Product datasheet

Anti-KDM5C / Jarid1C / SMCX antibody ab34718

Overview

Product name: Anti-KDM5C / Jarid1C / SMCX antibody
Description: Rabbit polyclonal to KDM5C / Jarid1C / SMCX
Host species: Rabbit
Tested applications: Suitable for: ChIP, IHC-FoFr, WB, ICC/IF
Species reactivity: Reacts with: Human

Immunogen: Synthetic peptide conjugated to KLH derived from within residues 1500 to the C-terminus of Human Jarid1C / SMCX. Read Abcam's proprietary immunogen policy (Peptide available as ab35501.)

Positive control: This antibody gave a positive signal in HEK 293 (Human embryonic kidney cell line), and Y79 (Human retinoblastoma cell line) Whole Cell Lysates. This antibody gave a positive signal in the following Methanol fixed cell lines: HeLa, Hek293, HepG2, and MCF-7. This antibody gave a positive signal in the following Formaldehyde fixed cell lines: HepG2, and MCF-7.

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer: Preservative: 0.02% Sodium Azide
Constituents: 1% BSA, PBS, pH 7.4
Purity: Immunogen affinity purified
Clonality: Polyclonal
Isotype: IgG

Applications

Our Abpromise guarantee covers the use of ab34718 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.
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'. Participates in transcriptional repression of neuronal genes by recruiting histone deacetylases and REST at neuron-restrictive silencer elements.

Tissue specificity
Expressed in all tissues examined. Highest levels found in brain and skeletal muscle.

Involvement in disease
Defects in KDM5C are the cause of mental retardation syndromic X-linked JARID1C-related (MRXSJ) [MIM:300534]. MRXSJ is characterized by significantly sub-average general intellectual functioning associated with impairments in adaptive behavior and manifested during the developmental period. MRXSJ patients manifest mental retardation associated with variable features such as slowly progressive spastic paraplegia, seizures, facial dysmorphism.

Sequence similarities
Belongs to the JARID1 histone demethylase family.
Contains 1 ARID domain.
Contains 1 JmjC domain.
Contains 1 JmjN domain.
Contains 2 PHD-type zinc fingers.

Domain
The first PHD-type zinc finger domain recognizes and binds H3-K9Me3.
Both the JmjC domain and the JmjN domain are required for enzymatic activity.

Cellular localization
Nucleus.

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<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tr>
<td>ChIP</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 19136938</td>
</tr>
<tr>
<td>IHC-FoFr</td>
<td>★★★★☆</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>WB</td>
<td>★★★★★</td>
<td>1/250. Detects a band of approximately 180 kDa (predicted molecular weight: 176 kDa). Abcam recommends using milk as the blocking agent.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>★★★★★</td>
<td>Use a concentration of 1 µg/ml.</td>
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Images
Lane 1: Wild-type HAP1 whole cell lysate (20 µg)
Lane 2: KDM5C knockout HAP1 whole cell lysate (20 µg)
Lane 3: HEK293 whole cell lysate (20 µg)
Lane 4: U2OS whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab34718 observed at 175 kDa. Red - loading control, ab18058, observed at 120 kDa.

ab34718 was shown to specifically recognize KDM5C in wild-type HAP1 cells as signal was lost at the expected MW in KDM5C knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and KDM5C knockout samples were subjected to SDS-PAGE. Ab34718 and ab18058 (Mouse anti Vinculin loading control) were incubated overnight at 4°C at 1/250 dilution and 1/20,000 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 1/20,000 dilution for 1 hour at room temperature before imaging.

PFA-fixed, 0.5% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells stained for KDM5C / Jarid1C / SMCX (green) using ab34718 at 1/200 dilution in ICC/IF. Counter-stained with DAPI in order to highlight the nucleus (red). Please refer to abreview for further experimental details.
ab34718 stained in Hela cells. Cells were fixed with 100% methanol (5min) at room temperature and incubated with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% triton for 1h at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab34718 at 5µg/ml and ab7291 (Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control) at 1/1000 dilution overnight at +4°C. The secondary antibodies were ab150120 (pseudo-colored red) and ab150081 (colored green) used at 1 µg/ml for 1 hour at room temperature. DAPI was used to stain the cell nuclei (colored blue) at a concentration of 1.43 µM for 1hour at room temperature.

**All lanes** : Anti-KDM5C / Jarid1C / SMCX antibody (ab34718) at 1 µg/ml

**Lane 1** : HEK293 (Human) Whole Cell Lysate

**Lane 2** : Y79 (Human retinoblastoma cell line) Whole Cell Lysate

Lysates/proteins at 20 µg per lane.

**Secondary**

**All lanes** : Goat Anti-Rabbit IgG H&L (HRP) preadsorbed at 1/50000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size**: 176 kDa

**Observed band size**: 180 kDa

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

**Additional bands at**: 45 kDa, 58 kDa. We are unsure as to the identity of these extra bands.
**Exposure time:** 20 minutes

This blot was produced using a 3-8% Tris Acetate gel under the TA buffer system. The gel was run at 150V for 60 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% Milk before being incubated with ab34718 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution ab133406.

Anti-KDM5C / Jarid1C / SMCX antibody (ab34718) at 1/250 dilution + HEK293 whole cell lysate (ab7902) at 20 µg

**Secondary**
IRDye 680 Conjugated Goat Anti-Rabbit IgG (H+L) at 1/10000 dilution

Performed under reducing conditions.

**Predicted band size:** 176 kDa
**Observed band size:** 176 kDa

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**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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