abcam

ab194286

Anti-KDM5A / Jarid1A / RBBP2 antibody [EPR18651]

Recombinant | RabMAb | Advanced Validation | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR18651
Purification technique	Affinity purification Protein A
Concentration	1.711 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Mouse
Dilution info	1/30
Notes	-

Species	Human

Dilution info	1/30
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/5000
Notes	-

Species	Human
Dilution info	1/5000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/2000
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	1/1500
Notes	-

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

ChIC/CUT&RUN-seq

Tested

Species	Human
Dilution info	5 µg
Notes	-

Species	Mouse
Dilution info	5 µg
Notes	-

ChIP-seq

Not recommended

Species	Human, Mouse
Dilution info	-
Notes	-

Target data

See full target information KDM5A

Function Histone demethylase that specifically demethylates 'Lys-4' of histone H3, thereby playing a central role in histone code. Does not demethylate histone H3 'Lys-9', H3 'Lys-27', H3 'Lys-36', H3 'Lys-79' or H4 'Lys-20'. Demethylates trimethylated and dimethylated but not monomethylated H3 'Lys-4'. Regulates specific gene transcription through DNA-binding on 5'-CCGCCC-3' motif (PubMed:18270511). May stimulate transcription mediated by nuclear receptors. Involved in transcriptional regulation of Hox proteins during cell differentiation (PubMed:19430464). May participate in transcriptional repression of cytokines such as CXCL12. Plays a role in the regulation of the circadian rhythm and in maintaining the normal periodicity of the circadian clock. In a histone

demethylase-independent manner, acts as a coactivator of the CLOCK-BMAL1-mediated transcriptional activation of PER1/2 and other clock-controlled genes and increases histone acetylation at PER1/2 promoters by inhibiting the activity of HDAC1 (By similarity). Seems to act as a transcriptional corepressor for some genes such as MT1F and to favor the proliferation of cancer cells (PubMed:27427228).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

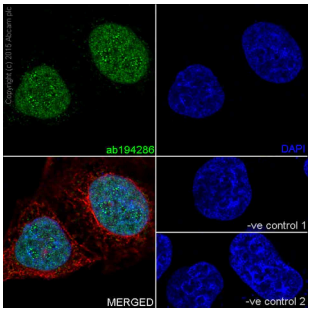
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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14 product images

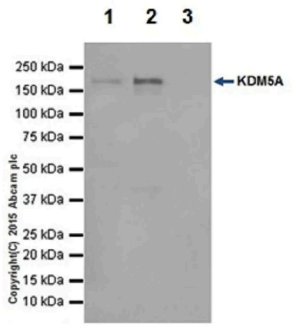


Immunocytochemistry/ Immunofluorescence - Anti-KDM5A / Jarid1A / RBBP2 antibody [EPR18651] (ab194286)

KDM5A / Jarid1A / RBBP2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-KDM5A / Jarid1A / RBBP2 antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling KDM5A / Jarid1A / RBBP2 with ab194286 at 1/2000 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 counter stain 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: ab194286 at 1/2000 dilution followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary 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 at 1/1000 dilution.



Immunoprecipitation - Anti-KDM5A / Jarid1A / RBBP2 antibody [EPR18651] (ab194286)

KDM5A / Jarid1A / RBBP2 was immunoprecipitated from 1mg of HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate with ab194286 at 1/100 dilution. Western blot was performed from the immunoprecipitate using ab194286 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.
Lane 1: HeLa whole cell lysate 10ug (Input).

Lane 2: ab194286 IP in HeLa whole cell lysate.

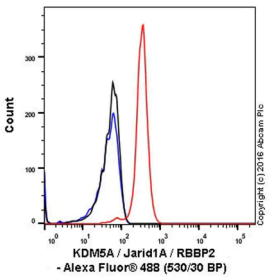
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab194286 in HeLa whole cell lysate.

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

Exposure time: 5 seconds.

