abcam

Product datasheet

Anti-MLKL antibody [EPR17514] ab184718

KO VALIDATED Recombinant RabMAb

5 Abreviews 64 References 8 Images

Overview

Product name	Anti-MLKL antibody [EPR17514]
Description	Rabbit monoclonal [EPR17514] to MLKL
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, WB, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HUVEC, HT-29 and HeLa whole cell lysates; Human fetal kidney lysate. IHC-P: Human tonsil and colonic adenocarcinoma tissues.
General notes	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**.

Properties

Form	Liquid
Storage instructions	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.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR17514
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab184718 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

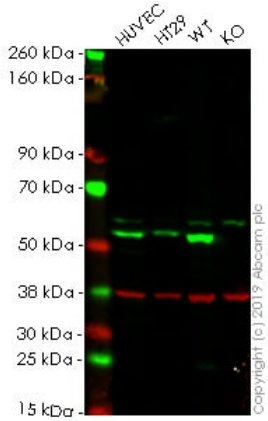
Application	Abreviews	Notes
ICC/IF	★★★★★ **(1)**	1/200.
WB	★★★★★ **(4)**	1/1000. Detects a band of approximately 54 kDa (predicted molecular weight: 54 kDa).
IHC-P		1/400. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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



Western blot - Anti-MLKL antibody [EPR17514] (ab184718)

All lanes : Anti-MLKL antibody [EPR17514] (ab184718) at 1/1000 dilution

Lane 1 : HUVEC cell lysate
Lane 2 : HT-29 cell lysate
Lane 3 : Wild-type HeLa cell lysate
Lane 4 : MLKL knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

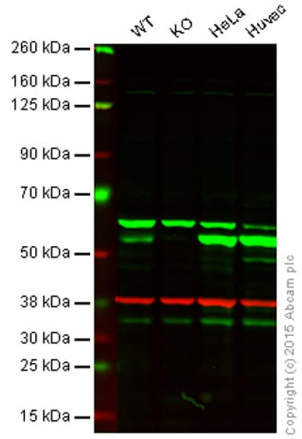
Performed under reducing conditions.

Predicted band size: 54 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab184718 observed at 54 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab184718 was shown to react with MLKL in wild-type HeLa cells.

Loss of signal was observed when knockout cell line **ab255408** (knockout cell lysate **ab263788**) was used. Wild-type and MLKL knockout samples were subjected to SDS-PAGE. ab184718 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-MLKL antibody [EPR17514] (ab184718)

All lanes : Anti-MLKL antibody [EPR17514] (ab184718) at 1/1000 dilution

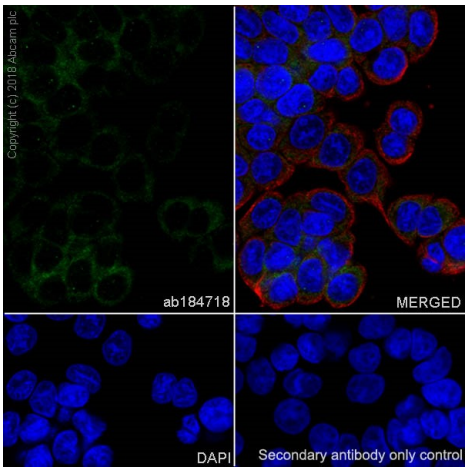
Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : MLKL knockout HAP1 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : Huvec cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 54 kDa

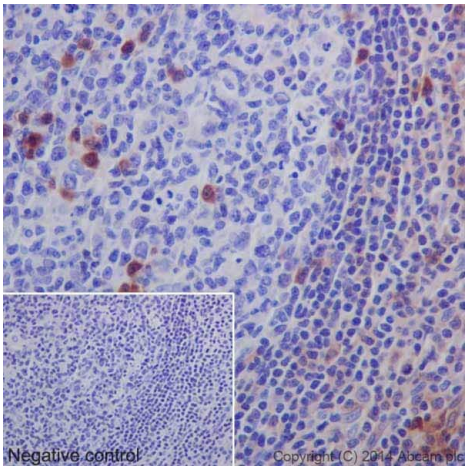
Lanes 1 - 4: Merged signal (red and green). Green - ab184718 observed at 55 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab184718 was shown to recognize MLKL in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when MLKL knockout samples were examined. Wild-type and MLKL knockout samples were subjected to SDS-PAGE. ab184718 and **ab8245** (loading control to GAPDH) were diluted to 1/1000 and 1/2000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-MLKL antibody [EPR17514] (ab184718)

