

Product datasheet

Anti-KDM2A antibody [EPR18602] ab191387

KO VALIDATED Recombinant RabMAb

9 References 12 Images

Overview

| | |
|----------------------------|---|
| Product name | Anti-KDM2A antibody [EPR18602] |
| Description | Rabbit monoclonal [EPR18602] to KDM2A |
| Host species | Rabbit |
| Tested applications | Suitable for: ICC/IF, IP, WB, IHC-P |
| Species reactivity | Reacts with: Mouse, Rat, Human |
| Immunogen | Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. |
| Positive control | WB: Human brain lysate; HeLa, Jurkat, K562, C6, PC-12, NIH/3T3 and RAW 264.7 whole cell lysates; rat brain lysate; mouse testis and brain lysates. IHC-P: Human, mouse and rat colon tissues. ICC/IF: HeLa and Jurkat cells. IP: Mouse brain whole cell lysate. |
| General notes | <p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p> |

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 | <p>pH: 7.2</p> <p>Preservative: 0.01% Sodium azide</p> <p>Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA</p> |
| Purity | Protein A purified |
| Clonality | Monoclonal |
| Clone number | EPR18602 |

Isotype

IgG

Applications

The Abpromise guarantee

Our [Abpromise guarantee](#) covers the use of ab191387 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 | | 1/1000. |
| IP | | 1/80. |
| WB | | 1/1000. Detects a band of approximately 133, 90 kDa (predicted molecular weight: 133 kDa). |
| IHC-P | | 1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |

Target

Function

Histone demethylase that specifically demethylates 'Lys-36' of histone H3, thereby playing a central role in histone code. Preferentially demethylates dimethylated H3 'Lys-36' residue while it has weak or no activity for mono- and tri-methylated H3 'Lys-36'. May also recognize and bind to some phosphorylated proteins and promote their ubiquitination and degradation. Required to maintain the heterochromatic state. Associates with centromeres and represses transcription of small non-coding RNAs that are encoded by the clusters of satellite repeats at the centromere. Required to sustain centromeric integrity and genomic stability, particularly during mitosis.

Tissue specificity

Widely expressed, with highest levels in brain, testis and ovary, followed by lung.

Sequence similarities

Belongs to the JHDM1 histone demethylase family.
Contains 1 CXXC-type zinc finger.
Contains 1 F-box domain.
Contains 1 JmjC domain.
Contains 6 LRR (leucine-rich) repeats.
Contains 1 PHD-type zinc finger.

Domain

The JmjC domain mediates demethylation activity and is required for satellite silencing. The CXXC zinc finger preferentially recognizes nonmethylated CpG DNA, and binding is blocked when the CpG DNA is methylated.

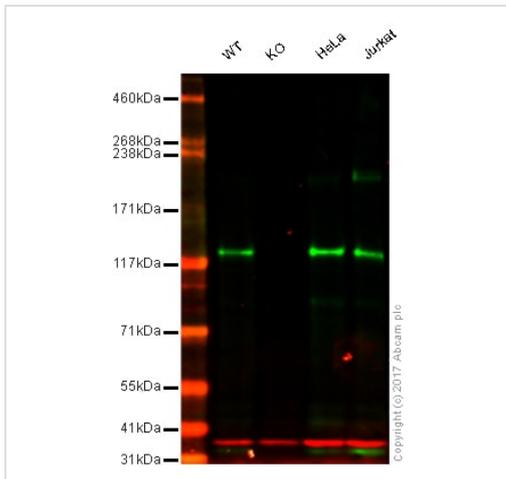
Post-translational modifications

Phosphorylated upon DNA damage, probably by ATM or ATR.

Cellular localization

Nucleus > nucleoplasm. Punctate expression throughout the nucleoplasm and enriched in the perinucleolar region. Specifically nucleates at CpG islands where its presence results in chromatin depleted in H3K36me2.

