

## Product Datasheet

### Anti-MLKL Antibody Picoband (orb1939981)

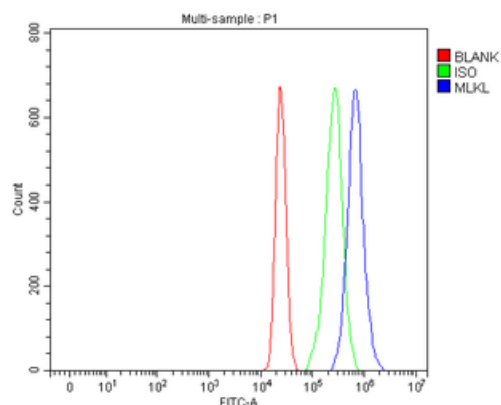
<b>Description</b>	Anti-MLKL Antibody. Tested in ELISA, IF, IHC, ICC, WB, Flow Cytometry applications. This antibody reacts with Human, Mouse, Rat.
<b>Species/Host</b>	Rabbit
<b>Reactivity</b>	Human, Mouse, Rat
<b>Conjugation</b>	Unconjugated
<b>Tested Applications</b>	ELISA, FC, ICC, IF, IHC, WB
<b>Immunogen</b>	E.coli-derived human MLKL recombinant protein (Position: R210-K464). Human MLKL shares 69.7% amino acid (aa) sequence identity with mouse MLKL.
<b>Form/Appearance</b>	Lyophilized
<b>Concentration</b>	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.
<b>Storage</b>	Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.
<b>Note</b>	For research use only
<b>Application notes</b>	Western blot, 0.25-0.5 µg/ml, Human, Mouse, Rat Immunohistochemistry, 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, -. Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml
<b>Isotype</b>	IgG
<b>Clonality</b>	Polyclonal
<b>MW</b>	50 kDa
<b>Uniprot ID</b>	<b>Q8NB16</b>
<b>Expiration Date</b>	12 months from date of receipt.

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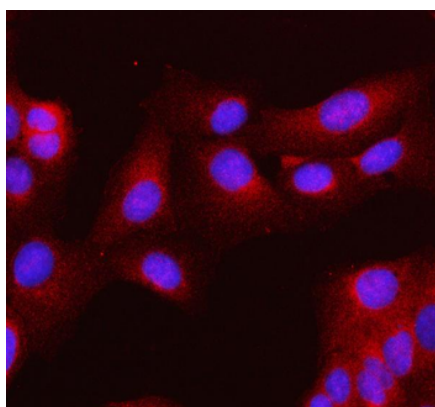
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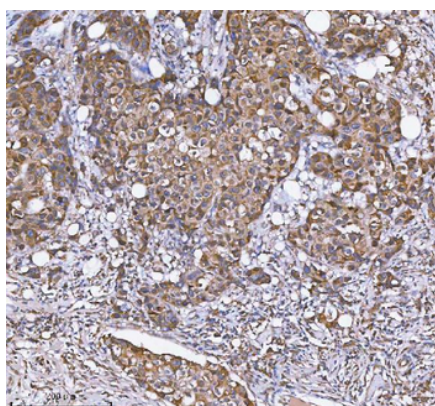
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Flow Cytometry analysis of MCF-7 cells using anti-MLKL antibody. Overlay histogram showing MCF-7 cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The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



IF analysis of MLKL using anti-MLKL antibody. MLKL was detected in an immunocytochemical section of U2OS cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5  $\mu\text{g}/\text{mL}$  rabbit anti-MLKL Antibody overnight at 4°C. Cy3 Conjugated Goat Anti-Rabbit IgG 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.



