abcam

Product datasheet

Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade ab129195

KO VALIDATED | Recombinant | RabMAb

4 Abreviews | 9 References | 18 Images

Overview

Product name	Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade
Description	Rabbit monoclonal [EPR6825] to KDM1/LSD1 - Nuclear Marker and ChIP Grade
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IP, IHC-P, ICC/IF, ChIP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human KDM1/LSD1 aa 50-150. The exact sequence is proprietary. Database link: O60341 (Peptide available as ab166919)
Positive control	WB: HAP1, 293T, HEK293, HeLa, Jurkat, PC3, C6, Raw 264.7, PC-12, and NIH 3T3 cell lysates. IHC-P: Human testis, rat kidney, and mouse colon tissues. ICC/IF: HAP1 and HeLa cells. Flow Cyt: HeLa cells. IP: Jurkat cell lysate. ChIP: HCT 116 (Human colorectal carcinoma epithelial cell).
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. **We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.**

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.

	Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR6825
Isotype	IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab129195 in the following tested applications.

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

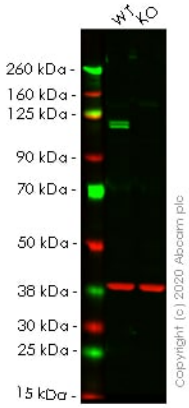
Application	Abreviews	Notes
Flow Cyt (Intra)		1/20. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★★ (2)	1/10000 - 1/50000. Detects a band of approximately 110 kDa (predicted molecular weight: 92 kDa).Can be blocked with KDM1/LSD1 peptide (ab166919).
IP		1/10 - 1/100.
IHC-P		1/50 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ICC/IF	★★★★☆ (2)	Use a concentration of 1 µg/ml.
ChIP		Use 5 µg for 25 µg of chromatin.

Target

Function Histone demethylase that demethylates both 'Lys-4' (H3K4me) and 'Lys-9' (H3K9me) of histone H3, thereby acting as a coactivator or a corepressor, depending on the context. Acts by oxidizing the substrate by FAD to generate the corresponding imine that is subsequently hydrolyzed. Acts as a corepressor by mediating demethylation of H3K4me, a specific tag for epigenetic transcriptional activation. Demethylates both mono- (H3K4me1) and di-methylated (H3K4me2) H3K4me. May play a role in the repression of neuronal genes. Alone, it is unable to demethylate H3K4me on nucleosomes and requires the presence of RCOR1/CoREST to achieve such activity. Also acts as a coactivator of androgen receptor (ANDR)-dependent transcription, by being recruited to ANDR target genes and mediating demethylation of H3K9me, a specific tag for epigenetic transcriptional repression. The presence of PRKCB in ANDR-containing complexes, which mediates phosphorylation of 'Thr-6' of histone H3 (H3T6ph), a specific tag that prevents demethylation H3K4me, prevents H3K4me demethylase activity of KDM1A. Demethylates di-methylated 'Lys-370' of p53/TP53 which prevents interaction of p53/TP53 with TP53BP1 and

	represses p53/TP53-mediated transcriptional activation. Demethylates and stabilizes the DNA methylase DNMT1. Required for gastrulation during embryogenesis.
Tissue specificity	Ubiquitously expressed.
Sequence similarities	Belongs to the flavin monoamine oxidase family. Contains 1 SWIRM domain.
Domain	The SWIRM domain may act as an anchor site for a histone tail.
Cellular localization	Nucleus.

Images



Western blot - Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195)

All lanes : Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : KDM1A knockout HeLa cell lysate

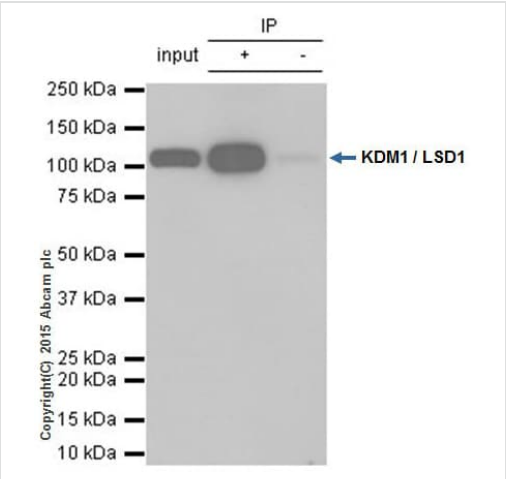
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 92 kDa
Observed band size: 110 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab129195 observed at 110 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab129195 was shown to react with KDM1/LSD1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab265790 (knockout cell lysate ab256965) was used. Wild-type HeLa and KDM1A knockout HeLa 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. ab129195 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 1000 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.

