Anti-MLKL Antibody [SA40-04]

ET1601-25

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IHC-P, IF-Cell, FC
Molecular Wt:	Predicted band size: 54 kDa
Clone number:	SA40-04

Description: Mixed lineage kinase domain like pseudokinase (MLKL) is a protein that in humans is encoded by the MLKL gene. This gene belongs to the protein kinase superfamily. The encoded protein contains a protein kinase-like domain; however, is thought to be inactive because it lacks several residues required for activity. This protein plays a critical role in tumor necrosis factor (TNF)-induced necroptosis, a programmed cell death process, via interaction with receptor-interacting protein 3 (RIP3), which is a key signaling molecule in necroptosis pathway. Inhibitor studies and knockdown of this gene inhibited TNF-induced necrosis.

Immunogen: Recombinant protein within Human MLKL aa 333-471 / 471.

Positive control: HUVEC cell lysate, HT-29 cell lysate, HeLa cell lysate, THP-1 cell lysate, U-937 cell lysate, K-562 cell lysate, NIH/3T3 cell lysate, HeLa, HT-29, human tonsil tissue.

Subcellular location: Cytoplasm, Cell membrane, Golgi apparatus.

Database links: SwissProt: Q8NB16 Human | Q9D2Y4 Mouse

Recommended Dilutions:

WB	1:5,000-1:10,000
IHC-P	1:500-1:2000
IF-Cell	1:100
FC	1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% 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

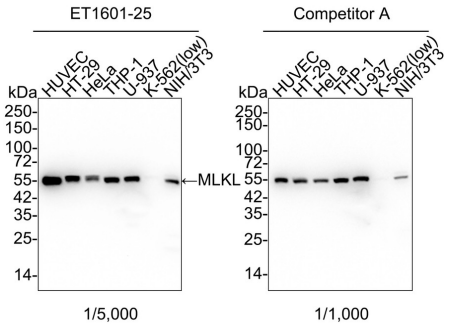

Fig1: Western blot analysis of MLKL on different lysates with Rabbit anti-MLKL antibody (ET1601-25) at 1/5,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: HUVEC cell lysate (20 µg/Lane)
Lane 2: HT-29 cell lysate (20 µg/Lane)
Lane 3: HeLa cell lysate (20 µg/Lane)
Lane 4: THP-1 cell lysate (20 µg/Lane)
Lane 5: U-937 cell lysate (20 µg/Lane)
Lane 6: K-562 cell lysate (low expression) (20 µg/Lane)
Lane 7: NIH/3T3 cell lysate (20 µg/Lane)

Predicted band size: 54 kDa
Observed band size: 54 kDa

Exposure time: 1 minute 2 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 (ET1601-25) at 1/5,000 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.

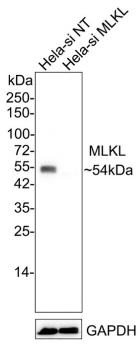

Fig2: Western blot analysis of MLKL on different lysates with Rabbit anti-MLKL antibody (ET1601-25) at 1/5,000 dilution.

Lane 1: Hela-si NT cell lysate (10 µg/Lane)
Lane 2: Hela-si MLKL cell lysate (10 µg/Lane)

Predicted band size: 54 kDa
Observed band size: 54 kDa

Exposure time: 3 minutes 20 seconds; ECL: K1801;
4-20% SDS-PAGE gel.

ET1601-25 was shown to specifically react with MLKL in Hela-si NT cells. No band was observed when Hela-si MLKL samples were tested. Hela-si NT and Hela-si MLKL samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1601-25, 1/5,000) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) were used in 5% BSA at 4 ℃ overnight. Goat Anti-rabbit IgG-HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

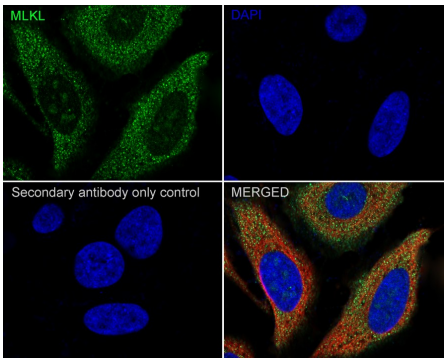

Fig3: Immunocytochemistry analysis of HeLa cells labeling MLKL with Rabbit anti-MLKL antibody (ET1601-25) at 1/100 dilution.

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-MLKL antibody (ET1601-25) 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.

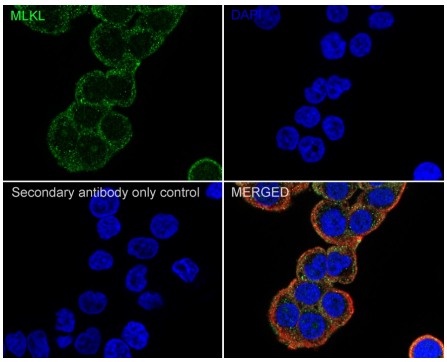

Fig4: Immunocytochemistry analysis of HT-29 cells labeling MLKL with Rabbit anti-MLKL antibody (ET1601-25) at 1/100 dilution.

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-MLKL antibody (ET1601-25) 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.

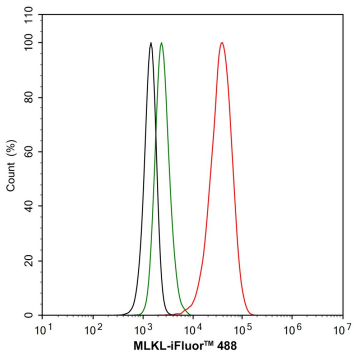

Fig5: Flow cytometric analysis of HT-29 cells labeling MLKL.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1601-25, 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).

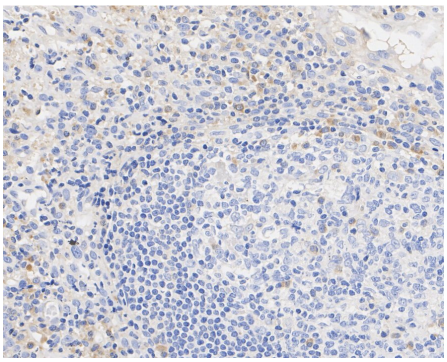

Fig6: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-MLKL antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1601-25, 1/2000) for 30 minutes 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.

Background References

1. Daub H., Olsen J.V., Bairlein M., et al. Kinase-selective enrichment enables quantitative phosphoproteomics of the kinome across the cell cycle. Mol. Cell 31:438-448(2008).
2. Murphy J.M., Lucet I.S., Hildebrand J.M., et al. Insights into the evolution of divergent nucleotide-binding mechanisms among pseudokinases revealed by crystal structures of human and mouse MLKL. Biochem. J. 457:369-377(2014).