All lanes:
Immunoprecipitation - Anti-KDM5A / Jarid1A / RBBP2 antibody [EPR18651] (ab194286)

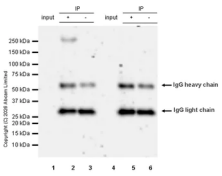
Predicted band size: 192 kDa



Flow Cytometry (Intracellular) - Anti-KDM5A / Jarid1A / RBBP2 antibody [EPR18651] (ab194286)

KDM5A / Jarid1A / RBBP2 Flow Cytometry (Intracellular) staining using rabbit Anti-KDM5A / Jarid1A / RBBP2 antibody

Intracellular Flow Cytometry analysis of NIH/3T3 (mouse embryo) cells labelling KDM5A / Jarid1A / RBBP2 (red) with purified ab194286 at dilution of 1/1500. The secondary antibody used was Alexa Fluorr® 488 goat-anti-rabbit IgG (1/2000). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. Isotype control antibody used was Rabbit Monoclonal IgG (black). The blue line shows cells without incubation with primary antibody and secondary antibody.



Immunoprecipitation - Anti-KDM5A / Jarid1A / RBBP2 antibody [EPR18651] (ab194286)

KDM5A / Jarid1A / RBBP2 was immunoprecipitated from 0.35 mg NIH/3T3 (mouse embryonic fibroblast) whole cell lysate with ab194286 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab194286 at 1/1000 dilution.

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

This blot was developed using a high-sensitivity ECL substrate, allowing for the detection of proteins in the mid-femtogram range.
All lanes:
Immunoprecipitation - Anti-KDM5A / Jarid1A / RBBP2 antibody [EPR18651] (ab194286) at 1/30 dilution

Lane 1:
3T3-L1 (mouse embryonic fibroblast) transfected with scrambled siRNA control whole cell lysate at 7 µg

Lane 2:
3T3-L1 transfected with scrambled siRNA control whole cell lysate

Lane 3:
Rabbit monoclonal IgG (ab172730) instead of ab194286 in 3T3-L1 transfected with scrambled siRNA control lysate

Lane 4:
3T3-L1 transfected with siRNA specifically targeting KDM5A whole cell lysate at 7 µg

Lane 5:
3T3-L1 transfected with siRNA specifically targeting KDM5A whole cell lysate

Lane 6:
Rabbit monoclonal IgG (ab172730) instead of ab194286 in 3T3-L1 transfected with siRNA specifically targeting KDM5A lysate

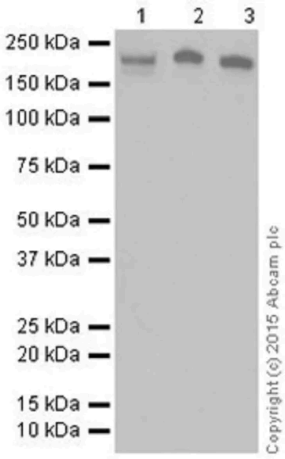
Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Developed using the ECL technique.

Observed band size: 200 kDa

Exposure time: 180s



Western blot - Anti-KDM5A / Jarid1A / RBBP2 antibody [EPR18651] (ab194286)

KDM5A / Jarid1A / RBBP2 Western blot staining using rabbit Anti-KDM5A / Jarid1A / RBBP2 antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-KDM5A / Jarid1A / RBBP2 antibody [EPR18651] (ab194286) at 1/5000 dilution

Lane 1:
LLC (Mouse lung carcinoma) whole cell lysate at 20 µg

Lane 2:
Mouse testis lysate at 20 µg

Lane 3:
HEK-293 (Human epithelial cells from embryonic kidney) whole cell lysate at 20 µg

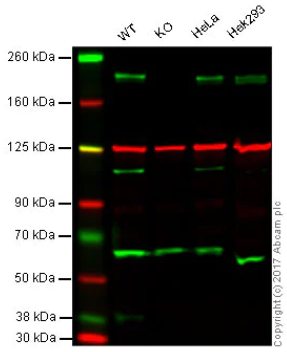
Secondary

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

Predicted band size: 192 kDa

Observed band size: 192 kDa

Exposure time: 30s



Western blot - Anti-KDM5A / Jarid1A / RBBP2 antibody [EPR18651] (ab194286)

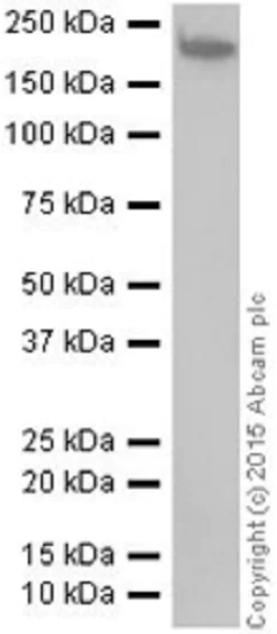
KDM5A / Jarid1A / RBBP2 Western blot staining using rabbit Anti-KDM5A / Jarid1A / RBBP2 antibody

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)
Lane 2: KDM5A knockout HAP1 whole cell lysate (20 µg)
Lane 3: HeLa whole cell lysate (20 µg)
Lane 4: HEK293 whole cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab194286 observed at 240 kDa. Red - loading control, ab18058, observed at 130 kDa.

ab194286 was shown to specifically recognize KDM5A in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when KDM5A knockout samples were examined. Wild-type and KDM5A knockout samples were subjected to SDS-PAGE. ab194286 and ab18058 (Mouse anti Vinculin loading control) were incubated overnight at 4°C at 1/2500 dilution and 1/10000 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 1/20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-KDM5A / Jarid1A / RBBP2 antibody [EPR18651] (ab194286)

Predicted band size: 192 kDa



Western blot - Anti-KDM5A / Jarid1A / RBBP2 antibody [EPR18651] (ab194286)

KDM5A / Jarid1A / RBBP2 Western blot staining of HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate using rabbit Anti-KDM5A / Jarid1A / RBBP2 antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-KDM5A / Jarid1A / RBBP2 antibody [EPR18651] (ab194286) at 1/5000 dilution

All lanes:
HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate at 20 µg

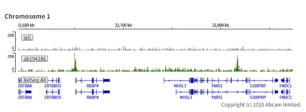
Secondary

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

Predicted band size: 161 kDa, 192 kDa, 22 kDa, 40 kDa, 43 kDa, 46 kDa

Observed band size: 162 kDa, 192 kDa, 43 kDa, 46 kDa

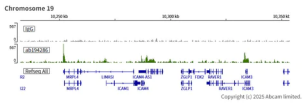
Exposure time: 10s



ChIC/CUT&RUN sequencing - Anti-KDM5A / Jarid1A / RBBP2 antibody [EPR18651] (ab194286)

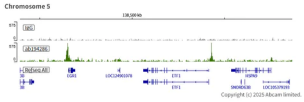
ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/mL, 2.5 x 10^5 K-562 (human chronic myelogenous leukemia lymphoblast) cells and 5 µg of ab194286 [EPR18651]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10

million reads. The negative IgG control ab172730 is also shown. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



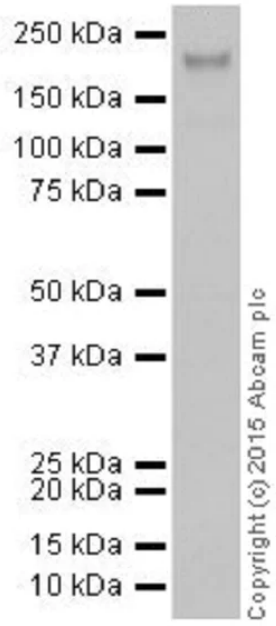
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Western blot - Anti-KDM5A / Jarid1A / RBBP2 antibody [EPR18651] (ab194286)

KDM5A / Jarid1A / RBBP2 Western blot staining of NIH/3T3 (Mouse embryonic fibroblast cells) whole cell lysate using rabbit Anti-KDM5A / Jarid1A / RBBP2 antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-KDM5A / Jarid1A / RBBP2 antibody [EPR18651] (ab194286) at 1/1000 dilution

All lanes:
NIH/3T3 (Mouse embryonic fibroblast cells) whole cell lysate at 10 µg

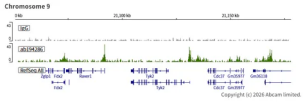
Secondary

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

Predicted band size: 192 kDa

Observed band size: 192 kDa

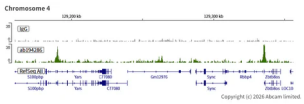
Exposure time: 10s



ChIC/CUT&RUN sequencing - Anti-KDM5A / Jarid1A / RBBP2 antibody [EPR18651] (ab194286)

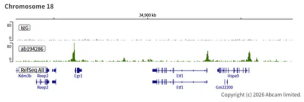
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