Ab184718 staining MLKL in HT-29 (Human colorectal adenocarcinoma epithelial cell) cells by Immunocytochemistry (ICC). Cells were fixed with 100% Methanol. Samples were incubated with primary antibody at 1/200 dilution (6.5µg/ml). An AlexaFluor® 488 Goat anti-Rabbit (**ab150077**) was used as the secondary antibody at 1/1000 dilution (2µg/ml). Ab195889 , Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) was used as the counterstain antibody (1/200 dilution, 2.5 µg/ml . DAPI was used as a nuclear counterstain. Confocal image showing cytoplasmic staining on HT-29 cell line.

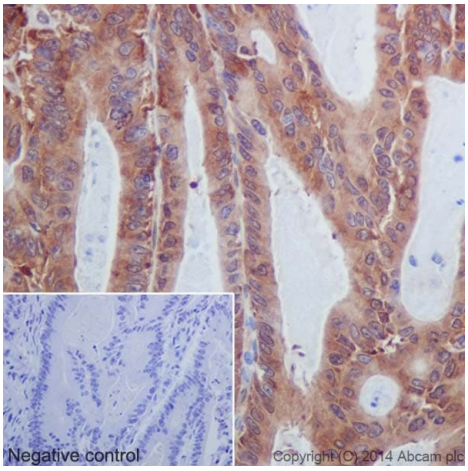


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MLKL antibody [EPR17514] (ab184718)

Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling MLKL with ab184718 at 1/400 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) secondary antibody (**ab97051**) at 1/500 dilution. Cytoplasmic staining on the lymphocytes of human tonsil is observed. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

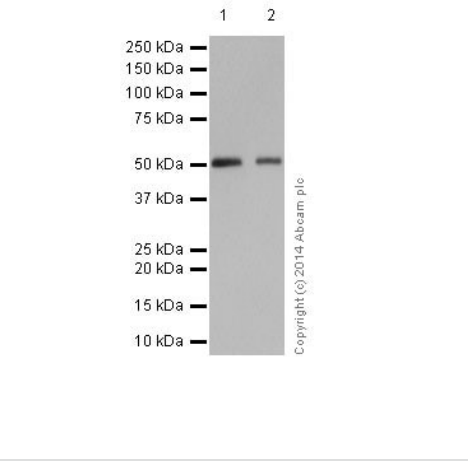


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MLKL antibody [EPR17514] (ab184718)

Immunohistochemical analysis of paraffin-embedded Human colonic adenocarcinoma tissue labeling MLKL with ab184718 at 1/400 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) secondary antibody (**ab97051**) at 1/500 dilution. Cytoplasmic staining on tumor cells of human colonic adenocarcinoma is observed. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-MLKL antibody [EPR17514] (ab184718)

All lanes : Anti-MLKL antibody [EPR17514] (ab184718) at 1/20000 dilution

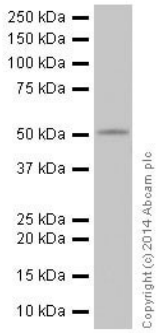
Lane 1 : HUVEC (Human umbilical vein endothelial cell line) whole cell lysate
Lane 2 : HT-29 (Human colorectal adenocarcinoma cells) whole cell lysate

Lysates/proteins at 20 µg per lane.

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

Predicted band size: 54 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-MLKL antibody [EPR17514] (ab184718)

Anti-MLKL antibody [EPR17514] (ab184718) at 1/1000 dilution + Human fetal kidney lysate at 10 µg

Secondary

Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 54 kDa

Observed band size: 54 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Anti-MLKL antibody [EPR17514] (ab184718)

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