# abcam

## Product datasheet

# Anti-KDM5A / Jarid1A / RBBP2 antibody [18E8] ab78322

# KO VALIDATED

### 10 References 4 Images

#### Overview

Product name Anti-KDM5A / Jarid1A / RBBP2 antibody [18E8]

**Description** Mouse monoclonal [18E8] to KDM5A / Jarid1A / RBBP2

Host species Mouse

Tested applications Suitable for: Flow Cyt (Intra), ICC/IF, WB

Species reactivity Reacts with: Mouse, Human

**Immunogen** Synthetic peptide corresponding to Human KDM5A/ Jarid1A/ RBBP2 aa 1416-1434.

Sequence:

**PRKQPRKSPLVPRSLEPPV** 

Database link: P29375

Run BLAST with
Run BLAST with

Positive control HeLa siRNA. HeLa RBP2 siRNA. Extracts from MCF7 cells, U2OS cells, NIH3T3 cells and J1

(mouse ES) cells. HeLa cells: Flow Cyt (Intra).

**General notes**Histone demethylase that specifically demethylates 'Lys-4' of histone H3, thereby playing a central

role in histone code.

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

#### **Properties**

Form Liquid

**Storage instructions** Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

Storage buffer pH: 6

Constituents: 50% Glycerol, PBS

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Purity Ion Exchange Chromatography

**Clonality** Monoclonal

Clone number18E8IsotypeIgG2aLight chain typekappa

#### **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab78322 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)		Use 1µg for 10 <sup>6</sup> cells.  ab170191 - Mouse monoclonal lgG2a, is suitable for use as an isotype control with this antibody.
ICC/IF		Use a concentration of 1 - 10 µg/ml.
WB		Use a concentration of 1 µg/ml. Predicted molecular weight: 196 kDa.

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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'. May stimulate transcription mediated by nuclear receptors. May be involved in transcriptional regulation of Hox proteins during cell differentiation. May participate in transcriptional repression

of cytokines such as CXCL12.

**Sequence similarities**Belongs to the JARID1 histone demethylase family.

Contains 1 ARID domain.
Contains 1 JmjC domain.
Contains 1 JmjN domain.

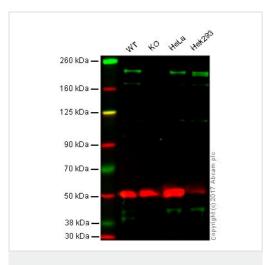
Contains 3 PHD-type zinc fingers.

**Domain** The GSGFP motif is required for the interaction with SUZ12.

Cellular localization Nucleus > nucleolus. Occupies promoters of genes involved in RNA metabolism and

mitochondrial function.

# **Images**



Western blot - Anti-KDM5A / Jarid1A / RBBP2 antibody [18E8] (ab78322)

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

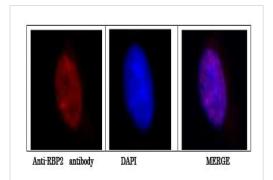
Lane 2: KDM5A knockout HAP1 whole cell lysate (20 µg)

Lane 3: HeLa whole cell lysate (20 µg)

Lane 4: HEK293 whole cell lysate (20 µg)

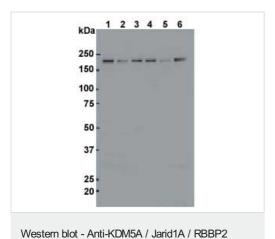
**Lanes 1 - 4:** Merged signal (red and green). Green - ab78322 observed at 240 kDa. Red - loading control, **ab176560**, observed at 50 kDa.

ab78322 was shown to specifically react with KDM5A in wild type HAP1 cells along with additional cross reactive bands. No bands were observed when KDM5A knockout samples were examined. Ab78322 and <a href="mailto:ab176560">ab176560</a> (Rabbit anti alpha Tubulin loading control) were incubated overnight at 4°C at 1 ug/ml and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed (<a href="mailto:ab216773">ab216773</a>) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed (<a href="mailto:ab216776">ab216776</a>) secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-KDM5A / Jarid1A / RBBP2 antibody [18E8] (ab78322)

ab78322 staining KDM5A / Jarid1A / RBBP2 [18E8] in HeLa cell line by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4 % paraformaldehyde, permeabilized with Triton X-100 0.25% in PBS and blocked with 1.5% BSA for 30 minutes. Samples were incubated with primary antibody (1/200 in PBS + 1% BSA) for overnight at 4°C. An Alexa Fluor®488-conjugated Goat anti-mouse IgG polyclonal (1/1000 in PBS + 1% BSA) was used as the secondary antibody and incubated for 1 hour at room temperature. DAPI was used to stain the nuclear DNA.



antibody [18E8] (ab78322)

All lanes : Anti-KDM5A / Jarid1A / RBBP2 antibody [18E8] (ab78322) at 1  $\mu$ g/ml

Lane 1: HeLa control siRNA

Lane 2: HeLa RBBP2 siRNA

Lane 3: Extracts from MCF7 cells

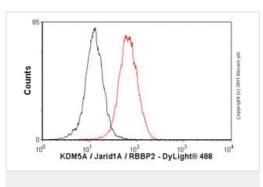
Lane 4: Extracts from U2OS cells

Lane 5 : Extracts from NIH3T3 cells

Lane 6: Extracts from J1 (mouse ES) cells

Developed using the ECL technique.

**Predicted band size:** 196 kDa **Observed band size:** 196 kDa



Flow Cytometry (Intracellular) - Anti-KDM5A / Jarid1A / RBBP2 antibody [18E8] (ab78322) Overlay histogram showing HeLa cells stained with ab78322 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab78322, 1µg/1x10<sup>6</sup> cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2a [ICIGG2A] (ab91361, 1µg/1x10<sup>6</sup> cells) used under the same conditions. Acquisition of >5,000 events was performed.

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