abcam

Product datasheet

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

重组 RabMAb

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概述

产品名称	Anti-MLKL (phospho S358)抗体[EPR9514]
描述	兔单克隆抗体[EPR9514] to MLKL (phospho S358)
宿主	Rabbit
特异性	Stimulation may be required to allow detection of the phosphorylated protein. Please see images below for recommended treatment conditions and positive controls.
经测试应用	**适用于:** WB, Dot blot, IHC-P
种属反应性	**与反应:** Human **不与反应:** Mouse, Rat
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab206929**)
阳性对照	WB: 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; human hepatocyte (treated with Smac/z0VAD) cell lysate. IHC-P: Human skin and melanoma tissue. Dot blot: MLKL (pS358) phospho peptide; MLKL (pT357/pS358) phospho peptide.
常规说明	This antibody was developed through collaboration with the lab of Xiaodong Wang at the National Institute of Biological Sciences, Beijing. Abcam recommended secondaries - Goat Anti-Rabbit HRP (**ab205718**) and Goat Anti-Rabbit Alexa Fluor® 488 (**ab150077**). Or search our wide range of secondary antibodies for use with your experiment. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR9514
同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab187091于以下的经测试应用

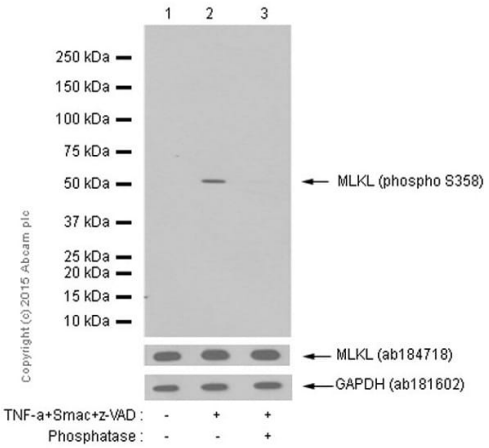
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB	★★★★☆ (7)	1/1000 - 1/2000. Predicted molecular weight: 54 kDa.Can be blocked with **Human MLKL (phospho S358) peptide (ab206929)**. **We recommend using 1% SDS Hot lysis method to prepare cell lysates.** For Lysate preparation protocol, please refer to the protocol book in the protocol section and/or **here (downloadable copy)**
Dot blot		1/1000.
IHC-P	★★★★☆ (4)	1/250 - 1/500. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. Not Suitable for Mouse and Rat

靶标

序列相似性	Belongs to the protein kinase superfamily. Contains 1 protein kinase domain.
结构域	The protein kinase domain is predicted to be catalytically inactive.

图片



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.

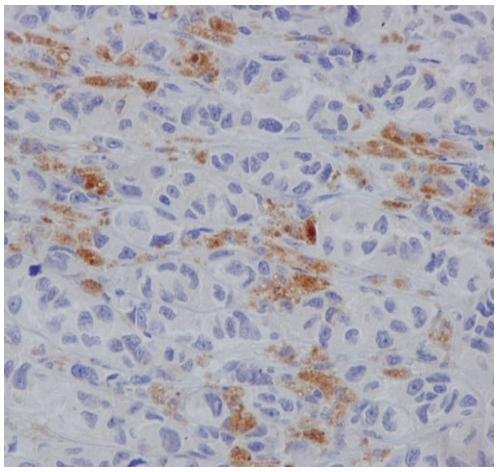
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

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).**

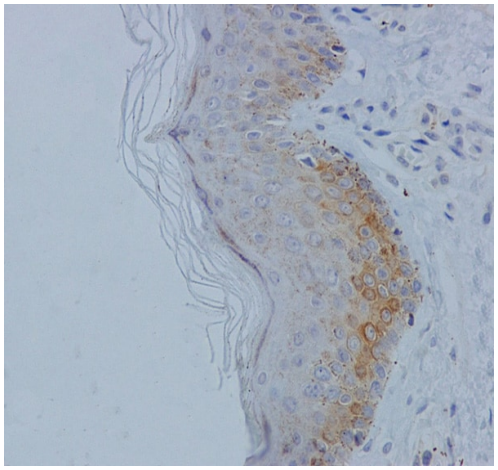


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MLKL (phospho S358) antibody [EPR9514] (ab187091)

