

Anti-KDM4A Antibody [PSH03-67]

HA722026



Product Type:	Recombinant Rabbit monoclonal IgG1, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 121 kDa
Clone number:	PSH03-67

Description: Lysine-specific demethylase 4A is an enzyme that in humans is encoded by the KDM4A gene. This gene is a member of the Jumonji domain 2 (JMJD2) family and encodes a protein with a JmjN domain, a JmjC domain, a JD2H domain, two TUDOR domains, and two PHD-type zinc fingers. This nuclear protein belongs to the alpha-ketoglutarate-dependent hydroxylase superfamily. It functions as a trimethylation-specific demethylase, converting specific trimethylated histone on histone H3 lysine 9 and 36 residues to the dimethylated form and lysine 9 dimethylated residues to monomethyl, and as a transcriptional repressor. Alterations in this gene have been found associated with chromosomal instability that leads to cancer.

Immunogen: Recombinant protein within human KDM4A aa 351-650 / 1,064.

Positive control: A549 cell lysate, HEK-293 cell lysate, HeLa cell lysate, HepG2 cell lysate, NIH/3T3 cell lysate, NCCIT, NIH/3T3, human colon tissue, mouse brain tissue, rat colon tissue.

Subcellular location: Nucleus.

Database links: SwissProt: O75164 Human | Q8BW72 Mouse
Entrez Gene: 313539 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:100
IHC-P	1:500
FC	1:1,000

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4°C. Store at +4°C short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20°C long term.

Purity: Protein A affinity purified.

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Orders:0086-571-88062880

Technical:0086-571-89986345

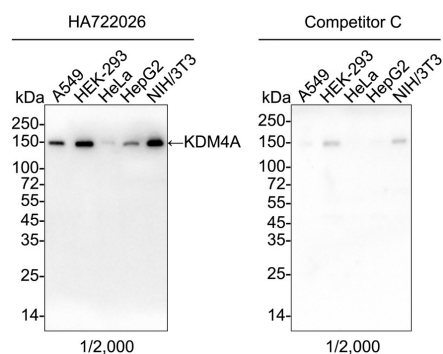
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Images

Fig1: Western blot analysis of KDM4A on different lysates with Rabbit anti-KDM4A antibody (HA722026) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution.

Lane 1: A549 cell lysate
Lane 2: HEK-293 cell lysate
Lane 3: HeLa cell lysate
Lane 4: HepG2 cell lysate
Lane 5: NIH/3T3 cell lysate



Lysates/proteins at 20 µg/Lane.

Predicted band size: 121 kDa
Observed band size: 150 kDa

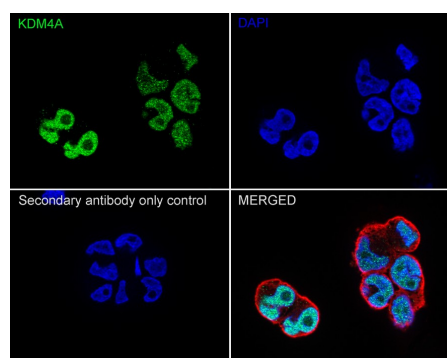
Exposure time: Lane 1-5 (left): 2 minutes 16 seconds; ECL: K1801;

Exposure time: Lane 1-5 (right): 3 minutes; ECL: K1802;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA722026) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution were used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig2: Immunocytochemistry analysis of NCCIT cells labeling KDM4A with Rabbit anti-KDM4A antibody (HA722026) at 1/100 dilution.



Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-KDM4A antibody (HA722026) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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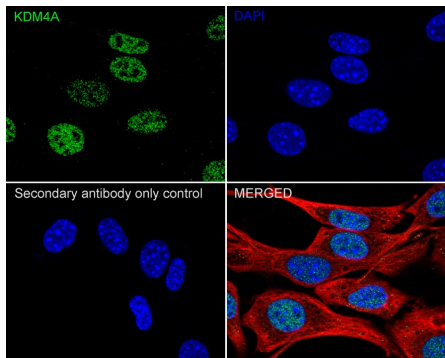
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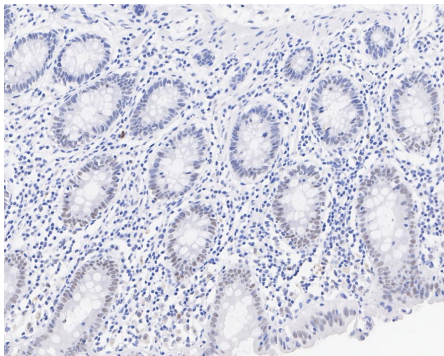
Fig3: Immunocytochemistry analysis of NIH/3T3 cells labeling KDM4A with Rabbit anti-KDM4A antibody (HA722026) at 1/100 dilution.



Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-KDM4A antibody (HA722026) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

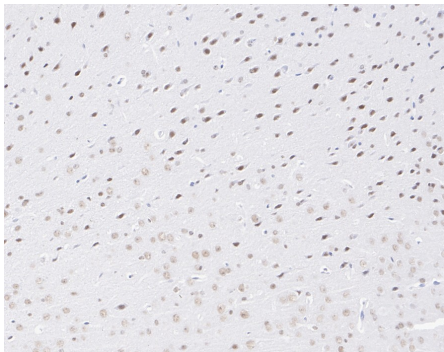
Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Fig4: Immunohistochemical analysis of paraffin-embedded human colon tissue with Rabbit anti-KDM4A antibody (HA722026) at 1/1,000 dilution.



The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA722026) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Fig5: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-KDM4A antibody (HA722026) at 1/1,000 dilution.



The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA722026) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

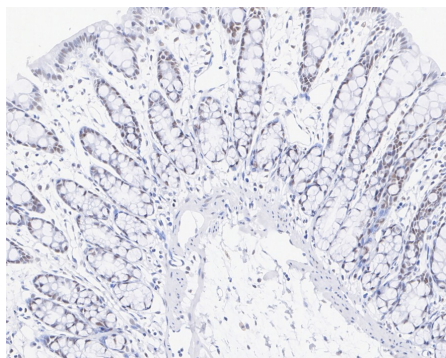


Fig6: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-KDM4A antibody (HA722026) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA722026) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

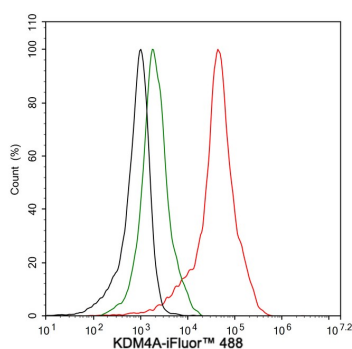


Fig7: Flow cytometric analysis of NCCIT cells labeling KDM4A.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722026, 1µg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Zhang W et al. Targeting KDM4A epigenetically activates tumor-cell-intrinsic immunity by inducing DNA replication stress. *Mol Cell*. 2021 May
2. Young NL et al. Mechanistic insights into KDM4A driven genomic instability. *Biochem Soc Trans*. 2021 Feb

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