Images



Western blot - Anti-KDM2A antibody [EPR18602] (ab191387)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)

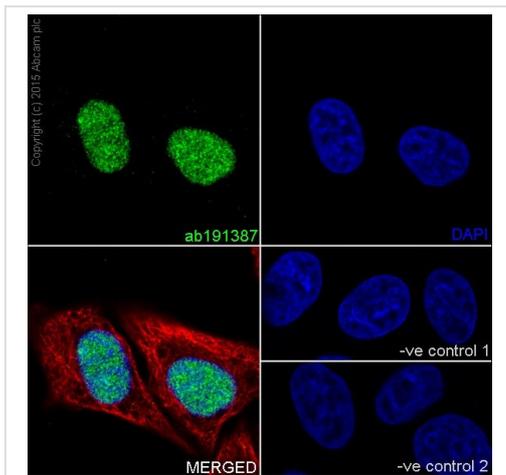
Lane 2: KDM2A knockout HAP1 whole cell lysate (20 µg)

Lane 3: HeLa whole cell lysate (20 µg)

Lane 4: Jurkat whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab191387 observed at 133 kDa. Red - loading control, ab9484, observed at 37 kDa.

ab191387 was shown to specifically react with KDM2A in wild-type HAP1 cells. No band was observed when KDM2A knockout cells were examined. Wild-type and KDM2A knockout samples were subjected to SDS-PAGE. Ab191387 and ab9484 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1.313 µg/ml and 1/20,000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20,000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-KDM2A antibody [EPR18602] (ab191387)

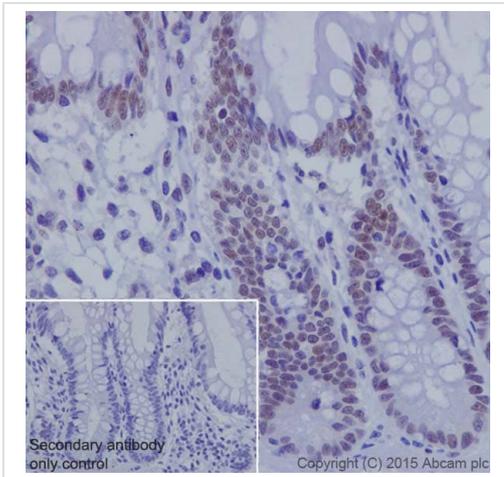
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling KDM2A with ab191387 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on HeLa cell line. The nuclear counterstain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab191387 at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary antibody at 1/1000 dilution.

-ve control 2: Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.

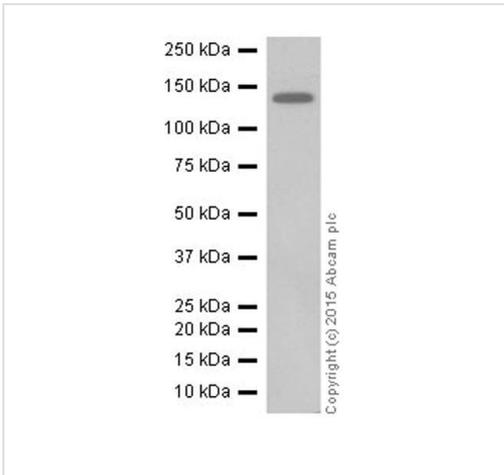


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KDM2A antibody [EPR18602] (ab191387)

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling KDM2A with ab191387 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on epithelium cells of human colon is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used 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-KDM2A antibody [EPR18602] (ab191387)

Anti-KDM2A antibody [EPR18602] (ab191387) at 1/5000 dilution + Human brain lysate at 10 µg

Secondary

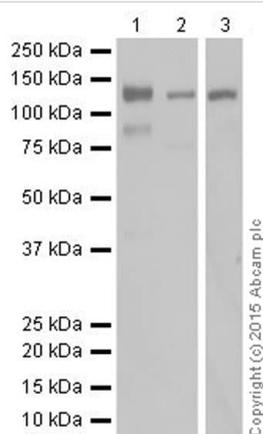
Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

Predicted band size: 133 kDa

Observed band size: 133 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-KDM2A antibody [EPR18602]
(ab191387)

All lanes : Anti-KDM2A antibody [EPR18602] (ab191387) at 1/1000 dilution

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Lane 3 : K562 (Human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

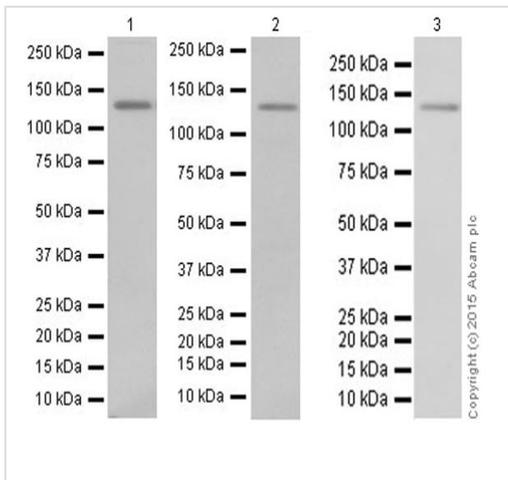
Predicted band size: 133 kDa

Observed band size: 133,90 kDa

Blocking/Dilution buffer: 5% NFDm/TBST.

Exposure time: Lane 1 and 2: 8 seconds; Lane 3: 15 seconds.

This antibody recognizes two isoforms. The predicted MW are 133kDa and 90kDa respectively.



Western blot - Anti-KDM2A antibody [EPR18602] (ab191387)

All lanes : Anti-KDM2A antibody [EPR18602] (ab191387) at 1/1000 dilution

Lane 1 : Rat brain lysate

Lane 2 : Mouse testis lysate

Lane 3 : Mouse brain lysate

Lysates/proteins at 10 µg per lane.

Secondary

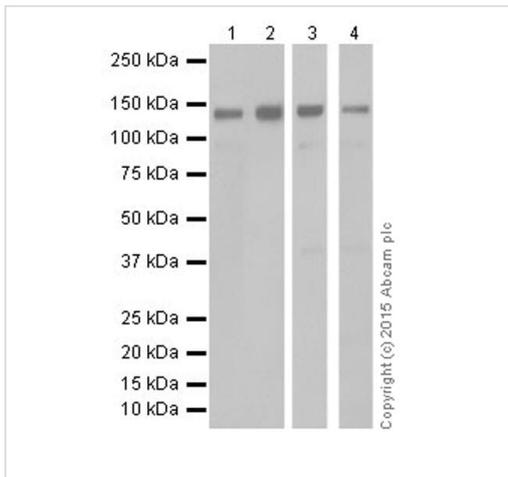
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 133 kDa

Observed band size: 133 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1 and 3: 3 minutes; Lane 2: 30 seconds.



Western blot - Anti-KDM2A antibody [EPR18602] (ab191387)

All lanes : Anti-KDM2A antibody [EPR18602] (ab191387) at 1/1000 dilution

Lane 1 : C6 (Rat glial tumor cell line) whole cell lysate

Lane 2 : PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate

Lane 3 : NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate

Lane 4 : RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

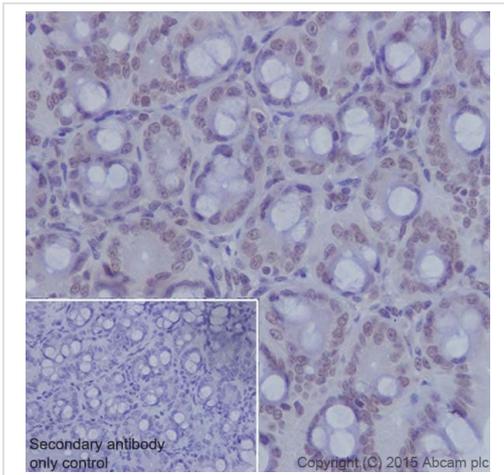
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 133 kDa

Observed band size: 133 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1 and 2: 15 seconds; Lane 3: 8 seconds; Lane 4: 30 seconds.