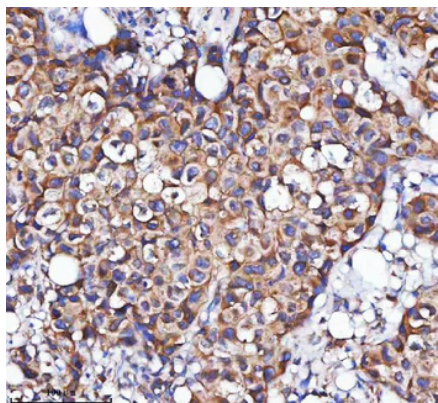
IHC analysis of MLKL using anti-MLKL antibody. MLKL was detected in a paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2  $\mu\text{g}/\text{ml}$  rabbit anti-MLKL Antibody 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 with DAB as the chromogen.

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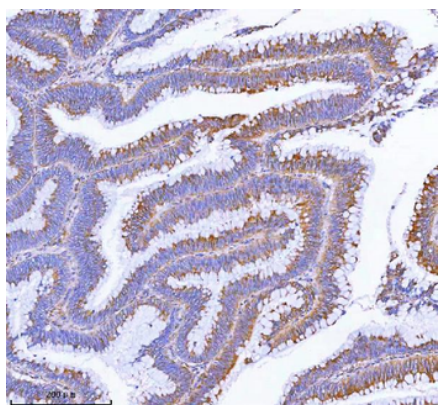
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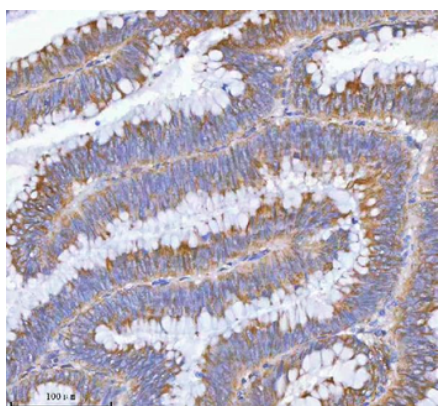
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IHC analysis of MLKL using anti-MLKL antibody. MLKL was detected in a paraffin-embedded section of human colon adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2  $\mu\text{g}/\text{ml}$  rabbit anti-MLKL Antibody 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 with DAB as the chromogen.



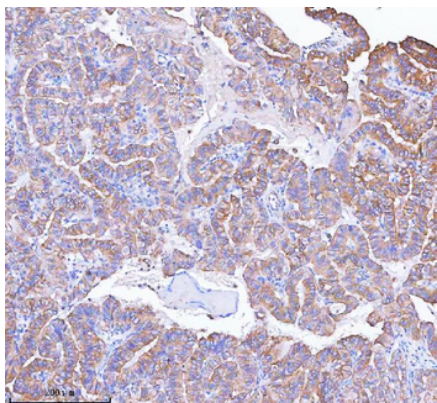
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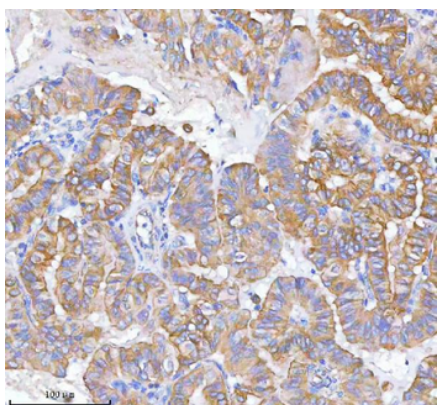
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IHC analysis of MLKL using anti-MLKL antibody. MLKL was detected in a paraffin-embedded section of human thyroid papillary carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 μg/ml rabbit anti-MLKL Antibody 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 with DAB as the chromogen.



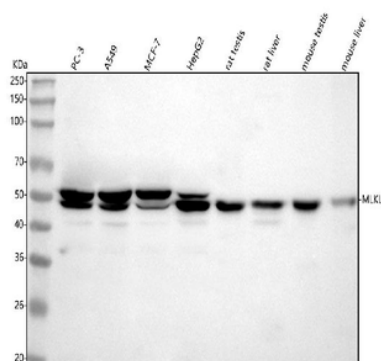
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Western blot analysis of MLKL using anti-MLKL antibody. Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. 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 at 0.5  $\mu$ g/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 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.

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