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

Antigen retrival 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**

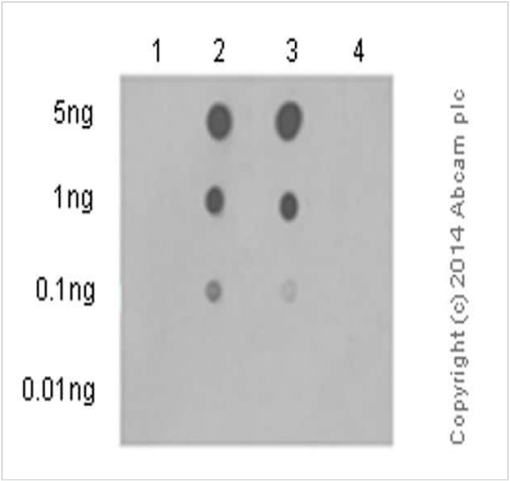


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MLKL (phospho S358) antibody [EPR9514] (ab187091)

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

Antigen retrival 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**



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

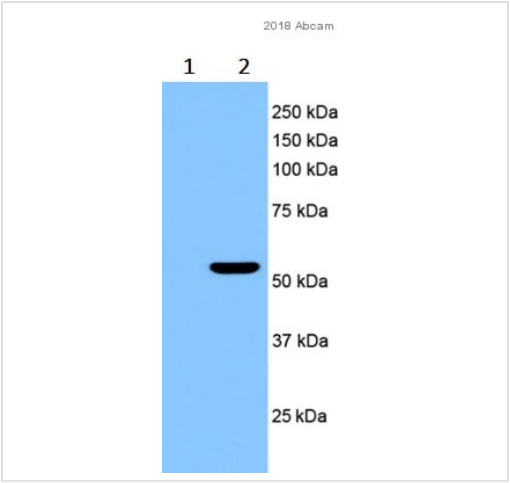
Dot blot analysis of MLKL peptides using ab187091 at 1/1000 dilution followed by Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated secondary antibody at 1/1000 dilution. Blocking and diluting buffer was 5% NFDM/TBST, exposure time 3 minuts.

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



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

Data courtesy of an anonymous AbReview

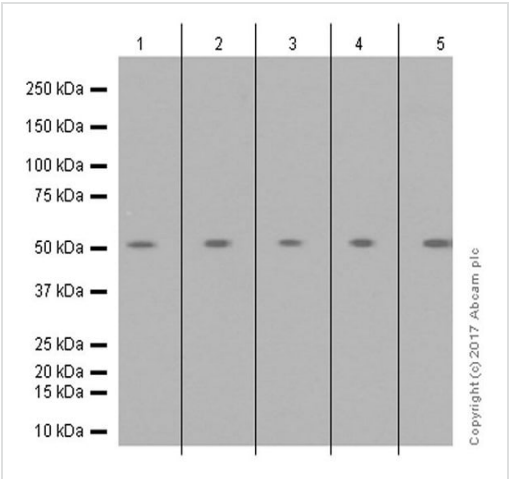
All lanes : Anti-MLKL (phospho S358) antibody [EPR9514] (ab187091) at 1000 cells

Lane 1 : Untreated human hepatocyte cell lysate
Lane 2 : Treated (10 ng/ml TNF-a+100 nM Smac mimetic+20 µM z-VAD 6 h) human hepatocyte cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit HRP at 1/5000 dilution

Predicted band size: 54 kDa



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

Sample: 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 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]

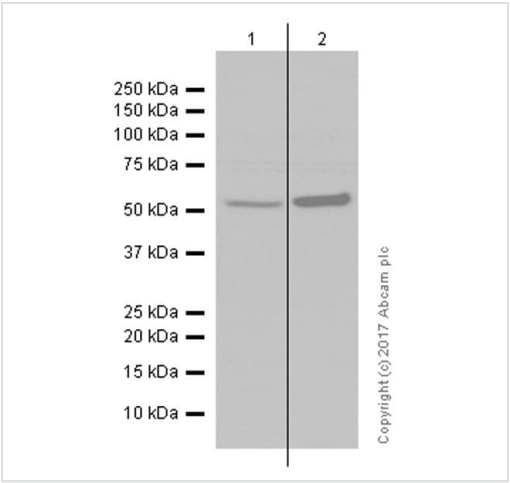
(ab187091) at 0.15 µg/ml (Batch produced in 2017)

Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 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).**



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

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.

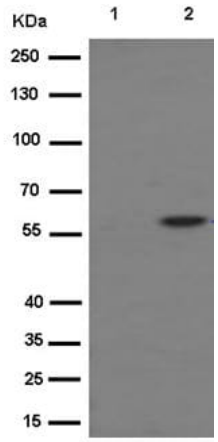
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

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).**



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

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).**

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

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