**Overview**

**Product name**
Anti-MLKL (phospho S345) antibody [EPR9515(2)]

**Description**
Rabbit monoclonal [EPR9515(2)] to MLKL (phospho S345)

**Host species**
Rabbit

**Specificity**
MLKL pS345 is a trigger for necroptosis. It is only detectable in infection/cellular damaged (PMID:29229989) or aging tissue (PMID: 28807105) but not in normal tissues.

**Tested applications**
Suitable for: ICC/IF, WB, IP, Dot blot

**Species reactivity**
Reacts with: Mouse

**Immunogen**
Synthetic peptide (the amino acid sequence is considered to be commercially sensitive) within Mouse MLKL aa 300-400 (phospho S345). The exact sequence is proprietary.

**Database link:** Q9D2Y4

**Positive control**
WB: L929 whole cell lysate treated with 20 ng/ml TNF alpha (ab9642), 100 nM Smac mimetic, and 20 µM z-VAD (ab120382) for 8h and then harvested. IP: L929 whole cell lysate treated with TNF alpha (ab9642) + Smac mimetic + z-VAD (ab120382) compound for 8h. ICC/IF: Mouse cardiomyocytes.

**General notes**
This antibody was developed through collaboration with the lab of Xiaodong Wang at the National Institute of Biological Sciences, Beijing.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

This product is a recombinant rabbit monoclonal antibody.

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**Properties**

**Form**
Liquid

**Storage instructions**

**Storage buffer**
Preservative: 0.01% Sodium azide
Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

**Purity**
Protein A purified
Clonality: Monoclonal
Clone number: EPR9515(2)
Isotype: IgG

Applications

Our Abpromise guarantee covers the use of ab196436 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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<td>ICC/IF</td>
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<td>WB</td>
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<td>1/1000. Detects a band of approximately 54 kDa (predicted molecular weight: 54 kDa).</td>
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<td>IP</td>
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<td>Dot blot</td>
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<td>1/1000.</td>
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Target

Sequence similarities
Belongs to the protein kinase superfamily.
Contains 1 protein kinase domain.

Domain
The protein kinase domain is predicted to be catalytically inactive.

Images

All lanes: Anti-MLKL (phospho S345) antibody [EPR9515(2)] (ab196436) at 1/1000 dilution

Lane 1: Untreated L-929 (Mouse connective tissue fibroblast cells) whole cell lysate
Lane 2: L-929 whole cell lysate treated with 20 ng/ml TNF alpha (ab9642), 100 nM Smac mimetic, and 20 µM z-VAD (ab120382) for 8 h and then harvested.

Lysates/proteins at 10 µg per lane.

Secondary
All lanes: Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 54 kDa
Observed band size: 54 kDa
**Exposure time:** 15 seconds

**Blocking and dilution buffer:** 5% NFDM/TBST.

Ab196436 staining MLKL in mouse cardiomyocytes by Immunocytochemistry. Samples were fixed with paraformaldehyde, permeabilized with 0.03% Triton x-100 and blocked with 1% BSA for 1 hour at room temperature. Samples were incubated with primary antibody at 1/100 dilution for 12 hours. An Alexa Fluor® anti-rabbit was used as a secondary antibody at 1/200 dilution.

MLKL (phospho S345) was immunoprecipitated from 1mg of L-929 (Mouse connective tissue fibroblast cells) whole cell lysate treated with 20 ng/ml TNF alpha (ab9642) + 100 nM Smac mimetic + 20 µM z-VAD compound (ab120382) for 8h using ab196436 at 1/150 dilution. Western blot was performed from the immunoprecipitate using ab196436 at 1/1000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution.

**Lane 1:** L-929 whole cell lysate treated with 20 ng/ml TNF alpha (ab9642) + 100 nM Smac mimetic+ 20 µM z-VAD compound (ab120382) for 8h; 10 µg (Input).

**Lane 2:** ab196436 IP in L-929 whole cell lysate treated with 20 ng/ml TNF alpha (ab9642) + 100 nM Smac mimetic+ 20 µM z-VAD compound (ab120382) for 8h.

**Lane 3:** Rabbit monoclonal IgG (ab172730) instead of ab196436 in L-929 whole cell lysate treated with 20 ng/ml TNF alpha (ab9642) + 100 nM Smac mimetic+ 20 µM z-VAD compound (ab120382) for 8h.

**Blocking and dilution buffer and concentration:** 5% NFDM/TBST.
All lanes: Anti-MLKL (phospho S345) antibody [EPR9515(2)] (ab196436) at 1/1000 dilution

Lane 1: L-929 treated with 20 ng/ml TNF alpha (ab9642), 100 nM Smac mimetic, and 20 µM z-VAD (ab120382) for 8 h, whole cell lysate
Lane 2: Mouse brain tissue lysate
Lane 3: Mouse colon tissue lysate
Lane 4: Mouse lung tissue lysate
Lane 5: Mouse retina tissue lysate
Lane 6: Mouse liver tissue lysate
Lane 7: Raw264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

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

Exposure time: 50 seconds

Blocking and diluting buffer: 5% NFDM/TBST.

MLKL pS345 is a trigger for necroptosis. It is only detectable in infection/cellular damaged (PMID:29229989) or aging tissue (PMID: 28807105) but not in normal tissues.
Dot blot analysis of MLKL (phospho S345) peptide (Lane 1), and non-phospho peptide (Lane 2), labeled using ab196436 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated secondary antibody at 1/1000 dilution.

**Blocking and dilution buffer:** 5% NFDM/TBST.

**Exposure time:** 3 minutes.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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