**Product datasheet**

**Anti-MLKL (phospho S358) antibody [EPR9514] ab187091**

**Overview**

**Product name**  
Anti-MLKL (phospho S358) antibody [EPR9514]

**Description**  
Rabbit monoclonal [EPR9514] to MLKL (phospho S358)

**Host species**  
Rabbit

**Specificity**  
Stimulation may be required to allow detection of the phosphorylated protein. Please see images below for recommended treatment conditions and positive controls.

**Tested applications**  
**Suitable for:** WB, Dot blot, IHC-P

**Species reactivity**  
**Reacts with:** Human  
**Does not react with:** Mouse, Rat

**Immunogen**  
Synthetic peptide (the amino acid sequence is considered to be commercially sensitive) within Human MLKL aa 350-450 (phospho S358). The exact sequence is proprietary.  
Database link: Q8NB16

**Positive control**  
HT-29 cell lysate treated with TNF alpha+ Smac mimetic+ z-VAD. HT-29 cells were treated with the indicated stimuli for 8 hours and then harvested. The final concentrations of 20 ng/ml TNF-a, 100 nM Smac mimetic, and 20 µM z-VAD were used to induce necrosis

**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.

**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: EPR9514
Isotype: IgG

Applications

Our Abpromise guarantee covers the use of ab187091 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dot blot</td>
<td></td>
<td>1/1000.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>★ ★ ★ ★ ‽</td>
<td>1/250 - 1/500. Not Suitable for Mouse and Rat</td>
</tr>
</tbody>
</table>

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

ab187091 at 1:250 staining MLKL (phospho S358) in Human melanoma tissue by immunohistochemistry (FFPE).

Antigen retrieval required on FFPE tissue: HIER using 10mM Citrate buffer pH 6.0, see recommended HIER protocol.

For additional IHC guideline, please see IHC resource page.
Western blot - Anti-MLKL (phospho S358) antibody [EPR9514] (ab187091)

All lanes: Anti-MLKL (phospho S358) antibody [EPR9514] (ab187091) at 1/1000 dilution

Lane 1: Untreated HT-29 (human colorectal adenocarcinoma) whole cell lysates 20µg

Lane 2: HT-29 (human colorectal adenocarcinoma) treated with TNF alpha+ Smac mimetic+ z-VAD whole cell lysates 20µg

Lane 3: HT-29 (human colorectal adenocarcinoma) treated with TNF alpha+ Smac mimetic + z-VAD and phosphatase whole cell lysates 20µg.

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

Blocking buffer and concentration: 5% NFDM/TBST, Diluting buffer and concentration: 5% NFDM /TBST, Exposure time: 1 minute

The lysate in this image is prepared by 1%SDS Hot Lysate buffer.

For Lysate preparation protocol, please refer to the protocol book in the protocol section and/or here (downloadable copy).
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MLKL (phospho S358) antibody [EPR9514] (ab187091) at 1:250 staining MLKL (phospho S358) in Human skin tissue by immunohistochemistry (FFPE).

Antigen retrieval required on FFPE tissue: HIER using 10mM Citrate buffer pH 6.0, see recommended HIER protocol

For additional IHC guideline, please see IHC resource page
All lanes: Anti-MLKL (phospho S358) antibody [EPR9514] (ab187091) at 1/5000 dilution

Lane 1: HT-29 (Human colorectal adenocarcinoma epithelial cell) treated with 20 ng/ml TNF-a, 100 nM Smac mimetic and 20 µM z-VAD for 6 hr. The lysate is directly prepared by 1xSDS loading buffer.

Lane 2: HT-29 (Human colorectal adenocarcinoma epithelial cell) treated with 20 ng/ml TNF-a, 100 nM Smac mimetic and 20 µM z-VAD for 8 hr. The lysate is prepared by 1%SDS Hot Lysate buffer method.

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: 3 minutes

Blocking and diluting buffer: 5% NFDM/TBST.

For 1%SDS Hot Lysate preparation protocol, please refer to the protocol book in the protocol section and/or here (downloadable copy).
Sample: HT-29 (Human colorectal adenocarcinoma epithelial cell) treated with 20 ng/ml TNF-α, 100 nM Smac mimetic and 20 μM z-VAD for 8 hours whole cell lysates 10 μg per lane.

Lane 1: Anti-MLKL (phospho S358) antibody [EPR9514] (ab187091) at 0.12 μg/ml (Batch produced in 2016)
Lane 2: Anti-MLKL (phospho S358) antibody [EPR9514] (ab187091) at 0.17 μg/ml (Batch produced in 2015)
Lane 3: Anti-MLKL (phospho S358) antibody [EPR9514] (ab187091) at 0.12 μg/ml (GR212667 - batch produced in 2014)
Lane 4: Anti-MLKL (phospho S358) antibody [EPR9514] (ab187091) at 0.16 μg/ml (The supernatant of the clone producing ab187091)
Lane 5: Anti-MLKL (phospho S358) antibody [EPR9514] (ab187091) at 0.15 μg/ml (Batch produced in 2017)

Secondary
Goat Anti-Rabbit IgG H&L (HRP) (ab97051), 1/20000 dilution

Blocking and diluting buffer: 5% NFDM/TBST.

The lysate in this image is prepared by 1%SDS Hot Lysate buffer.

For Lysate preparation protocol, please refer to the protocol book in the protocol section and/or here (downloadable copy).
All lanes: Anti-MLKL (phospho S358) antibody [EPR9514] (ab187091) at 1/2000 dilution

Lane 1: Untreated HT-29 lysate
Lane 2: HT-29 cell lysate treated with TNF alpha+ Smac mimetic+ z-VAD

Lysates/proteins at 10 µg per lane.

Secondary
All lanes: Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 54 kDa

Details on WB tested positive control samples: HT-29 cells were treated with the indicated stimuli for 8 hr and then harvested. The final concentrations of 20 ng/ml TNF-a, 100 nM Smac mimetic, and 20 µM z-VAD were used to induce necrosis.

The lysate in this image is prepared by 1%SDS Hot Lysate buffer.

For Lysate preparation protocol, please refer to the protocol book in the protocol section and/or here (downloadable copy).
Dot blot analysis of MLKL peptides using ab187091 at 1/1000 dilution followed by Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated secondary antibody at 1/1000 dilution. Blocking and diluting buffer was 5% NFDM/TBST, exposure time 3 minutes.

Lane 1: MLKL (pT357) phospho peptide
Lane 2: MLKL (pS358) phospho peptide
Lane 3: MLKL (pT357/pS358) phospho peptide
Lane 4: MLKL non-phospho peptide

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

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors