abcam

Product datasheet

Anti-MTH1 antibody [EPR15934] ab197028

KO VALIDATED Recombinant RabMAb

2 References 7 Images

Overview

Product name	Anti-MTH1 antibody [EPR15934]
Description	Rabbit monoclonal [EPR15934] to MTH1
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, Flow Cyt, ICC/IF
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat
Immunogen	Recombinant fragment within Human MTH1 aa 50-150. The exact sequence is proprietary. Database link: P36639
Positive control	WB: HEK-293T, HAP1, HeLa, Jurkat and A549 cell lysates. Human fetal thymus lysates. IHC: Human Spleen tissue. ICC/IF: PC-3 cells.
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. Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough. Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility. We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee. In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for. We are also updating the applications & species that this product has been "predicted to work with," however this information is not covered by our Abpromise guarantee.

Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, as well as customer reviews and Q&As.

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 (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR15934
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab197028** in the following tested applications.

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

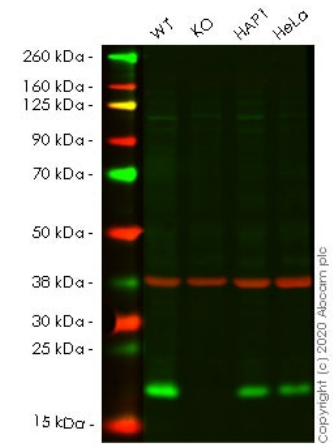
Application	Abreviews	Notes
WB		1/2000. Detects a band of approximately 18 kDa (predicted molecular weight: 23 kDa).
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt		Use at an assay dependent concentration.
ICC/IF		1/250.

Target

Function	Antimutagenic. Acts as a sanitizing enzyme for oxidized nucleotide pools, thus suppressing cell dysfunction and death induced by oxidative stress. Hydrolyzes 8-oxo-dGTP, 8-oxo-dATP and 2-OH-dATP, thus preventing misincorporation of oxidized purine nucleoside triphosphates into DNA and subsequently preventing A:T to C:G and G:C to T:A transversions. Able to hydrolyze also the corresponding ribonucleotides, 2-OH-ATP, 8-oxo-GTP and 8-oxo-ATP. Does not play a role in U8 snoRNA decapping activity. Binds U8 snoRNA.
Tissue specificity	Widely expressed with highest expression in thymus, testis, embryo and proliferating blood lymphocytes.

Sequence similarities	Belongs to the Nudix hydrolase family. Contains 1 nudix hydrolase domain.
Developmental stage	In peripheral blood lymphocytes, expressed at much higher levels in proliferating cells than in resting cells.
Post-translational modifications	The N-terminus is blocked.
Cellular localization	Cytoplasm. Mitochondrion matrix and Cytoplasm. Mitochondrion matrix. Nucleus. Mostly present in cytoplasm. Variant Met-124 has decreased efficiency in translocation to mitochondria.

Images



Western blot - Anti-MTH1 antibody [EPR15934] (ab197028)

All lanes : Anti-MTH1 antibody [EPR15934] (ab197028) at 1/2000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : NUDT1 knockout HEK-293T cell lysate
Lane 3 : HAP1 cell lysate
Lane 4 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

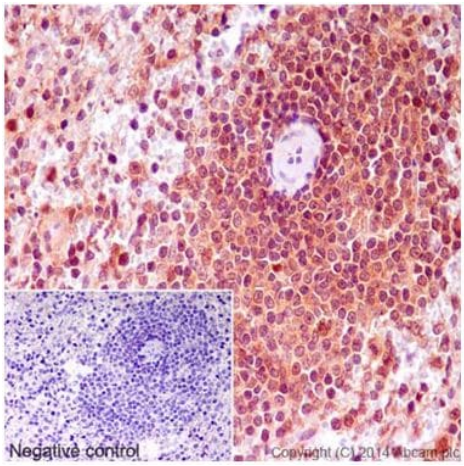
Performed under reducing conditions.

Predicted band size: 23 kDa
Observed band size: 18 kDa
why is the actual band size different from the predicted?

Lanes 1- 4: Merged signal (red and green). Green - ab197028 observed at 18 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab197028 was shown to react with MTH1 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line ab266400 (knockout cell lysate ab257565) was used. Wild-type HEK-293T and NUDT1 knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab197028 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 2000 dilution and a 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)

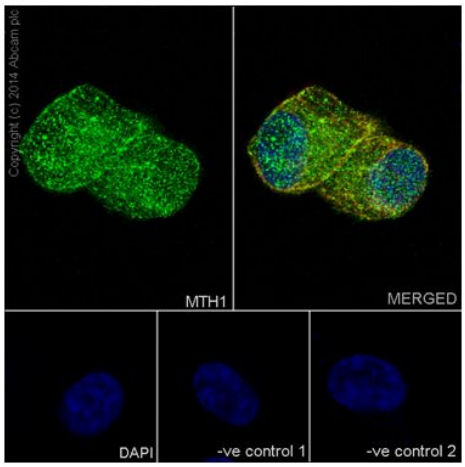
secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MTH1 antibody [EPR15934] (ab197028)

