Anti-Histone H3 (di methyl K27) Antibody [PSH05-88]

HA722486

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, ChIP
Molecular Wt:	Predicted band size: 15 kDa
Clone number:	PSH05-88

Description: The nucleosome, made up of four core histone proteins (H2A, H2B, H3, and H4), is the primary building block of chromatin. Originally thought to function as a static scaffold for DNA packaging, histones have now been shown to be dynamic proteins, undergoing multiple types of post-translational modifications, including acetylation, phosphorylation, methylation, and ubiquitination. Histone methylation is a major determinant for the formation of active and inactive regions of the genome and is crucial for the proper programming of the genome during development. Arginine methylation of histones H3 (Arg2, 17, 26) and H4 (Arg3) promotes transcriptional activation and is mediated by a family of protein arginine methyltransferases (PRMTs), including the co-activators PRMT1 and CARM1 (PRMT4). In contrast, a more diverse set of histone lysine methyltransferases has been identified, all but one of which contain a conserved catalytic SET domain originally identified in the Drosophila Su(var)3-9, Enhancer of zeste, and Trithorax proteins. Lysine methylation occurs primarily on histones H3 (Lys4, 9, 27, 36, 79) and H4 (Lys20) and has been implicated in both transcriptional activation and silencing. Methylation of these lysine residues coordinates the recruitment of chromatin modifying enzymes containing methyl-lysine binding modules such as chromodomains (HP1, PRC1), PHD fingers (BPTF, ING2), tudor domains (53BP1), and WD-40 domains (WDR5). The discovery of histone demethylases, such as PADI4, LSD1, JMJD1, JMJD2, and JHDM1, has shown that methylation is a reversible epigenetic marker.

Immunogen: Synthetic peptide corresponding to the amino terminus of histone H3 in which Lys27 is di-methylated.

Positive control: HeLa cell lysate, SH-SY5Y cell lysate, HCT 116 cell lysate, C6 cell lysate, MCF7 cell lysate, RAW264.7 cell lysate, C2C12 cell lysate, NIH/3T3 cell lysate, MCF7, NIH/3T3, C6.

Subcellular location: Nucleus, Chromosome.

Database links: SwissProt: P68431 Human | P84243 Human | Q16695 Human | Q6NXT2 Human | Q71DI3 Human | P68433 Mouse | P84228 Mouse | Q6LED0 Rat

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:100
ChIP	Use 0.5~2 μg for 25 μg of chromatin.

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

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

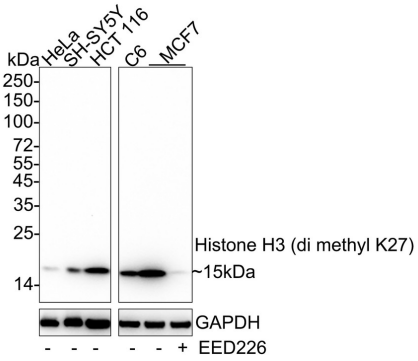

Fig1: Western blot analysis of Histone H3 (di methyl K27) on different lysates with Rabbit anti-Histone H3 (di methyl K27) antibody (HA722486) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: SH-SY5Y cell lysate
Lane 3: HCT 116 cell lysate
Lane 4: C6 cell lysate
Lane 5: MCF7 cell lysate
Lane 6: MCF7 treated with 10μM EED226 for 72 hours cell lysate

Lysates/proteins at 20 μg/Lane.

Predicted band size: 15 kDa
Observed band size: 15 kDa

Exposure time: 30 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 (HA722486) at 1/1,000 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.

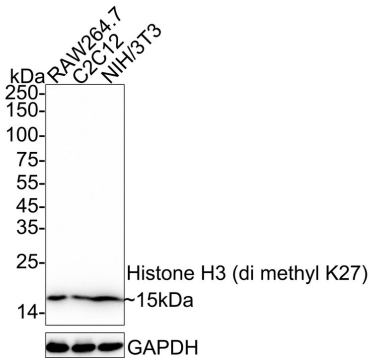

Fig2: Western blot analysis of Histone H3 (di methyl K27) on different lysates with Rabbit anti-Histone H3 (di methyl K27) antibody (HA722486) at 1/1,000 dilution.

Lane 1: RAW264.7 cell lysate (20 μg/Lane)
Lane 2: C2C12 cell lysate (20 μg/Lane)
Lane 3: NIH/3T3 cell lysate (20 μg/Lane)

Predicted band size: 15 kDa
Observed band size: 15 kDa

Exposure time: 12 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 (HA722486) at 1/1,000 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.

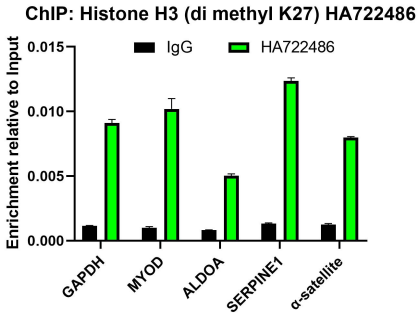

Fig3: Chromatin immunoprecipitations were performed with cross-linked chromatin from MCF7 cells and either Histone H3 (di methyl K27) (HA722486) or Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

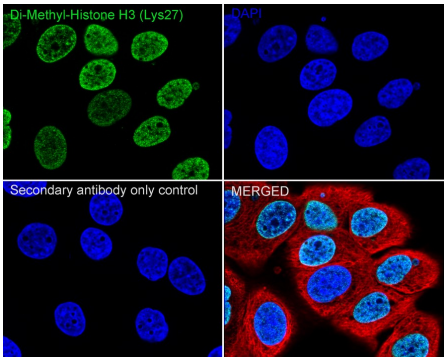

Fig4: Immunocytochemistry analysis of MCF7 cells labeling Histone H3 (di methyl K27) with Rabbit anti-Histone H3 (di methyl K27) antibody (HA722486) at 1/100 dilution.

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-Histone H3 (di methyl K27) antibody (HA722486) 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 (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.

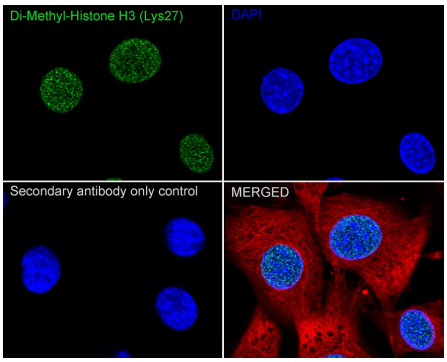

Fig5: Immunocytochemistry analysis of NIH/3T3 cells labeling Histone H3 (di methyl K27) with Rabbit anti-Histone H3 (di methyl K27) antibody (HA722486) at 1/100 dilution.

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-Histone H3 (di methyl K27) antibody (HA722486) 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 (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.

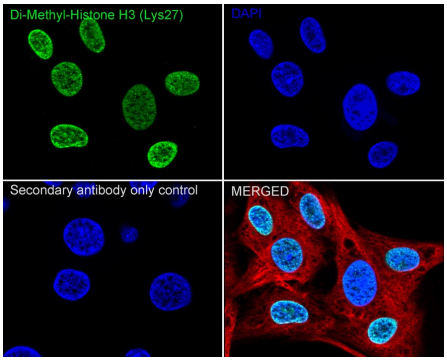

Fig6: Immunocytochemistry analysis of C6 cells labeling Histone H3 (di methyl K27) with Rabbit anti-Histone H3 (di methyl K27) antibody (HA722486) at 1/100 dilution.

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-Histone H3 (di methyl K27) antibody (HA722486) 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 (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.

Background References

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2. Kubicek, S. et al. (2006) Ernst Schering Res Found Workshop, 1-27.
3. Lin, W. and Dent, S.Y. (2006) Curr Opin Genet Dev 16, 137-42.
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