abcam

Product datasheet

Anti-KDM1/LSD1 antibody [EPR6825] - BSA and Azide free ab224270

敲除验证 重组 RabMAb

3 References 14 图像

概述

产品名称	Anti-KDM1/LSD1抗体[EPR6825] - BSA and Azide free
描述	兔单克隆抗体[EPR6825] to KDM1/LSD1 - BSA and Azide free
宿主	Rabbit
经测试应用	**适用于:** ChIP, Flow Cyt (Intra), ChIC/CUT&RUN-seq, ICC/IF, WB, IHC-P, IP
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	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 (intra): HeLa cells. IP: Jurkat cell lysate. ChIP: HCT 116 (Human colorectal carcinoma epithelial cell). ChIC/CUT&RUN-Seq: HeLa cells.
常规说明	ab224270 is the carrier-free version of **ab129195**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C. Do Not Freeze.
存储溶液	pH: 7.2 Constituent: PBS
无载体	**是**
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR6825
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用**ab224270**于以下的**经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ChIP		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 110 kDa (predicted molecular weight: 92 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
IP		Use at an assay dependent concentration.

靶标

功能 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.

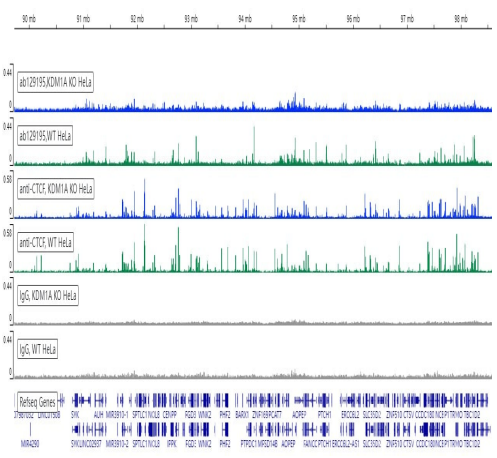
组织特异性 Ubiquitously expressed.

序列相似性 Belongs to the flavin monoamine oxidase family.
Contains 1 SWIRM domain.

结构域 The SWIRM domain may act as an anchor site for a histone tail.

细胞定位 Nucleus.

图片



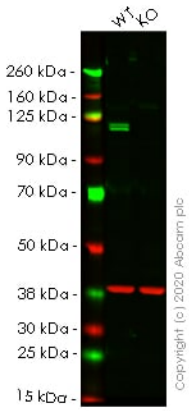
ChIC/CUT&RUN sequencing - Anti-KDM1/LSD1 antibody [EPR6825] - BSA and Azide free (ab224270)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL. 2.5X10^5 of Human wild-type HeLa cell line (**ab255928**) or KDM1A (LSD1) knockout HeLa cell line (**ab265790**) were used along with 5µg of **ab129195** [EPR6825]. Assay Quality Control was conducted using 5µg Anti-CTCF(**ab188408**) on the same cell lines. 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.

Additional screenshots of mapped reads can be downloaded **here**.

The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.

This data was developed using the same antibody clone in a different buffer formulation (**ab129195**).



Western blot - Anti-KDM1/LSD1 antibody [EPR6825] - BSA and Azide free (ab224270)

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.

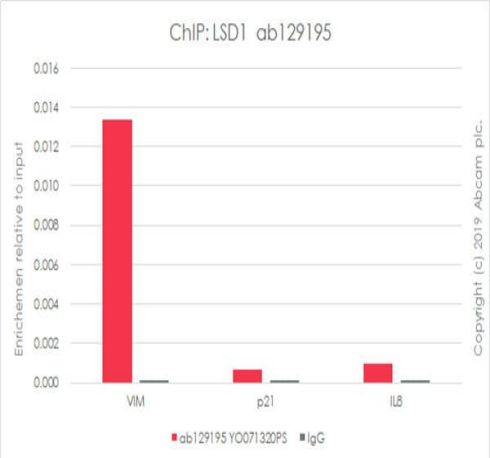
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (**ab129195**).

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.

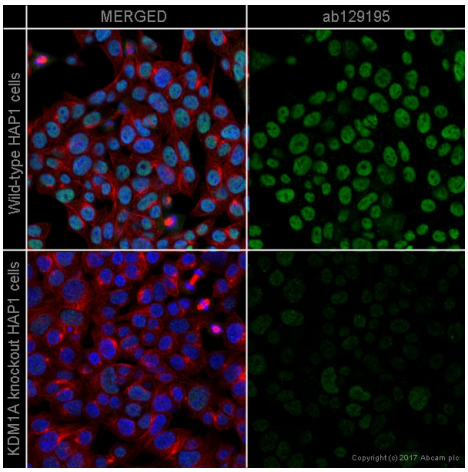
ab129195 was shown to react with KDM1/LSD1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab265790** (knockout cell lysate **ab256965**) was used. Wild-type HeLa and KDM1 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 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.