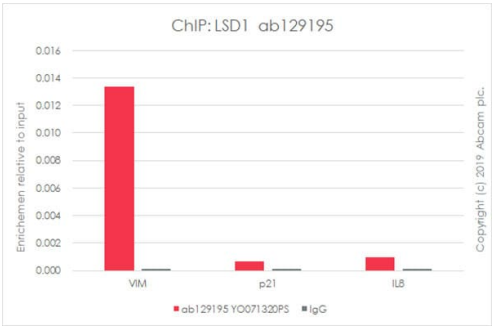


Immunoprecipitation - Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195)

ab129195 (purified) at 1/20 immunoprecipitating KDM1/LSD1 in 10 µg Jurkat cell lysate (Lanes 1 and 2, observed at 110 kDa). Lane 3 - Rabbit monoclonal IgG (ab172730).

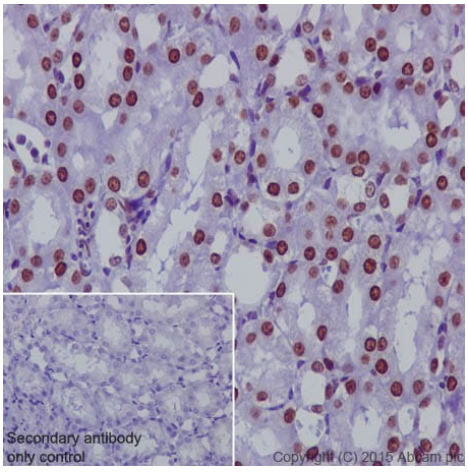
For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10,000 dilution.

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



ChIP - Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195)

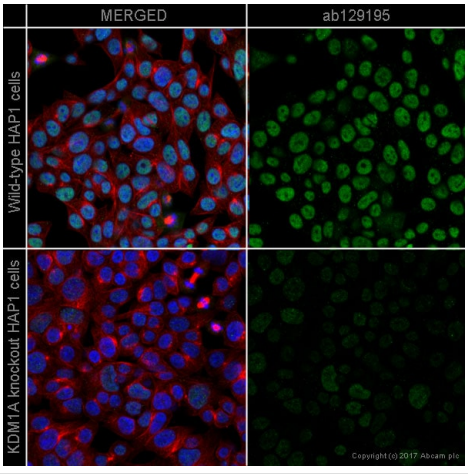
Chromatin was prepared from HCT 116 cells according to the Abcam X-ChIP protocol. Cells were fixed with 1% formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 5µg of ab129195 (red), and 20µl of protein A/G sepharose beads slurry (10µl of sepharose A beads + 10µl of sepharose G beads). 5µg of rabbit normal IgG was added to the beads control (grey). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195)

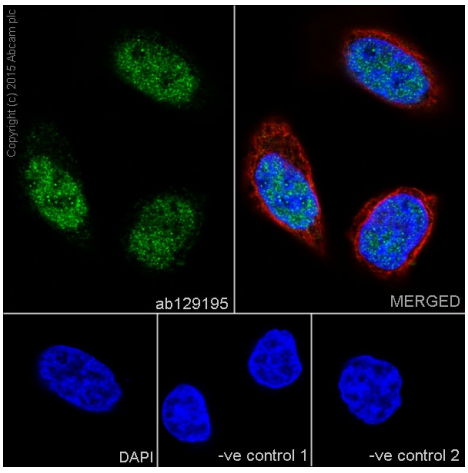
Immunohistochemical staining of paraffin embedded rat kidney with purified ab129195 at a working dilution of 1/50. The secondary antibody used is ab97051, a goat anti-rabbit IgG (H&L) at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0.

PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



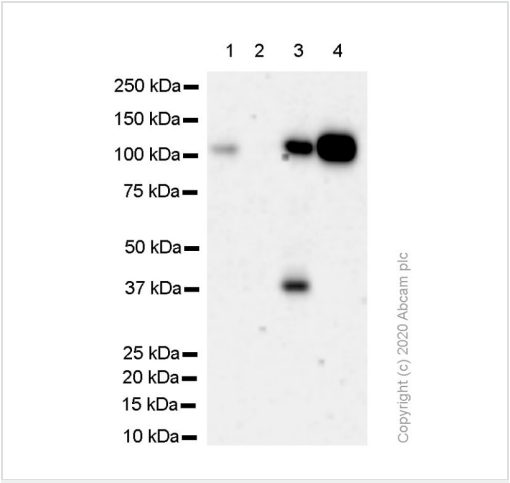
Immunocytochemistry/ Immunofluorescence - Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195)

ab129195 staining KDM1A/LSD1 in wild-type HAP1 cells (top panel) and KDM1A knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), 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 with ab129195 at 1µg/ml concentration and ab195889 at 1/250 dilution (shown in pseudo colour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.



Immunocytochemistry/ Immunofluorescence - Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195)

Immunofluorescence staining of HeLa cells with purified ab129195 at a working dilution of 1/100, counter-stained with DAPI. The secondary antibody was Alexa Fluor® 488 goat anti-rabbit (ab150077), used at a dilution of 1/1000. ab7291, a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with ab150120 (Alexa Fluor® 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified ab129195 was used at a dilution of 1/500 followed by an Alexa Fluor® 594 goat anti-mouse antibody (ab150120) at a dilution of 1/500. For negative control 2, ab7291 (mouse anti-tubulin) was used at a dilution of 1/500 followed by an Alexa Fluor® 488 goat anti-rabbit antibody (ab150077) at a dilution of 1/400.



Western blot - Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195)

All lanes : ab129195 at 1/1000 dilution

Lane 1 : Mouse brain lysates
Lane 2 : Mouse heart lysates
Lane 3 : Mouse liver lysates
Lane 4 : Mouse spleen lysates

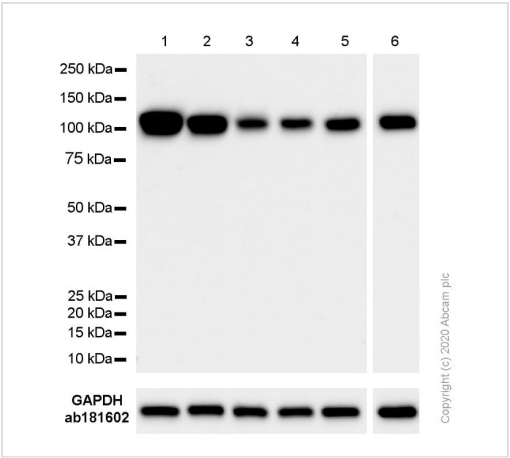
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1/20000 dilution

Predicted band size: 92 kDa
Observed band size: 110 kDa

Exposure time: 180 seconds

Blocking/diluting buffer and concentration: 5% NFDM/TBST



Western blot - Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195)

All lanes : ab129195 at 1/1000 dilution

Lane 1 : Jurkat (Human T cell leukemia T lymphocyte) whole cell lysates
Lane 2 : HCT 116 (Human colorectal carcinoma epithelial cell) whole cell lysates
Lane 3 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates
Lane 4 : C2C12 (Mouse myoblasts myoblast) whole cell lysates
Lane 5 : Mouse skeletal muscle lysates
Lane 6 : C6 (Rat glial tumor glial cell) whole cell lysates

Lysates/proteins at 20 µg per lane.

