Anti-UHRF1 Antibody [PSH10-63] - BSA and Azide free

HA751366

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Monkey
Applications:	WB, IF-Cell, FC, IP, ChIP
Molecular Wt:	Predicted band size: 90 kDa
Clone number:	PSH10-63

Description: Ubiquitin-like, containing PHD and RING finger domains, 1, also known as UHRF1, is a protein which in humans is encoded by the UHRF1 gene. This gene encodes a member of a subfamily of RING-finger type E3 ubiquitin ligases. The protein binds to hemi-methylated DNA during S-phase and recruits the main DNA methyltransferase protein, DNMT1, to regulate chromatin structure and gene expression. Its expression peaks at late G1 phase and continues during G2 and M phases of the cell cycle. It plays a major role in the G1/S transition, and functions in the p53-dependent DNA damage checkpoint. Multiple transcript variants encoding different isoforms have been found for this gene.

Immunogen: Recombinant protein within human UHRF1 aa 1-350.

Positive control: HCT 116 cell lysate, HL-60 cell lysate, HepG2 cell lysate, Jurkat cell lysate, F9 cell lysate, NIH/3T3 cell lysate, COS-1 cell lysate, HCT 116, HL-60.

Subcellular location: Nucleus.

Database links: SwissProt: Q96T88 Human | Q8VDF2 Mouse | Q7TPK1 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:10,000
FC	1:1,000
IP	1-2μg/sample
ChIP	Use 0.5~2 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

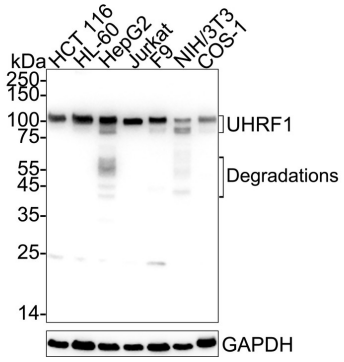

Fig1: Western blot analysis of UHRF1 on different lysates with Rabbit anti-UHRF1 antibody (HA751366) at 1/2,000 dilution.

Lane 1: HCT 116 cell lysate
Lane 2: HL-60 cell lysate
Lane 3: HepG2 cell lysate
Lane 4: Jurkat cell lysate
Lane 5: F9 cell lysate
Lane 6: NIH/3T3 cell lysate
Lane 7: COS-1 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 90 kDa
Observed band size: 90-100 kDa

Exposure time: 10 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 (HA751366) 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.

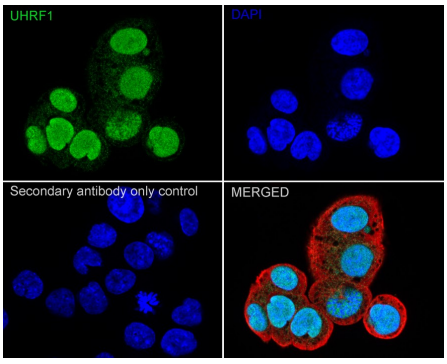

Fig2: Immunocytochemistry analysis of HCT 116 cells labeling UHRF1 with Rabbit anti-UHRF1 antibody (HA751366) at 1/10,000 dilution.

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-UHRF1 antibody (HA751366) at 1/10,000 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 (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.

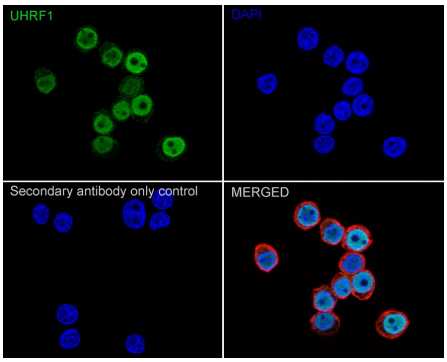

Fig3: Immunocytochemistry analysis of HL-60 cells labeling UHRF1 with Rabbit anti-UHRF1 antibody (HA751366) at 1/10,000 dilution.

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-UHRF1 antibody (HA751366) at 1/10,000 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 (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.

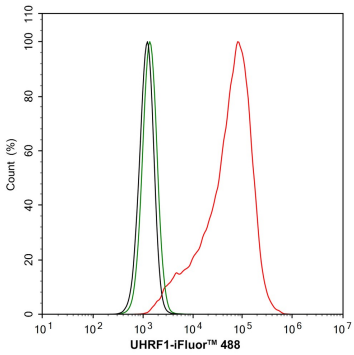

Fig4: Flow cytometric analysis of HCT 116 cells labeling UHRF1.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA751366, 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).

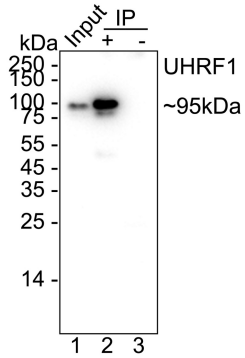

Fig5: UHRF1 was immunoprecipitated from 0.2 mg HL-60 cell lysate with HA751366 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA751366 at 1/1,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HL-60 cell lysate (input)
Lane 2: HA751366 IP in HL-60 cell lysate
Lane 3: Rabbit IgG instead of HA751366 in HL-60 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 9 seconds; ECL: K1801

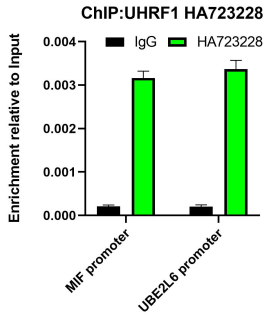

Fig6: Chromatin immunoprecipitations were performed with cross-linked chromatin from HCT 116 cells with UHRF1 (HA751366) or Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

Background References

1. Xu X et al. Nuclear UHRF1 is a gate-keeper of cellular AMPK activity and function. Cell Res. 2022 Jan
2. Kostyrko K et al. UHRF1 is a mediator of KRAS driven oncogenesis in lung adenocarcinoma. Nat Commun. 2023 Jul