ChIP - Anti-KDM1/LSD1 antibody [EPR6825] - BSA and Azide free (ab224270)

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).

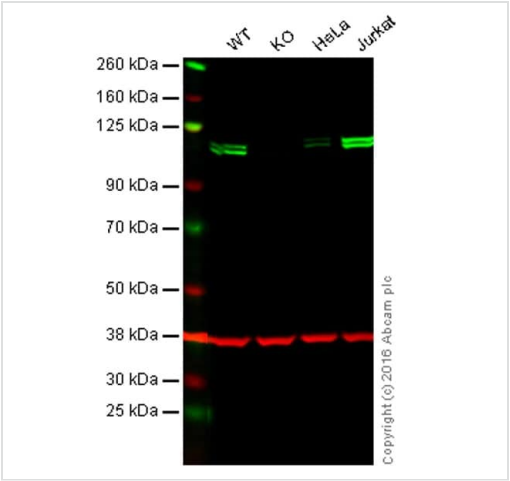
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab129195**).



Immunocytochemistry/ Immunofluorescence - Anti-KDM1 / LSD1 antibody [EPR6825] - BSA and Azide free (ab224270)

This ICC data was generated using the same anti-KDM1/LSD! antibody clone [EPR6825] in a different buffer formulation (cat# **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.



Western blot - Anti-KDM1/LSD1 antibody [EPR6825] - BSA and Azide free (ab224270)

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

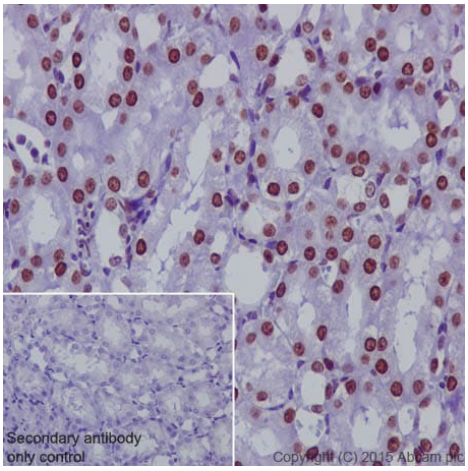
Lysates/proteins at 20 µg per lane.

Predicted band size: 92 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab129195**).

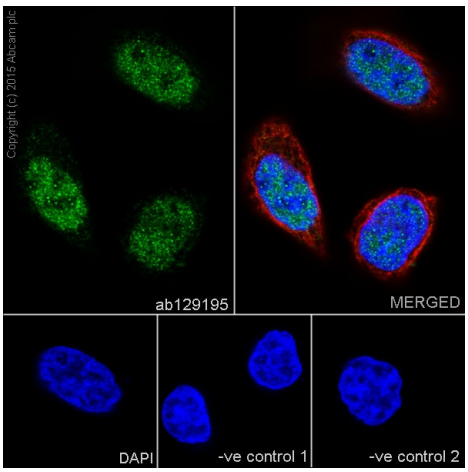
Lanes 1 - 4: Merged signal (red and green). Green - **ab129195** observed at 110 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KDM1 / LSD1 antibody [EPR6825] - BSA and Azide free (ab224270)

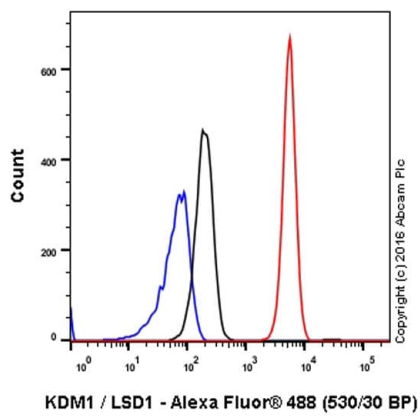
Immunohistochemical staining of paraffin embedded rat kidney with purified **ab129195** at a working dilution of 1/50. The secondary antibody used is **ab97051**, a goat anti-rabbit IgG (H&L) at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab129195**).



Immunocytochemistry/ Immunofluorescence - Anti-KDM1 / LSD1 antibody [EPR6825] - BSA and Azide free (ab224270)

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.

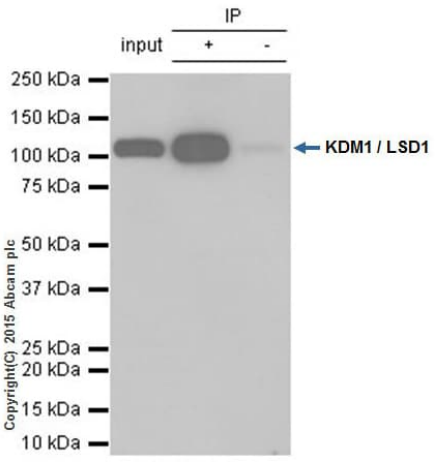
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab129195**).



Flow Cytometry (Intracellular) - Anti-KDM1/LSD1 antibody [EPR6825] - BSA and Azide free (ab224270)

Intracellular 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.

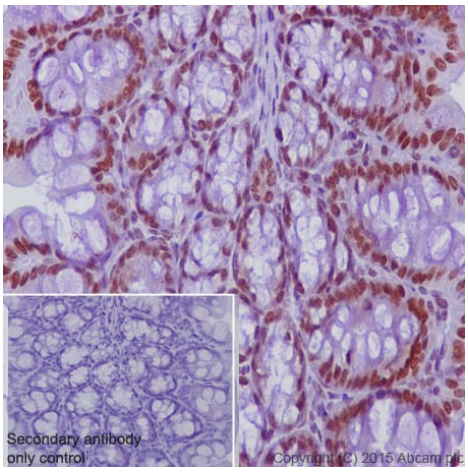
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab129195**).



Immunoprecipitation - Anti-KDM1 / LSD1 antibody [EPR6825] - BSA and Azide free (ab224270)

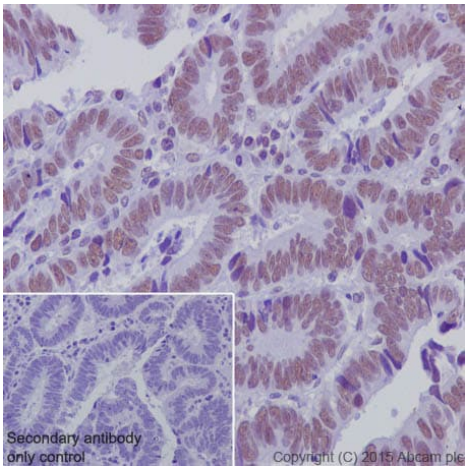
ab129195 (purified) at 1/20 immunoprecipitating KDM1/LSD1 in 10 µg Jurkat cell lysate (Lanes 1 and 2, observed at 110 kDa). Lane 3 - Rabbit monoclonal IgG (**ab172730**). For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10,000 dilution. Blocking buffer and concentration: 5% NFDM/TBST Dilution buffer and concentration: 5% NFDM/TBST

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab129195**).



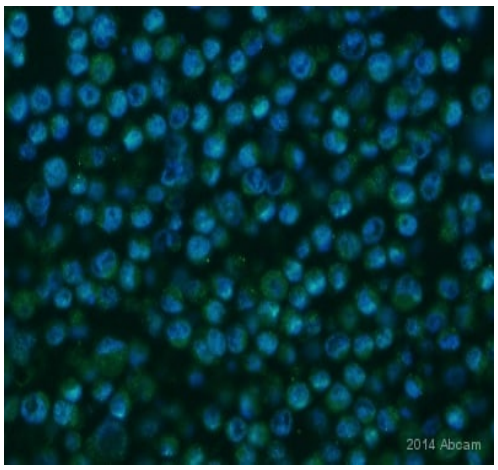
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KDM1 / LSD1 antibody [EPR6825] - BSA and Azide free (ab224270)

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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab129195**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KDM1 / LSD1 antibody [EPR6825] - BSA and Azide free (ab224270)

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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab129195**).

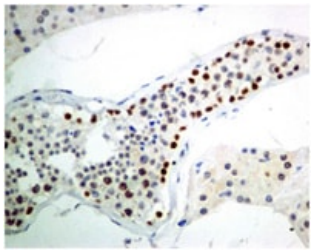


Immunocytochemistry/ Immunofluorescence - Anti-KDM1/LSD1 antibody [EPR6825] - BSA and Azide free (ab224270)

This image is courtesy of an anonymous Abreview.

Unpurified **ab129195** staining KDM1/LSD1 in human 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab129195**).

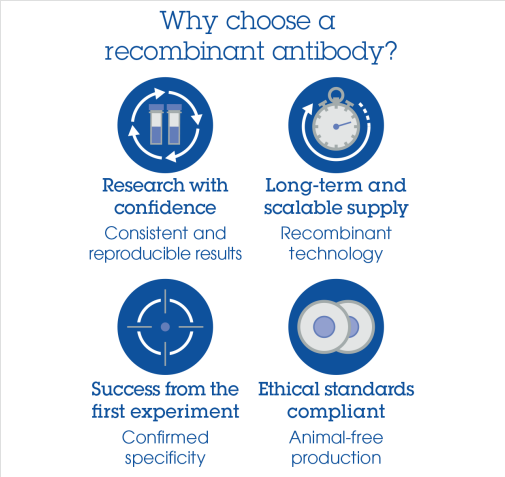


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KDM1 / LSD1 antibody [EPR6825] - BSA and Azide free (ab224270)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab129195**).

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



Anti-KDM1/LSD1 antibody [EPR6825] - BSA and Azide free (ab224270)

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