BOSTER BIOLOGICAL TECHNOLOGY
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888-466-3604 | support@bosterbio.com | www.bosterbio.com

Anti-MLKL Antibody Picoband®

Catalog Number: A00535-3

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.

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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 Na2HPO4.
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.

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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 ug/ml, Human, Mouse, Rat Immunohistochemistry(Paraffin-embedded Section), 2-5 ug/ml, Human Immunocytochemistry/Immunofluorescence, 5 ug/ml, Human Flow Cytometry (Fixed), 1-3 ug/1x10^6 cells, Human ELISA, 0.1-0.5 ug/ml, - Note: For flow cytometry (FCM), suitable for fixed cells only. Not suitable for FACS or surface flow cytometry using live cells.

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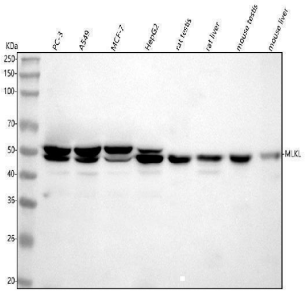
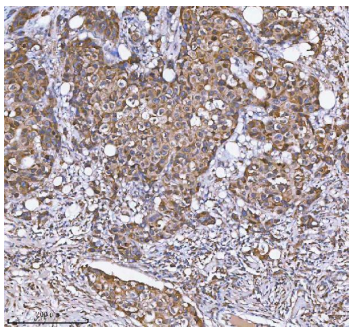
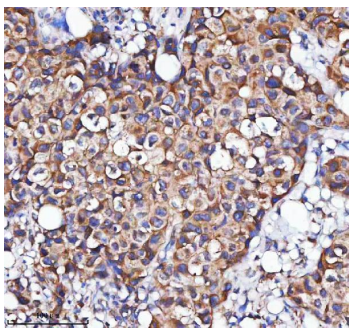
Anti-MLKL Antibody Picoband® (A00535-3) Images



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).

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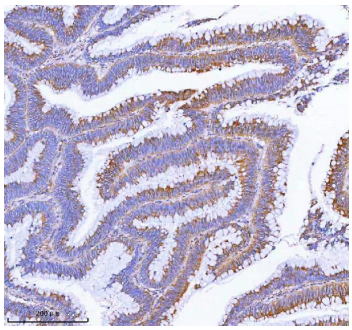
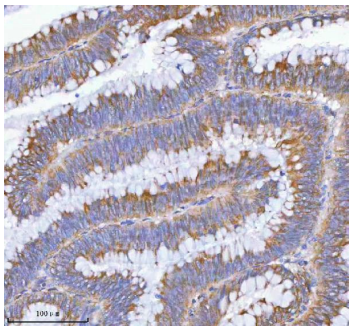
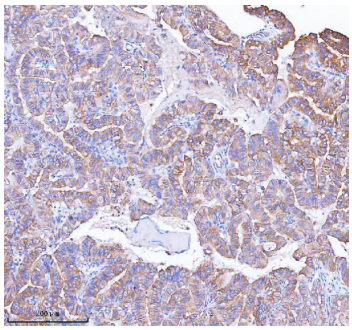


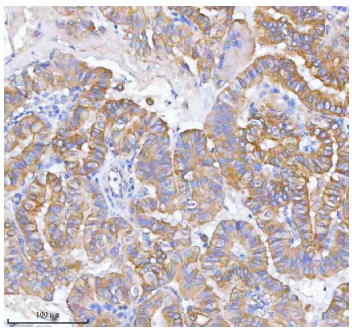
MLKL was detected in a paraffin-embedded section of human colon adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-MLKL 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.



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.



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.

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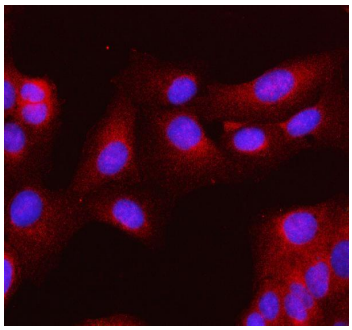
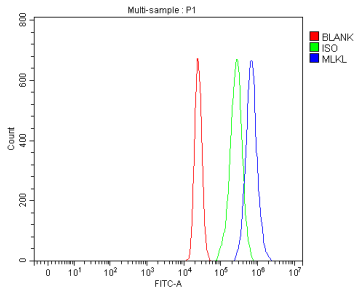


The cells were blocked with 10% goat serum. And then incubated with 5 ug/mL rabbit anti-MLKL Antibody (A00535-3) overnight at 4°C. Cy3 Conjugated Goat Anti-Rabbit IgG (BA1032) was used as secondary antibody at 1:500 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. 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/1x106 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x106 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x106) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

Anti-MLKL Antibody

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