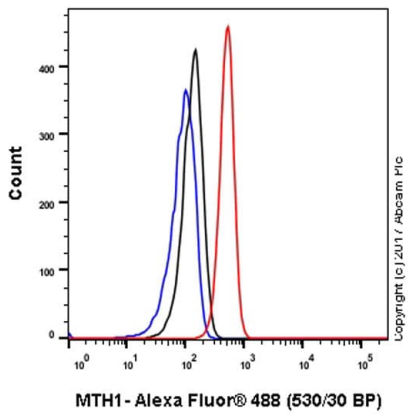
Immunohistochemical analysis of paraffin-embedded Human spleen tissue labeling MTH1 with ab197028 at 1/100 dilution followed by ab97051 Goat Anti-Rabbit IgG H&L (HRP) at 1/500 dilution. Cytoplasm and nuclear staining on Human spleen is observed. Counter stained with Hematoxylin. Negative control: Used PBS instead of primary antibody.

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



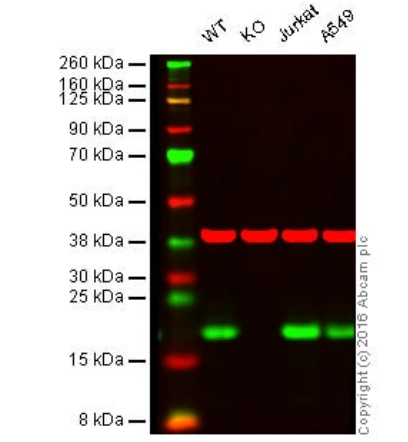
Immunocytochemistry/ Immunofluorescence - Anti-MTH1 antibody [EPR15934] (ab197028)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized PC-3 cells (Human prostate cancer cell line) labeling MTH1 with ab197028 at 1/250 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/500 dilution (green). Cytoplasm and nuclear staining on PC-3 cell line is observed. The nuclear counterstain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).
The negative controls are as follows:-
-ve control 1 - ab197028 at 1/250 dilution followed by ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2. - ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution followed by ab150077 (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.



Flow Cytometry - Anti-MTH1 antibody [EPR15934] (ab197028)

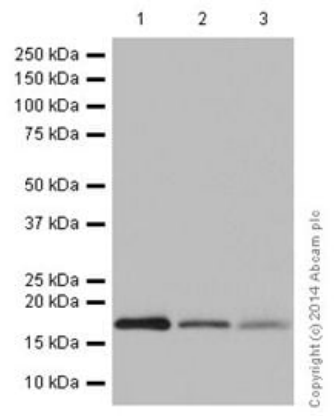
Flow Cytometry analysis of PC-3 (human prostate adenocarcinoma) cells labeling MTH1 with purified ab188474 at 1/230 (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077)(1/2000 dilution) was used as the secondary antibody. Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.



Western blot - Anti-MTH1 antibody [EPR15934] (ab197028)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: MTH1 knockout HAP1 cell lysate (20 µg)
Lane 3: Jurkat cell lysate(20 µg)
Lane 4: A549 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab197028 observed at 18 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab197028 was shown to specifically react with MTH1 when MTH1 knockout samples were used. Wild-type and MTH1 knockout samples were subjected to SDS-PAGE. ab197028 at a dilution of 1/2000 and ab8245 (loading control to GAPDH) diluted at 1/10000 were 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 1/10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-MTH1 antibody [EPR15934] (ab197028)

All lanes : Anti-MTH1 antibody [EPR15934] (ab197028) at 1/2000 dilution

Lane 1 : Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysate
Lane 2 : A549 (Human lung carcinoma) whole cell lysate
Lane 3 : Human fetal thymus lysate

Lysates/proteins at 20 µg per lane.

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

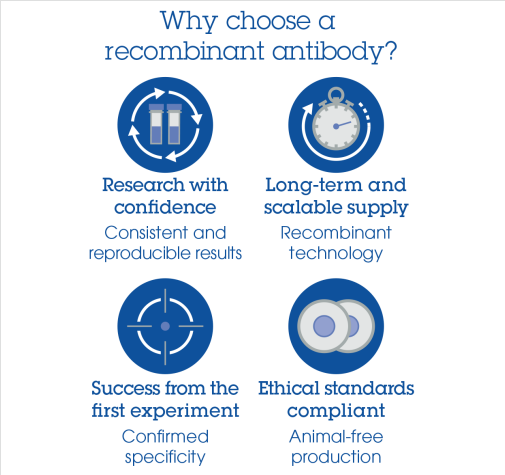
Developed using the ECL technique.

Predicted band size: 23 kDa
Observed band size: 18 kDa why is the actual band size different from the predicted?

Exposure time: 3 minutes

The expression profile observed is consistent with what has been described in the literature (PMID: 11296483).The observed band may be isoform p18.

Blocking/dilution buffer: 5% NFDM/TBST.



Anti-MTH1 antibody [EPR15934] (ab197028)

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