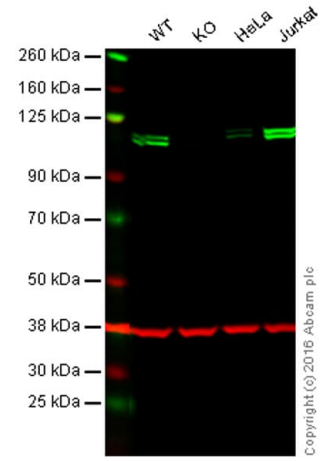
Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/20000 dilution

Predicted band size: 92 kDa

Exposure time: 20 seconds

Blocking/diluting buffer and concentration: 5% NFDM/TBST

Observed band: 110kd



Western blot - Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195)

All lanes : Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195) at 1/10000 dilution

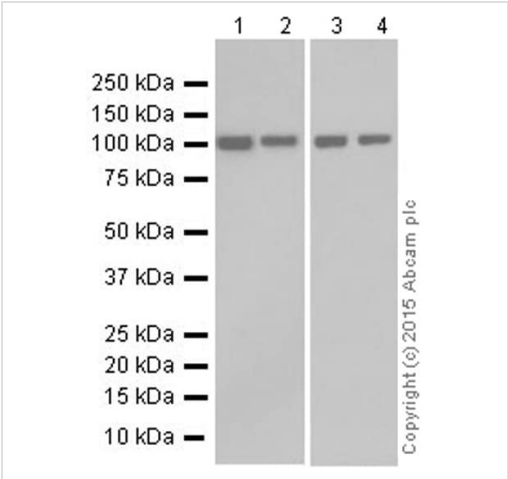
Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : KMD1 / LSD1 knockout HAP1 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 92 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab129195 observed at 110 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab129195 was shown to specifically react with KMD1 / LSD1 in wild-type HAP1 cells. No band was observed when KMD1 / LSD1 knockout samples were used. Wild-type and KMD1 / LSD1 knockout samples were subjected to SDS-PAGE. ab129195 and ab8245 (loading control to GAPDH) were both diluted 1/10,000 and 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 1/10,000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195)

All lanes : Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195) at 1/10000 dilution (purified)

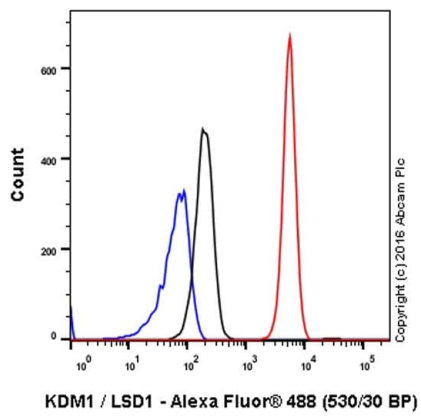
Lane 1 : C6 whole cell lysate
Lane 2 : Raw 264.7 whole cell lysate
Lane 3 : PC-12 whole cell lysate
Lane 4 : NIH/3T3 whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

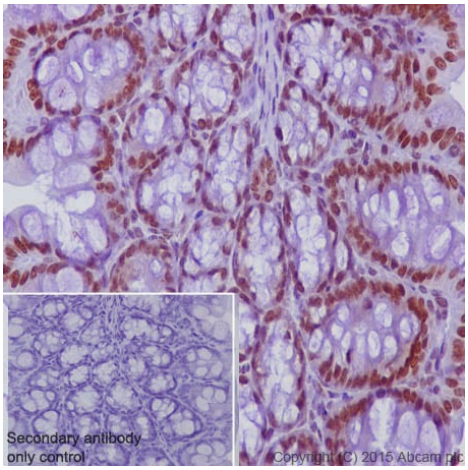
Predicted band size: 92 kDa
Observed band size: 110 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.



Flow Cytometry (Intracellular) - Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195)

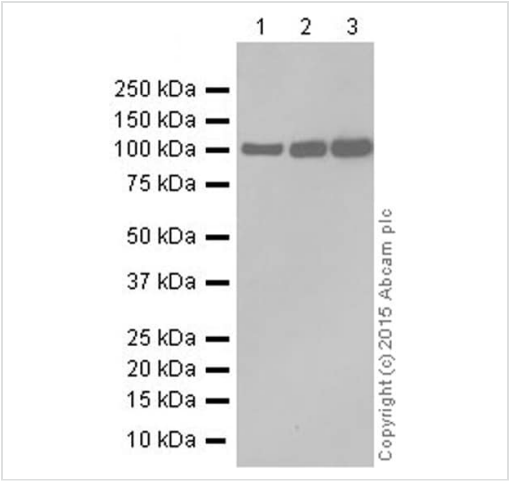
Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling KDM1/LSD1 with purified ab129195 at 1/20 dilution (10ug/mL) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195)

Immunohistochemical staining of paraffin embedded mouse colon with purified ab129195 at a working dilution of 1/50. The secondary antibody used is ab97051, a goat anti-rabbit IgG (H&L) at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0.

PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Western blot - Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195)

All lanes : Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195) at 1/10000 dilution (purified)

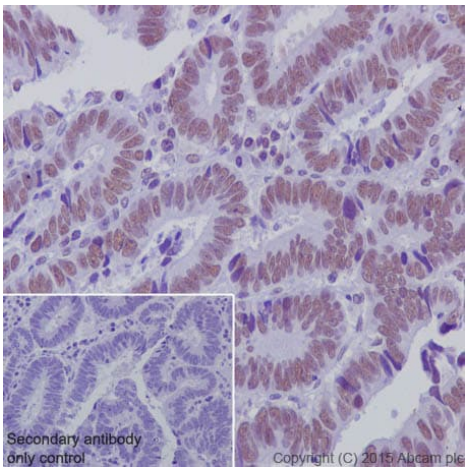
Lane 1 : HEK293 whole cell lysate
Lane 2 : HeLa whole cell lysate
Lane 3 : Jurkat whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 92 kDa
Observed band size: 110 kDa

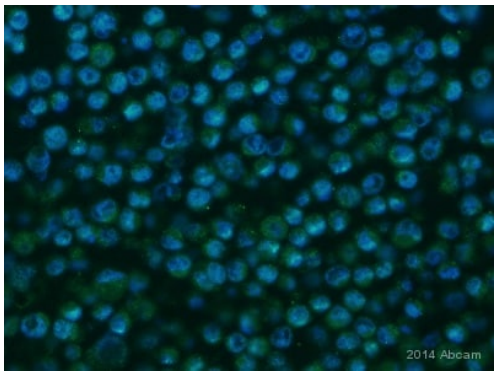
Blocking/Dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195)

Immunohistochemical staining of paraffin embedded human stomach carcinoma with purified ab129195 at a working dilution of 1/50. The secondary antibody used is ab97051, a goat anti-rabbit IgG (H&L) at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0.

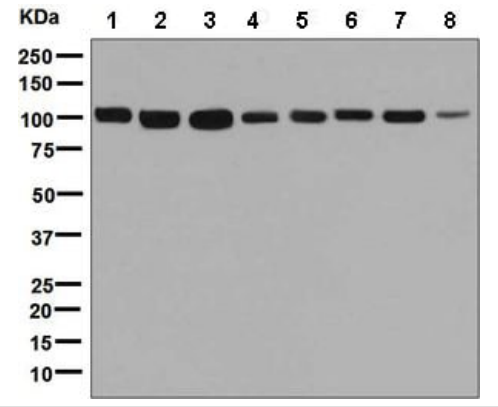
PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Immunocytochemistry/ Immunofluorescence - Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195)

This image is courtesy of an anonymous Abreview

Unpurified ab129195 staining KDM1/LSD1 in paraffin-embedded A549 lung cancer cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed using the HOPE technique and permeabilized with 0.05% Tween. Samples were incubated with primary antibody (1/100) for 45 minutes at 25°C. An Alexa Fluor®488-conjugated Donkey anti-mouse IgG polyclonal (1/200) was used as the secondary antibody.



Western blot - Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195)

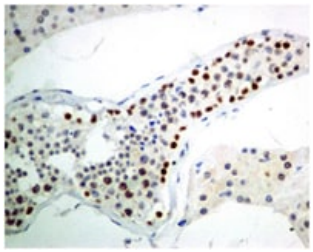
All lanes : Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195) at 1/10000 dilution (unpurified)

Lane 1 : 293T cell lysate
Lane 2 : HeLa cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : PC3 cell lysate
Lane 5 : C6 cell lysate
Lane 6 : RAW264.7 cell lysate
Lane 7 : PC12 cell lysate
Lane 8 : NIH 3T3 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat anti-Rabbit HRP at 1/2000 dilution

Predicted band size: 92 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195)

Unpurified ab129195, at 1/100, staining KDM1/LSD1 in paraffin embedded human testis tissue by Immunohistochemistry.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195)

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors