Anti-JMJD6 Antibody [PSH15-03]

HA723703

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Monkey
Applications:	WB, IF-Cell, IHC-P, FC, IP, ChIP
Molecular Wt:	Predicted band size: 46 kDa
Clone number:	PSH15-03

Description: Bifunctional arginine demethylase and lysyl-hydroxylase JMJD6 is an enzyme that in humans is encoded by the JMJD6 gene. This gene encodes a nuclear protein with a JmjC domain. JmjC domain-containing proteins belong to the alpha-ketoglutarate-dependent hydroxylase superfamily. They are predicted to function as protein hydroxylases or histone demethylases. This protein was first identified as a putative phosphatidylserine receptor involved in phagocytosis of apoptotic cells. Subsequent studies suggest that the protein may cross-react with a monoclonal antibody that recognizes the phosphatidylserine receptor and does not directly function in the clearance of apoptotic cells. Multiple transcript variants encoding different isoforms have been found for this gene. On a physiological level JMJD6 has a role in angiogenesis, the process of vessel formation, whereas further roles of JMJD6 in pathophysiological processes were implicated, such as mammary tumorigenesis. Here, elevated JMJD6 level were found in breast cancer associated with aggressiveness and metastasis in mice.

Immunogen: Recombinant protein within human JMJD6 aa 1-360.

Positive control: 293T cell lysate, PANC-1 cell lysate, HeLa cell lysate, SH-SY5Y cell lysate, COS-1 cell lysate, Neuro-2a cell lysate, PC-12 cell lysate, PC-12, human colon cancer tissue, human gastric carcinoma tissue, human lung carcinoma tissue.

Subcellular location: Nucleus, nucleoplasm, nucleolus, Cytoplasm.

Database links: SwissProt: Q6NYC1 Human | Q9ERI5 Mouse | Q6AYK2 Rat

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:100
IHC-P	1:8,000
FC	1:1,000
IP	1-2μg/sample
ChIP	Use 5 μg for 25 μg of chromatin.

Storage Buffer: 1*PBS (pH7.4), 0.1% BSA, 40% Glycerol, 0.2% Proclean 950.

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

Purity: Protein A affinity purified.

Images

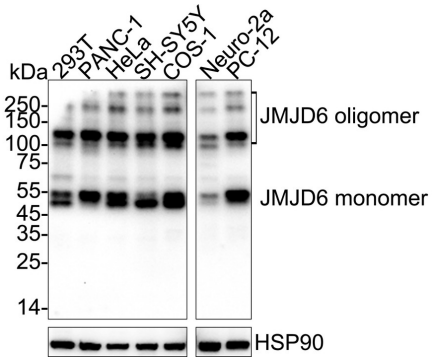

Fig1: Western blot analysis of JMJD6 on different lysates with Rabbit anti-JMJD6 antibody (HA723703) at 1/5,000 dilution.

Lane 1: 293T cell lysate
Lane 2: PANC-1 cell lysate
Lane 3: HeLa cell lysate
Lane 4: SH-SY5Y cell lysate
Lane 5: COS-1 cell lysate
Lane 6: Neuro-2a cell lysate
Lane 7: PC-12 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 46 kDa
Observed band size: 50/110-260 kDa

Exposure time: 2 minutes; ECL: K1801;

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 (HA723703) at 1/5,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

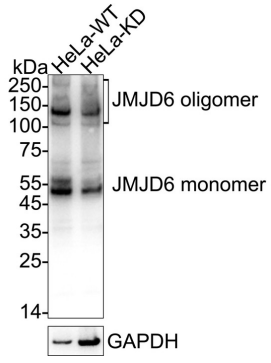

Fig2: Western blot analysis of JMJD6 on different lysates with Rabbit anti-JMJD6 antibody (HA723703) at 1/5,000 dilution.

Lane 1: HeLa-parental cell lysate
Lane 2: HeLa-JMJD6 KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 46 kDa
Observed band size: 50/110-260 kDa

Exposure time: 59 seconds; ECL: K1801;

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 (HA723703) at 1/5,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

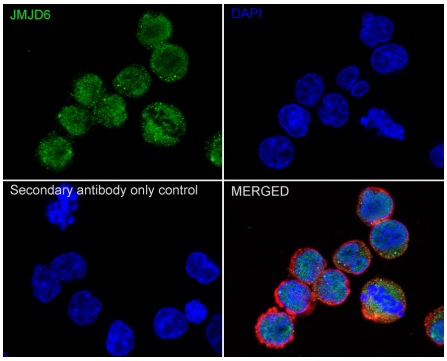

Fig3: Immunocytochemistry analysis of PC-12 cells labeling JMJD6 with Rabbit anti-JMJD6 antibody (HA723703) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-JMJD6 antibody (HA723703) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. 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 (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

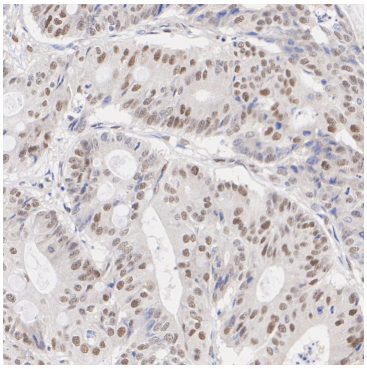

Fig4: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue with Rabbit anti-JMJD6 antibody (HA723703) at 1/8,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA723703) at 1/8,000 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.

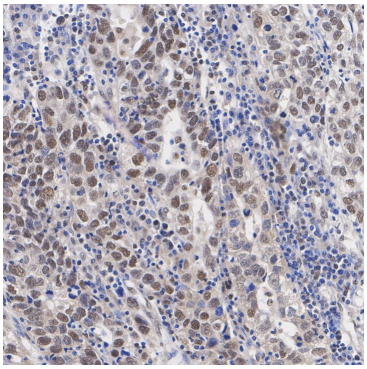

Fig5: Immunohistochemical analysis of paraffin-embedded human gastric carcinoma tissue with Rabbit anti-JMJD6 antibody (HA723703) at 1/8,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA723703) at 1/8,000 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.

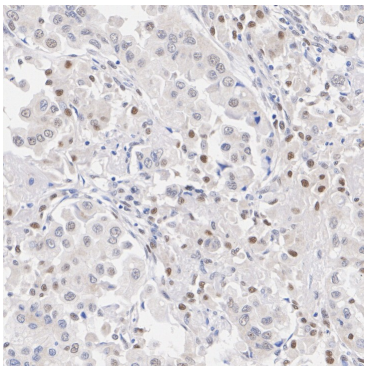

Fig6: Immunohistochemical analysis of paraffin-embedded human lung carcinoma tissue with Rabbit anti-JMJD6 antibody (HA723703) at 1/8,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA723703) at 1/8,000 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.

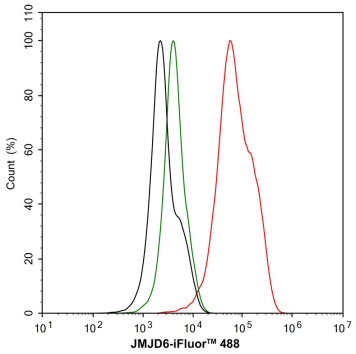

Fig7: Flow cytometric analysis of PC-12 cells labeling JMJD6.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA723703, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ 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℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

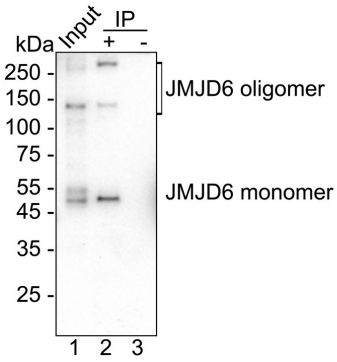

Fig8: JMJD6 was immunoprecipitated from 0.2 mg HeLa cell lysate with HA723703 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA723703 at 1/5,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: HA723703 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of HA723703 in HeLa cell lysate

Blocking/Dilution buffer: primary antibody dilution (K1803)
Exposure time: 3 minutes; ECL: K1801

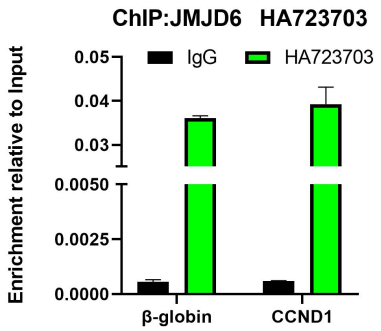

Fig9: Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells with JMJD6 (HA723703) or Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

Background References

1. Chen S et al. JMJD6 in tumor-associated macrophage regulates macrophage polarization and cancer progression via STAT3/IL-10 axis. Oncogene. 2023 Sep
2. Zhou J et al. An oncogenic JMJD6-DGAT1 axis tunes the epigenetic regulation of lipid droplet formation in clear cell renal cell carcinoma. Mol Cell. 2022 Aug