

## Anti-MLKL Antibody Picoband®

Catalog Number: A00535-3-carrier-free

### About MLKL

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. Alternatively spliced transcript variants have been described for this gene.

### Overview

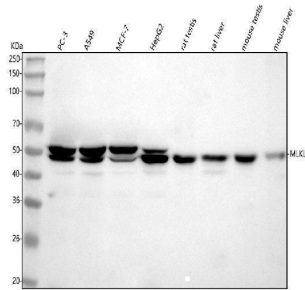
Product Name	Anti-MLKL Antibody Picoband®
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-MLKL Antibody Picoband® catalog # A00535-3. Tested in ELISA, IF, IHC, ICC, WB, Flow Cytometry applications. This antibody reacts with Human, Mouse, Rat. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	ELISA, Flow Cytometry, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl, 0.2 mg Na <sub>2</sub> HPO <sub>4</sub> .
Storage Instructions	At -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freezing and thawing.
Host	Rabbit
Uniprot ID	Q8NB16

### Technical Details

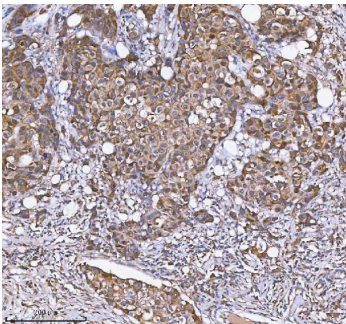
Immunogen	E.coli-derived human MLKL recombinant protein (Position: R210-K464). Human MLKL shares 69.7% amino acid (aa) sequence identity with mouse MLKL.
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and ICC.
Cross Reactivity	No cross reactivity with other proteins.
Isotype	IgG

Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.25-0.5 µg/ml, Human, Mouse, Rat Immunohistochemistry(Paraffin-embedded Section), 2-5 µg/ml, Human Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human Flow Cytometry (Fixed), 1-3 µg/1x10 <sup>6</sup> cells, Human ELISA, 0.1-0.5 µg/ml, -

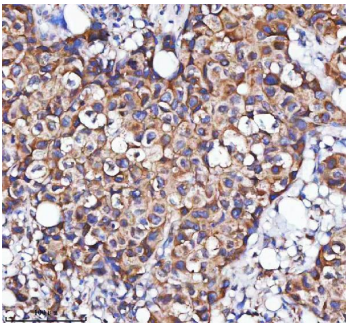
## Anti-MLKL Antibody Picoband® (A00535-3-carrier-free) Images



Western blot analysis of MLKL using anti-MLKL antibody (A00535-3). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human PC-3 whole cell lysates, Lane 2: human A549 whole cell lysates, Lane 3: human MCF-7 whole cell lysates, Lane 4: human HepG2 whole cell lysates, Lane 5: rat testis tissue lysates, Lane 6: rat liver tissue lysates, Lane 7: mouse testis tissue lysates, Lane 8: mouse liver tissue lysates. After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-MLKL antigen affinity purified polyclonal antibody (Catalog # A00535-3) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for MLKL at approximately 50 kDa. The expected band size for MLKL is at 54 kDa.

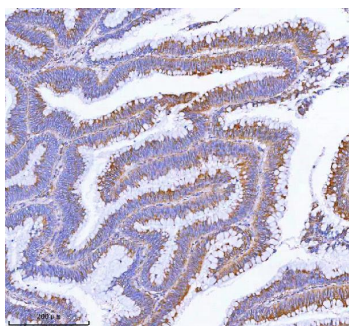


IHC analysis of MLKL using anti-MLKL antibody (A00535-3). MLKL was detected in a paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-MLKL Antibody (A00535-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

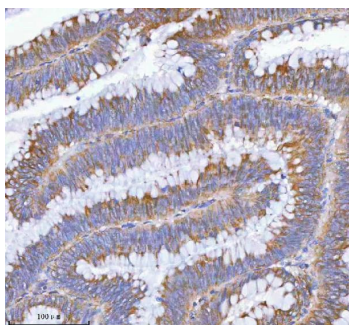


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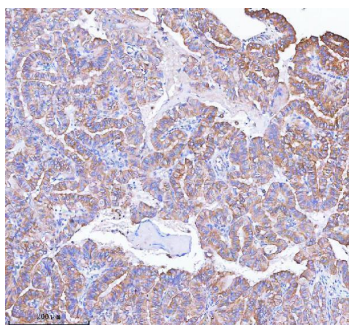
IHC analysis of MLKL using anti-MLKL antibody (A00535-3). MLKL was detected in a paraffin-embedded section of



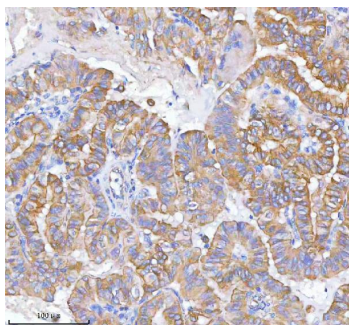
human colon adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-MLKL Antibody (A00535-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.



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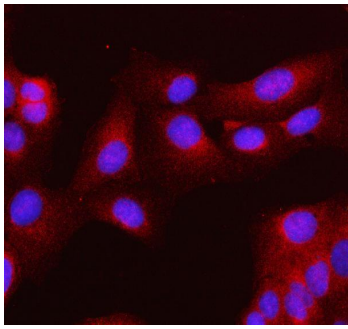


IHC analysis of MLKL using anti-MLKL antibody (A00535-3). MLKL was detected in a paraffin-embedded section of human thyroid papillary carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-MLKL Antibody (A00535-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

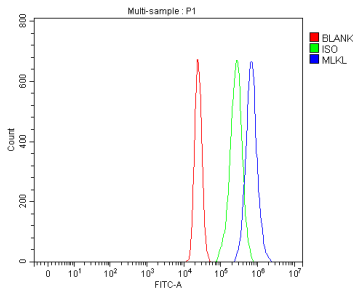


IHC analysis of MLKL using anti-MLKL antibody (A00535-3). MLKL was detected in a paraffin-embedded section of human thyroid papillary carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-MLKL Antibody (A00535-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

IF analysis of MLKL using anti-MLKL antibody (A00535-3). MLKL was detected in an immunocytochemical section of U2OS cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then



incubated with 5 ug/mL rabbit anti-MLKL Antibody (A00535-3) overnight at 4°C. Cy3 Conjugated Goat Anti-Rabbit IgG (BA1032) was used as secondary antibody at 1:500 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



Flow Cytometry analysis of MCF-7 cells using anti-MLKL antibody (A00535-3). Overlay histogram showing MCF-7 cells stained with A00535-3 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-MLKL Antibody (A00535-3, 1 ug/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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### Anti-MLKL Antibody

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