Anti-KDM2B Antibody [PSH05-38] - BSA and Azide free

HA750997

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC, IF-Tissue, IP, ChIP
Molecular Wt:	Predicted band size: 150 kDa
Clone number:	PSH05-38

Description: This gene encodes a member of the F-box protein family which is characterized by an approximately 40 amino acid motif, the F-box. The F-box proteins constitute one of the four subunits of ubiquitin protein ligase complex called SCFs (SKP1-cullin-F-box), which function in phosphorylation-dependent ubiquitination. The F-box proteins are divided into 3 classes: Fbws containing WD-40 domains, Fbls containing leucine-rich repeats, and Fbxs containing either different protein-protein interaction modules or no recognizable motifs. The protein encoded by this gene belongs to the Fbls class. Multiple alternatively spliced transcript variants have been found for this gene, but the full-length nature of some variants has not been determined. As part of the ncPRC1.1 complex, KDM2B was found to be rapidly and transiently recruited to sites of DNA damage in a PARP1- and TIMELESS-dependent manner to promote mono-ubiquitylation of histone H2A on K119 with concomitant local decrease of H2A levels and an increase of H2A.Z. These events promote transcriptional repression at DNA lesions, double strand break signaling, and homologous recombination repair. The activity of the ncPRC1.1 complex at DNA lesions was necessary for the proper recruitment of the two canonical PRC1 complexes (cPRC1.2 and cPRC1.4), defined by their PCGF subunits, MEL18 and BMI1 respectively. Therefore, recruitment of the ncPRC1.1 complex represents an early and critical regulatory step in homologous recombination repair.

Immunogen:	Recombinant protein within mouse KDM2B aa 681-920 / 1,309.
Positive control:	HeLa cell lysate, HepG2 cell lysate, 293T cell lysate, NIH/3T3 cell lysate, C2C12 cell lysate, PC-12 cell lysate, C6 cell lysate, human brain tissue lysate, mouse brain tissue lysate, mouse colon tissue lysate, rat brain tissue lysate, HeLa, NIH/3T3, human brain tissue, human colon tissue, mouse brain tissue, mouse skin tissue, rat brain tissue.
Subcellular location:	Nucleus, nucleolus, Chromosome.
Database links:	SwissProt: Q8NHM5 Human | Q6P1G2 Mouse Entrez Gene: 304495 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:2,000
IHC-P	1:200-1:1,000
FC	1:1,000
IF-Tissue	1:50-1:200
IP	1-2μg/sample
ChIP	Use 5 μg for 25 μg of chromatin.

Storage Buffer:	1*PBS (pH7.4).
Storage Instruction:	Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.
Purity:	Protein A affinity purified.

Images

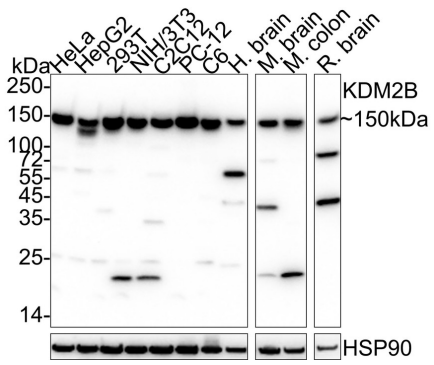

Fig1: Western blot analysis of KDM2B on different lysates with Rabbit anti-KDM2B antibody (HA750997) at 1/2,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: HepG2 cell lysate (20 µg/Lane)
Lane 3: 293T cell lysate (20 µg/Lane)
Lane 4: NIH/3T3 cell lysate (20 µg/Lane)
Lane 5: C2C12 cell lysate (20 µg/Lane)
Lane 6: PC-12 cell lysate (20 µg/Lane)
Lane 7: C6 cell lysate (20 µg/Lane)
Lane 8: Human brain tissue lysate (40 µg/Lane)
Lane 9: Mouse brain tissue lysate (40 µg/Lane)
Lane 10: Mouse colon tissue lysate (40 µg/Lane)
Lane 11: Rat brain tissue lysate (40 µg/Lane)

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

Exposure time: 25 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 (HA750997) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

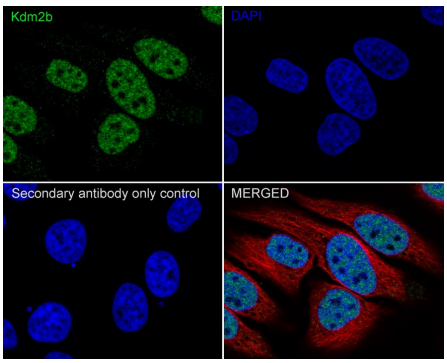

Fig2: Immunocytochemistry analysis of HeLa cells labeling KDM2B with Rabbit anti-KDM2B antibody (HA750997) at 1/2,000 dilution.

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-KDM2B antibody (HA750997) at 1/2,000 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 (M1305-2, 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.

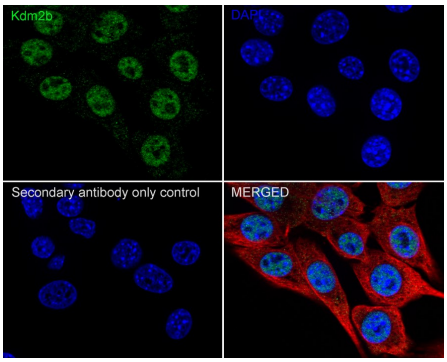

Fig3: Immunocytochemistry analysis of NIH/3T3 cells labeling KDM2B with Rabbit anti-KDM2B antibody (HA750997) at 1/2,000 dilution.

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-KDM2B antibody (HA750997) at 1/2,000 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 (M1305-2, 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.

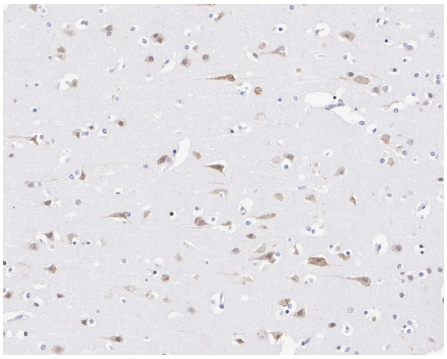

Fig4: Immunohistochemical analysis of paraffin-embedded human brain tissue with Rabbit anti-KDM2B antibody (HA750997) at 1/200 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (HA750997) at 1/200 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.

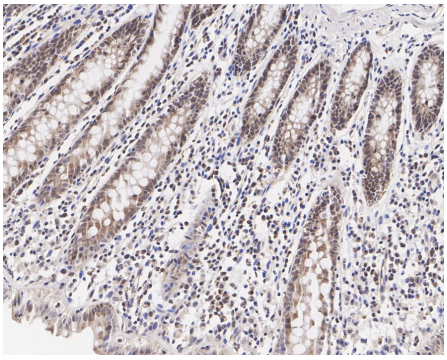

Fig5: Immunohistochemical analysis of paraffin-embedded human colon tissue with Rabbit anti-KDM2B antibody (HA750997) at 1/200 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (HA750997) at 1/200 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.

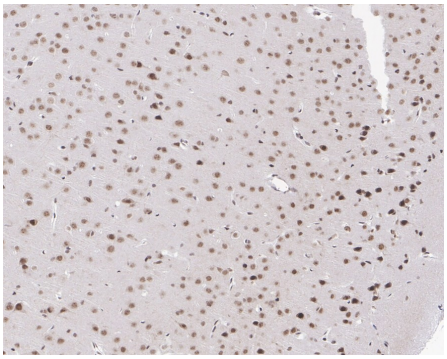

Fig6: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-KDM2B antibody (HA750997) at 1/1,000 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (HA750997) 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.

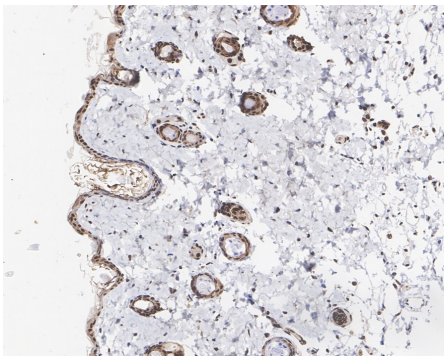

Fig7: Immunohistochemical analysis of paraffin-embedded mouse skin tissue with Rabbit anti-KDM2B antibody (HA750997) at 1/200 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (HA750997) at 1/200 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.

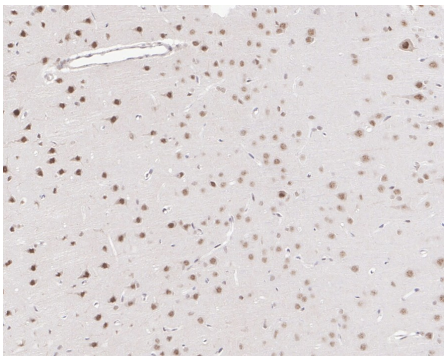

Fig8: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-KDM2B antibody (HA750997) at 1/1,000 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (HA750997) 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.

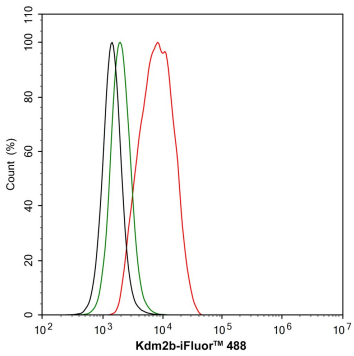

Fig9: Flow cytometric analysis of HeLa cells labeling KDM2B.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750997, 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).

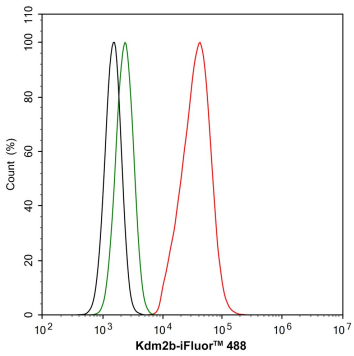

Fig10: Flow cytometric analysis of NIH/3T3 cells labeling KDM2B.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750997, 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).

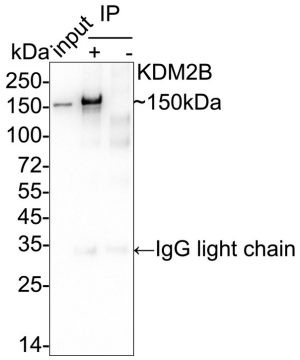

Fig11: KDM2B was immunoprecipitated from 0.2 mg MDA-MB-231 cell lysate with HA750997 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA750997 at 1/2,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: MDA-MB-231 cell lysate (input)
Lane 2: HA750997 IP in MDA-MB-231 cell lysate
Lane 3: Rabbit IgG instead of HA750997 in MDA-MB-231 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 10 seconds; ECL: K1802

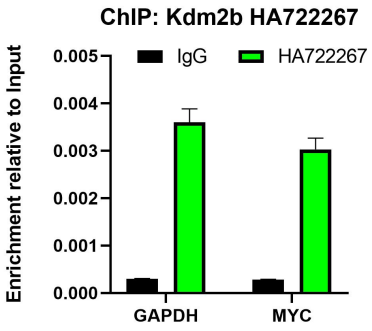

Fig12: Chromatin immunoprecipitations were performed with cross-linked chromatin from MDA-MB-231 cells with KDM2B (HA750997) or Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

Background References

1. van Jaarsveld RH et al. Delineation of a KDM2B-related neurodevelopmental disorder and its associated DNA methylation signature. Genet Med. 2023 Jan
2. Zhang B et al. KDM2B regulates hippocampal morphogenesis by transcriptionally silencing Wnt signaling in neural progenitors. Nat Commun. 2023 Oct