abcam

Product datasheet

Anti-MLKL antibody [EPR17514] - BSA and Azide free ab211045

KO VALIDATED Recombinant RabMAb

7 Images

Overview

Product name	Anti-MLKL antibody [EPR17514] - BSA and Azide free
Description	Rabbit monoclonal [EPR17514] to MLKL - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, IHC-P, WB
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	ab211045 is the carrier-free version of **ab184718**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR17514
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab211045 in the following tested applications.

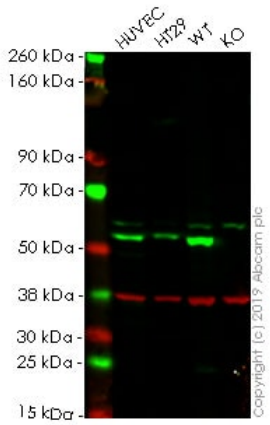
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 54 kDa (predicted molecular weight: 54 kDa).

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] - BSA and Azide free (ab211045)

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.

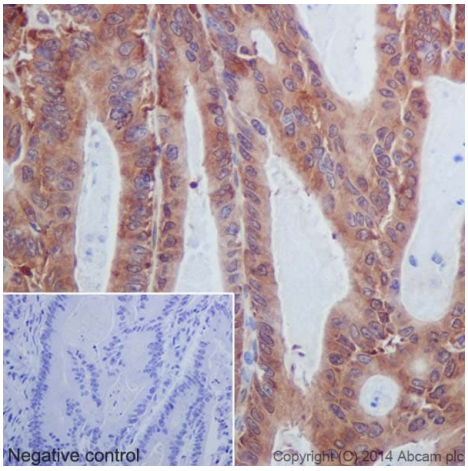
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (**ab184718**).

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.



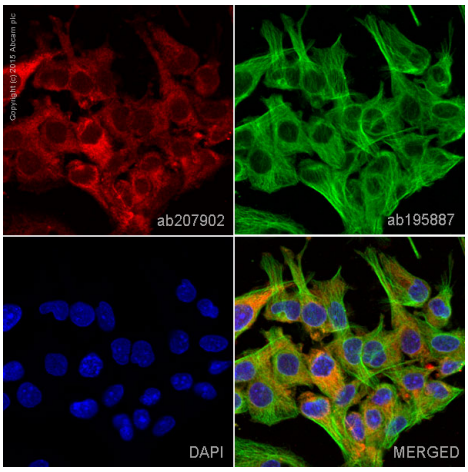
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MLKL antibody [EPR17514] - BSA and Azide free (ab211045)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab184718**).

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

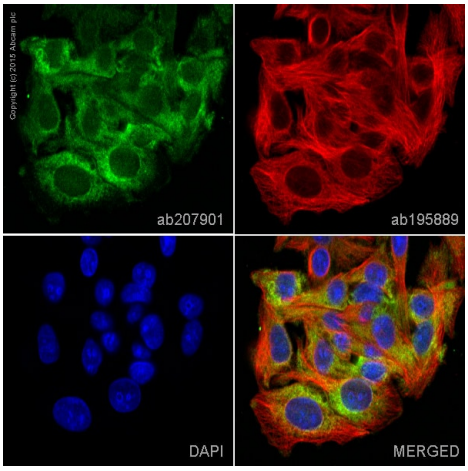


Immunocytochemistry/ Immunofluorescence - Anti-MLKL antibody [EPR17514] - BSA and Azide free (ab211045)

Clone EPR17514 (ab211045) has been successfully conjugated by Abcam. This image was generated using Anti-MLKL antibody [EPR17514] (Alexa Fluor® 647). Please refer to **ab207902** for protocol details.

ab207902 staining MLKL in SW480 cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab207902** at a 1/50 dilution (shown in red) and **ab195887**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at a 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

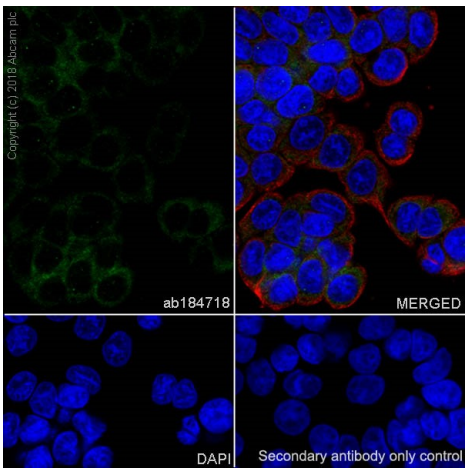


Immunocytochemistry/ Immunofluorescence - Anti-MLKL antibody [EPR17514] - BSA and Azide free (ab211045)

Clone EPR17514 (ab211045) has been successfully conjugated by Abcam. This image was generated using Anti-MLKL antibody [EPR17514] (Alexa Fluor® 488). Please refer to **ab207901** for protocol details.

ab207901 staining MLKL in SW480 cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab207901** at a 1/100 dilution (shown in green) and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at a 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

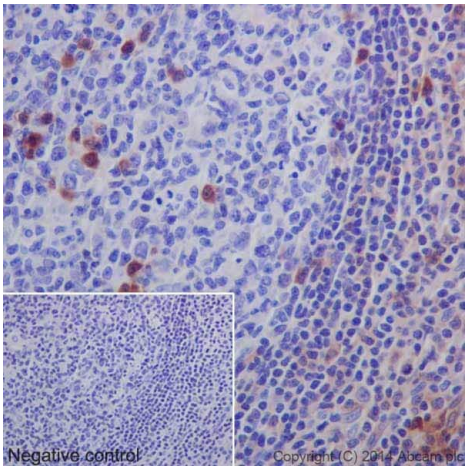
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Anti-MLKL antibody [EPR17514] - BSA and Azide free (ab211045)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab184718**).



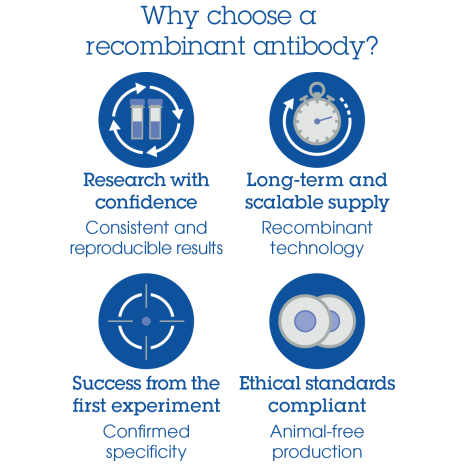
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MLKL antibody [EPR17514] - BSA and Azide free (ab211045)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab184718**).

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



Anti-MLKL antibody [EPR17514] - BSA and Azide free (ab211045)

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