abcam

Product datasheet

Anti-KDM3A / JHDM2A antibody [EPR18875] ab191389

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-KDM3A / JHDM2A antibody [EPR18875]
Description	Rabbit monoclonal [EPR18875] to KDM3A / JHDM2A
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, IP
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Untreated HEK-293 whole cell lysate; HEK-293, HeLa and Jurkat treated with 100 µM CoCl2 for 24 hours whole cell lysates; Ramos whole cell lysate. IHC-P: Human testis, bladder cancer and lung cancer tissues. IP: Ramos whole cell lysate.
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 (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR18875

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab191389 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)**	1/1000. Detects a band of approximately 147, 100, 75 kDa (predicted molecular weight: 147 kDa).
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/30.

Target

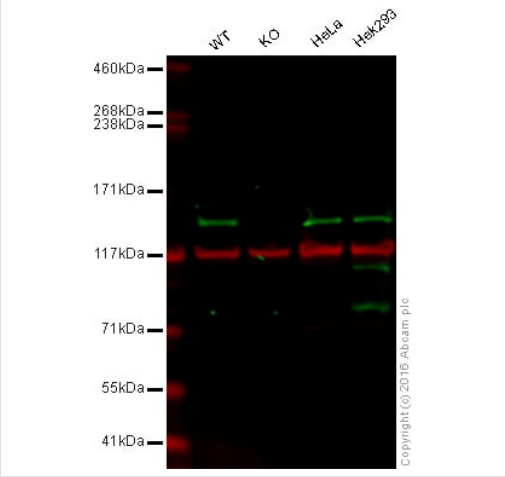
Function Histone demethylase that specifically demethylates 'Lys-9' of histone H3, thereby playing a central role in histone code. Preferentially demethylates mono- and dimethylated H3 'Lys-9' residue, with a preference for dimethylated residue, while it has weak or no activity on trimethylated H3 'Lys-9'. Demethylation of Lys residue generates formaldehyde and succinate. Involved in hormone-dependent transcriptional activation, by participating in recruitment to androgen-receptor target genes, resulting in H3 'Lys-9' demethylation and transcriptional activation. Involved in spermatogenesis by regulating expression of target genes such as PRM1 and TMP1 which are required for packaging and condensation of sperm chromatin. Involved in obesity resistance through regulation of metabolic genes such as PPARA and UCP1.

Sequence similarities Belongs to the JHDM2 histone demethylase family.
Contains 1 JmjC domain.

Domain The JmjC domain and the C6-type zinc-finger are required for the demethylation activity. Leu-Xaa-Xaa-Leu-Leu (LXXLL) motifs are known to mediate the association with nuclear receptors.

Cellular localization Cytoplasm. Nucleus. Nuclear in round spermatids. When spermatids start to elongate, localizes to the cytoplasm where it forms distinct foci which disappear in mature spermatozoa.

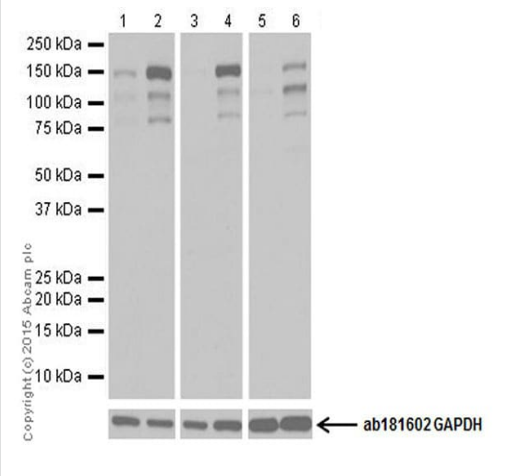
Images



Western blot - Anti-KDM3A / JHDM2A antibody [EPR18875] (ab191389)

Lane 1: Wild type HAP1 whole cell lysate (40 µg)
Lane 2: KDM3A knockout HAP1 whole cell lysate (40 µg)
Lane 3: HeLa whole cell lysate (40 µg)
Lane 4: Hek293 whole cell lysate (40 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab191389 observed at 150 kDa. Red - loading control, **ab18058**, observed at 130 kDa.

ab191389 was shown to specifically react with KDM3A when KDM3A knockout samples were used. Wild-type and KDM3A knockout samples were subjected to SDS-PAGE. Ab191389 and **ab18058** (Mouse anti Vinculin loading control) were incubated overnight at 4°C at 500 dilution and 1/10000 dilution respectively. Blots were developed with 800CW Goat anti Rabbit and 680CW Goat anti Mouse secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-KDM3A / JHDM2A antibody [EPR18875] (ab191389)

All lanes : Anti-KDM3A / JHDM2A antibody [EPR18875] (ab191389) at 1/1000 dilution

Lane 1 : Untreated HEK-293 (Human epithelial cell line from embryonic kidney), whole cell lysate
Lane 2 : HEK-293 (Human epithelial cell line from embryonic kidney) treated with 100 µM CoCl2 for 24 hours, whole cell lysate
Lane 3 : Untreated HeLa (Human epithelial cell line from cervix adenocarcinoma), whole cell lysate
Lane 4 : HeLa (Human epithelial cell line from cervix adenocarcinoma) treated with 100 µM CoCl2 for 24 hours, whole cell lysate
Lane 5 : Untreated Jurkat (Human T cell leukemia cell line from peripheral blood), whole cell lysate
Lane 6 : Jurkat (Human T cell leukemia cell line from peripheral blood) treated with 100 µM CoCl2 for 24 hours, whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

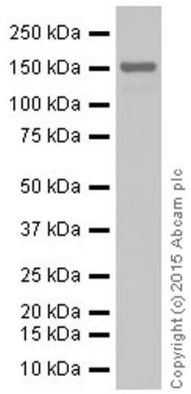
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 147 kDa

Observed band size: 100,147,75 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1 and 2: 15 seconds; Lane 3 and 4: 30 seconds; Lane 5 and 6: 5 seconds.



Western blot - Anti-KDM3A / JHDM2A antibody [EPR18875] (ab191389)

Anti-KDM3A / JHDM2A antibody [EPR18875] (ab191389) at 1/1000 dilution + Ramos (Human Burkitt's lymphoma cell line) whole cell lysate

Secondary

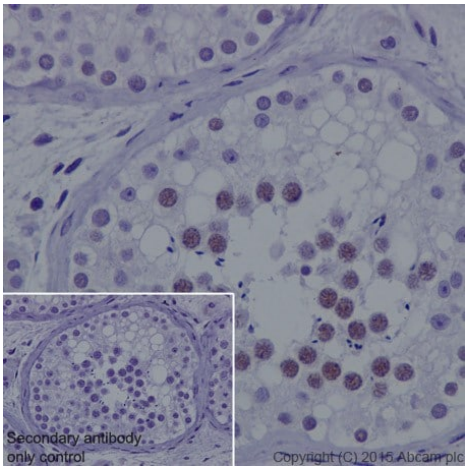
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 147 kDa

Observed band size: 147 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KDM3A / JHDM2A antibody [EPR18875] (ab191389)

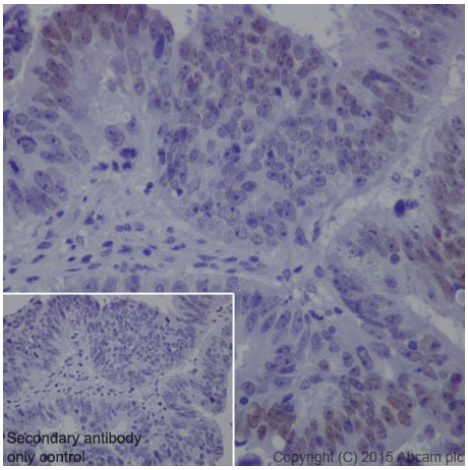
Immunohistochemical analysis of paraffin-embedded human testis tissue labeling KDM3A / JHDM2A with ab191389 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Nuclear staining on spermatogenic cells of human testis 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-KDM3A / JHDM2A antibody [EPR18875] (ab191389)

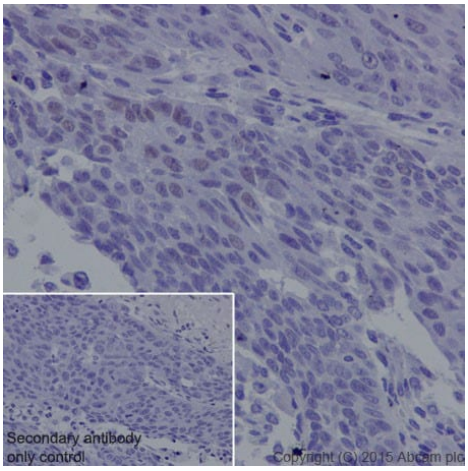
Immunohistochemical analysis of paraffin-embedded human bladder cancer tissue labeling KDM3A / JHDM2A with ab191389 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Weak nuclear staining on cancer cells of human bladder cancer 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-KDM3A / JHDM2A antibody [EPR18875] (ab191389)

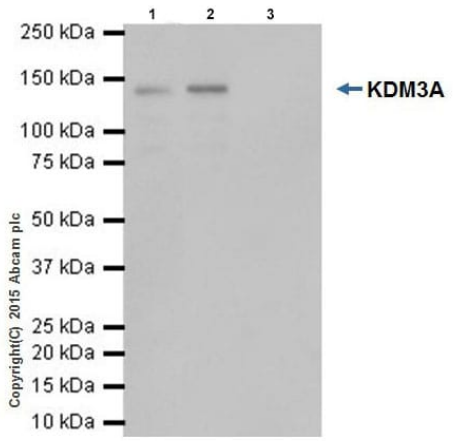
Immunohistochemical analysis of paraffin-embedded human lung cancer tissue labeling KDM3A / JHDM2A with ab191389 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Weak nuclear staining on cancer cells of human lung cancer 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.



Immunoprecipitation - Anti-KDM3A / JHDM2A antibody [EPR18875] (ab191389)

KDM3A / JHDM2A was immunoprecipitated from 1mg of Ramos (Human Burkitt's lymphoma cell line) whole cell lysate with ab191389 at 1/30 dilution.

Western blot was performed from the immunoprecipitate using ab191389 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

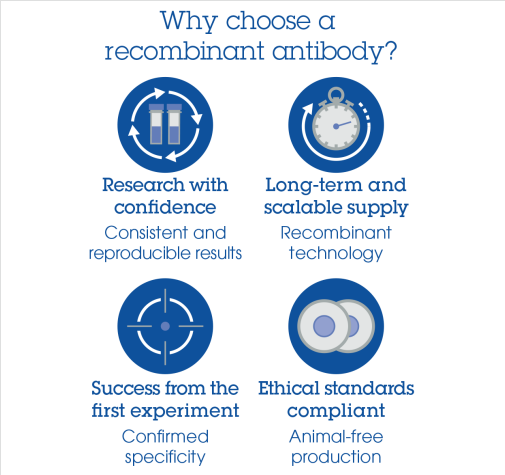
Lane 1: Ramos whole cell lysate 10µg (Input).

Lane 2: ab191389 IP in Ramos whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab191389 in Ramos whole cell lysate.

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

Exposure time: 10 seconds.



Anti-KDM3A / JHDM2A antibody [EPR18875]

(ab191389)

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