Anti-Phospho-MLKL (S345) Antibody [JM92-37] - BSA and Azide free

HA750433

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Mouse
Applications:	WB, IHC-P, IHC-Fr
Molecular Wt:	Predicted band size: 54 kDa
Clone number:	JM92-37

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. High levels of this protein and RIP3 are associated with inflammatory bowel disease in children.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser345 of mouse MLKL.

Positive control: L929 treated with 20μM Z-VAD for 3.5 hours then added 100nM SM-164 and 20ng/ml TNF-α for 3 hours cell lysate, mouse spleen tissue, mouse lung tissue, mouse liver tissue, mouse colon tissue.

Subcellular location: Cell membrane, Cytoplasm, Nucleus.

Database links: SwissProt: Q9D2Y4 Mouse

Recommended Dilutions:

WB	1:1,000
IHC-P	1:50-1:100
IHC-Fr	1:100

Storage Buffer: 1*PBS (pH7.4).

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

Purity: Protein A affinity purified.

Images

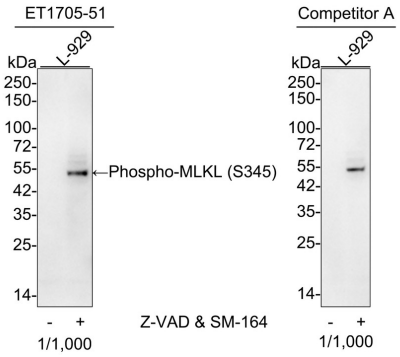

Fig1: Western blot analysis of Phospho-MLKL (S345) on different lysates with Rabbit anti-Phospho-MLKL (S345) antibody (HA750433) at 1/1,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: L-929 cell lysate
Lane 2: L929 treated with 20μM Z-VAD for 3.5 hours, then added 100nM SM-164 and 20ng/ml TNF-α for 3 hours cell lysate

Lysates/proteins at 20 μg/Lane.

Predicted band size: 54 kDa
Observed band size: 54 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 (HA750433) at 1/1,000 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.

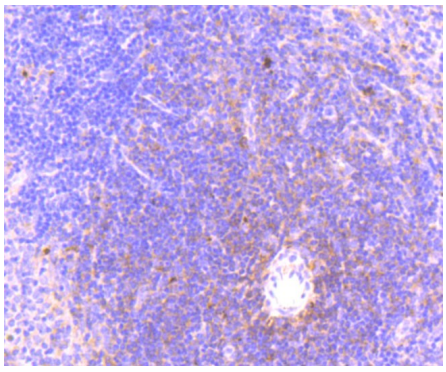

Fig2: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue using anti-Phospho-MLKL (S345) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750433, 1/50) 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.

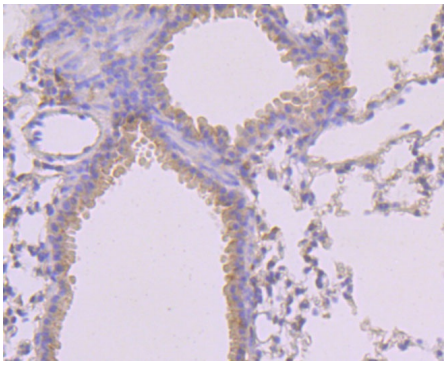

Fig3: Immunohistochemical analysis of paraffin-embedded mouse lung tissue using anti-Phospho-MLKL (S345) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750433, 1/50) 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.

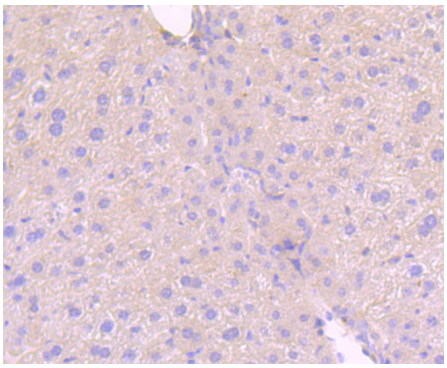

Fig4: Immunohistochemical analysis of paraffin-embedded mouse liver tissue using anti-Phospho-MLKL (S345) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750433, 1/50) 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.

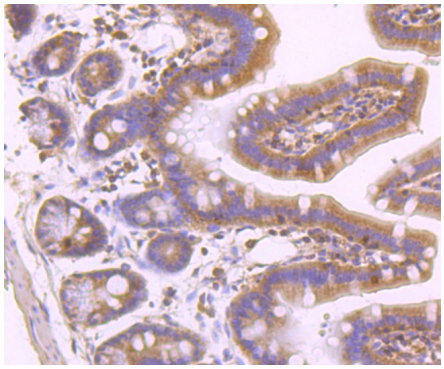

Fig5: Immunohistochemical analysis of paraffin-embedded mouse colon tissue using anti-Phospho-MLKL (S345) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750433, 1/50) 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.

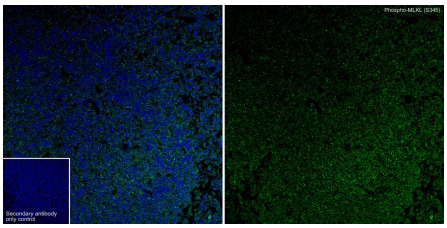

Fig6: Immunofluorescence analysis of frozen mouse spleen tissue with Rabbit anti-Phospho-MLKL (S345) antibody (HA750433) at 1/100 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA750433, green) at 1/100 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

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

Background References

1. Martens S et al. MLKL in cancer: more than a necroptosis regulator. Cell Death Differ. 2021 Jun
2. Liu S et al. MLKL polymerization-induced lysosomal membrane permeabilization promotes necroptosis. Cell Death Differ. 2024 Jan