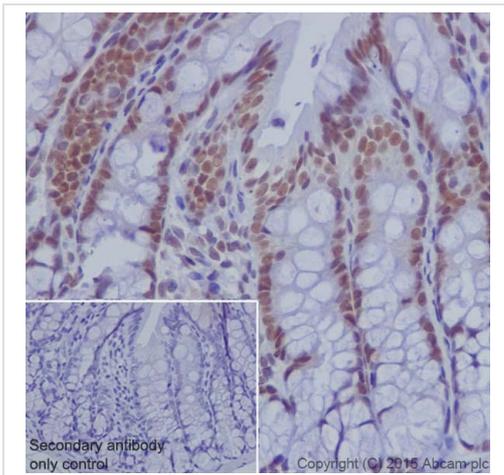


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KDM2A antibody [EPR18602] (ab191387)

Immunohistochemical analysis of paraffin-embedded mouse colon tissue labeling KDM2A with ab191387 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on epithelium cells of mouse colon is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used 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.

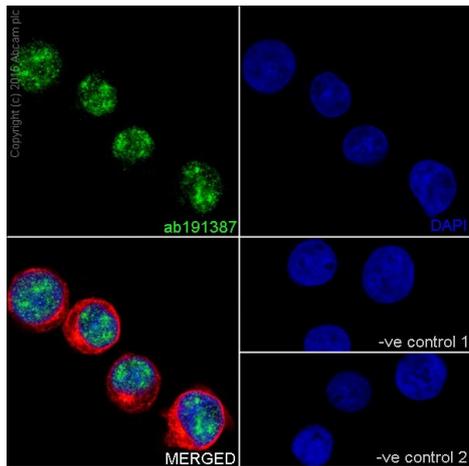


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KDM2A antibody [EPR18602] (ab191387)

Immunohistochemical analysis of paraffin-embedded rat colon tissue labeling KDM2A with ab191387 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on epithelium cells of rat colon is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used 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.



Immunocytochemistry/ Immunofluorescence - Anti-KDM2A antibody [EPR18602] (ab191387)

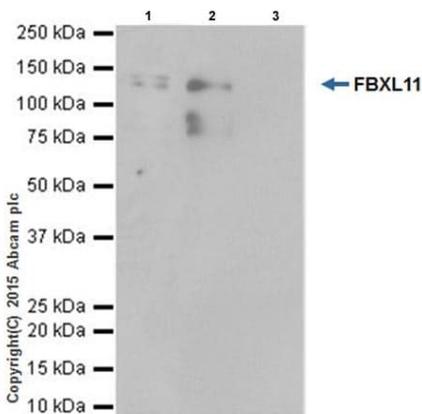
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Jurkat (Human T cell leukemia cell line from peripheral blood) cells labeling KDM2A with ab191387 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on Jurkat cell line. The nuclear counterstain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab191387 at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary antibody at 1/1000 dilution.

-ve control 2: Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.



Immunoprecipitation - Anti-KDM2A antibody [EPR18602] (ab191387)

KDM2A was immunoprecipitated from 1mg of mouse brain whole cell lysate with ab191387 at 1/100 dilution. Western blot was performed from the immunoprecipitate using ab191387 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

Lane 1: Mouse brain whole cell lysate 10µg (Input).

Lane 2: ab191387 IP in Mouse brain whole cell lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab191387 in Mouse brain whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDm/TBST.

Exposure time: 30 seconds.

Why choose a recombinant antibody?



Research with confidence
Consistent and reproducible results



Long-term and scalable supply
Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

Anti-KDM2A antibody [EPR18602] (ab191387)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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