untreated / treated with λpp with Rabbit anti-Phospho-MNK1 (T250 + T255) antibody (HA723960) at 1/200 dilution.

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 (HA723960) at 1/200 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.

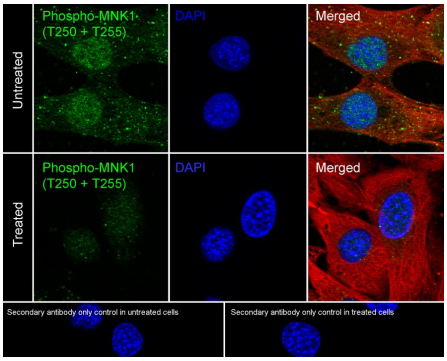

Fig3: Immunocytochemistry analysis of NIH/3T3 cells untreated / treated with λpp labeling Phospho-MNK1 (T250 + T255) with Rabbit anti-Phospho-MNK1 (T250 + T255) antibody (HA723960) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-Phospho-MNK1 (T250 + T255) antibody (HA723960) 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 (HA601187, 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.

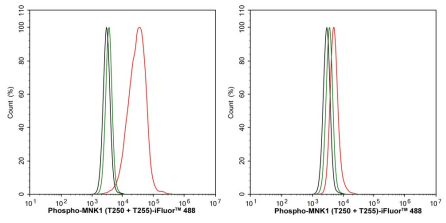

Fig4: Flow cytometric analysis of HeLa cells untreated (left) / treated with λpp (right) labeling Phospho-MNK1 (T250 + T255).

Cells were fixed and permeabilized. Then stained with the primary antibody (HA723960, 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).

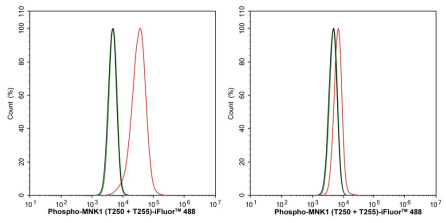

Fig5: Flow cytometric analysis of NIH/3T3 cells untreated (left) / treated with λpp (right) labeling Phospho-MNK1 (T250 + T255).

Cells were fixed and permeabilized. Then stained with the primary antibody (HA723960, 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. Preston SEJ et al. Blocking tumor-intrinsic MNK1 kinase restricts metabolic adaptation and diminishes liver metastasis. Sci Adv. 2024 Sep
2. Guo Q et al. Correction: MNK1/NODAL Signaling Promotes Invasive Progression of Breast Ductal Carcinoma In Situ. Cancer Res. 2024 Apr