**Product name**
Anti-JMJD6 antibody

**Description**
Rabbit polyclonal to JMJD6

**Host species**
Rabbit

**Tested applications**
Suitable for: ICC/IF, WB

**Species reactivity**
Reacts with: Mouse, Rat

Predicted to work with: Human

**Immunogen**
Synthetic peptide corresponding to Human JMJD6 aa 350 to the C-terminus conjugated to keyhole limpet haemocyanin.

(Peptide available as [ab64574](#))

**Positive control**
WB: THP1 and HEK-293 cell lysates; Rat Liver, Mouse Liver, Mouse Heart tissue lysates.
ICC/IF: HeLa cells.

**Form**
Liquid

**Storage instructions**
Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.

**Storage buffer**
- pH: 7.40
- Preservative: 0.02% Sodium azide
- Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

**Purity**
Immunogen affinity purified

**Clonality**
Polyclonal

**Isotype**
IgG

**Applications**
Dioxygenase that can both act as a histone arginine demethylase and a lysyl-hydroxylase. Acts as a lysyl-hydroxylase that catalyzes 5-hydroxylation on specific lysine residues of target proteins such as U2AF2/U2AF65 and LUC7L2. Acts as a regulator of RNA splicing by mediating 5-hydroxylation of U2AF2/U2AF65, affecting the pre-mRNA splicing activity of U2AF2/U2AF65. In addition to peptidyl-lysine 5-dioxygenase activity, may act as a RNA hydroxylase, as suggested by its ability to bind single strand RNA. Also acts as an arginine demethylase which demethylates histone H3 at ‘Arg-2’ (H3R2me) and histone H4 at ‘Arg-3’ (H4R3me), thereby playing a role in histone code. However, histone arginine demethylation may not constitute the primary activity in vivo. Has no histone lysine demethylase activity. Required for differentiation of multiple organs during embryogenesis. Acts as a key regulator of hematopoietic differentiation: required for angiogenic sprouting by regulating the pre-mRNA splicing activity of U2AF2/U2AF65. Seems to be necessary for the regulation of macrophage cytokine responses.

Tissue specificity
Highly expressed in the heart, skeletal muscle and kidney. Expressed at moderate or low level in brain, placenta, lung, liver, pancreas, spleen, thymus, prostate, testis and ovary. Up-regulated in many patients with chronic pancreatitis. Expressed in nursing thymic epithelial cells.

Sequence similarities
Belongs to the JMJD6 family. Contains 1 JmjC domain.

Domain
The nuclear localization signal motifs are necessary and sufficient to target it into the nucleus.

Cellular localization
Nucleus > nucleoplasm. Mainly found throughout the nucleoplasm outside of regions containing heterochromatic DNA, with some localization in nucleolus. During mitosis, excluded from the nucleus and reappears in the telophase of the cell cycle.

Target

Function
Dioxygenase that can both act as a histone arginine demethylase and a lysyl-hydroxylase. Acts as a lysyl-hydroxylase that catalyzes 5-hydroxylation on specific lysine residues of target proteins such as U2AF2/U2AF65 and LUC7L2. Acts as a regulator of RNA splicing by mediating 5-hydroxylation of U2AF2/U2AF65, affecting the pre-mRNA splicing activity of U2AF2/U2AF65. In addition to peptidyl-lysine 5-dioxygenase activity, may act as a RNA hydroxylase, as suggested by its ability to bind single strand RNA. Also acts as an arginine demethylase which demethylates histone H3 at ‘Arg-2’ (H3R2me) and histone H4 at ‘Arg-3’ (H4R3me), thereby playing a role in histone code. However, histone arginine demethylation may not constitute the primary activity in vivo. Has no histone lysine demethylase activity. Required for differentiation of multiple organs during embryogenesis. Acts as a key regulator of hematopoietic differentiation: required for angiogenic sprouting by regulating the pre-mRNA splicing activity of U2AF2/U2AF65. Seems to be necessary for the regulation of macrophage cytokine responses.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICC/IF</td>
<td>Use a concentration of 1 µg/ml.</td>
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<tr>
<td>WB</td>
<td>Use a concentration of 1 µg/ml. Detects a band of approximately 50 kDa (predicted molecular weight: 46 kDa).</td>
<td></td>
</tr>
</tbody>
</table>

Target

Function

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Images
**All lanes**: Anti-JMJD6 antibody (ab64575) at 1 µg/ml

**Lane 1**: THP1 cell lysate

**Lane 2**: Wild-type HEK293 cell lysate

**Lane 3**: JMJD6 knockout HEK-293 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size**: 46 kDa

**Observed band size**: 50 kDa

*why is the actual band size different from the predicted?*

**Lanes 1 - 3**: Merged signal (red and green). Green - ab64575 observed at 50 kDa. Red - loading control, ab8245 (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab64575 was shown to react with JMJD6 in wild-type HEK-293 cells in western blot. Loss of signal was observed when JMJD6 knockout cell lysate (ab257490) was used. Wild-type and JMJD6 knockout HEK-293 cell lysates were subjected to SDS-PAGE. Membranes were blocked in non-mammalian (TBS-based) blocking solution before incubation with ab64575 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at 1 µg/ml and a 1 in 20000 dilution respectively. Blots were incubated 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 in 20000 dilution for 1 hour at room temperature before imaging.
**Immunocytochemistry/ Immunofluorescence - Anti-JMJD6 antibody (ab64575)**

ICC/IF image of ab64575 stained HeLa cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab64575, 1µg/ml) overnight at +4°C. The secondary antibody (green) was ab96899, DyLight® 488 goat anti-rabbit IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM. This antibody also gave a positive result in 100% methanol fixed (5 min) Hek293, HepG2 and MCF7 cells at 1µg/ml, and in 4% formaldehyde fixed (10 min) Hek293, HepG2 and MCF7 cells at 1µg/ml.

**All lanes**: Anti-JMJD6 antibody (ab64575) at 1 µg/ml

- **Lane 1**: Liver (Rat) Tissue Lysate
- **Lane 2**: Liver (Mouse) Tissue Lysate
- **Lane 3**: Heart (Mouse) Tissue Lysate

Lysates/proteins at 10 µg per lane.

**Secondary**

All lanes: Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Performed under reducing conditions.

**Predicted band size**: 46 kDa

**Observed band size**: 50 kDa

*why is the actual band size different from the predicted?*

**Additional bands at**: 26 kDa, 30 kDa, 43 kDa (possible isoform).

We are unsure as to the identity of these extra bands.

**Exposure time**: 16 minutes

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