Anti-Histone H3 (acetyl K27) Antibody [A6D6] HA600048

Product Type:	Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC, ChIP
Molecular Wt:	Predicted band size: 15 kDa
Clone number:	A6D6

Description: Eukaryotic histones are basic and water soluble nuclear proteins that form hetero-octameric nucleosome particles by wrapping 146 base pairs of DNA in a left-handed super-helical turn sequentially to form chromosomal fibers. Two molecules of each of the four core histones (H2A, H2B, H3 and H4) form the octamer, which is comprised of two H2A-H2B dimers and two H3-H4 dimers, forming two nearly symmetrical halves by tertiary structure. Histones are subject to posttranslational modification by enzymes primarily on their N-terminal tails, but also in their globular domains. Human and mouse Histone H4 are subject to methylation at Lys 20, a modification that may be necessary for select DNA transactions or chromatin state transitions.

Immunogen: Synthetic peptide within human Histone H3 aa 20-40 (acetyl K27).

Positive control: NIH/3T3 treated with 1μM TSA for 18 hours cell lysate, PC-12 cell lysate, 293 cell lysate, MCF-7 cell lysate, NIH/3T3 cell lysate, HeLa cell lysates, HeLa treated with Trichostatin A whole cell lysates, HUVEC, HeLa, human tonsil tissue.

Subcellular location: Nucleus, Chromosome.

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

Recommended Dilutions:

WB	1:500-1:2,000
IF-Cell	1:100
IHC-P	1:600
FC	1:500
ChIP	Use 0.5~2 μg for 25 μg of chromatin.

Storage Buffer: PBS (pH 7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

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 G affinity purified.

Images

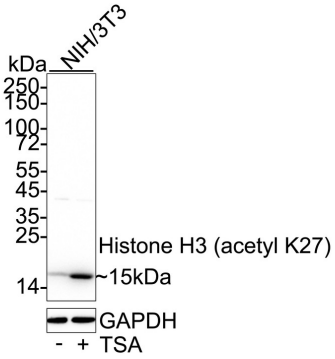

Fig1: Western blot analysis of Histone H3 (acetyl K27) on different lysates with Mouse anti-Histone H3 (acetyl K27) antibody (HA600048) at 1/1,000 dilution.

Lane 1: NIH/3T3 cell lysate
Lane 2: NIH/3T3 treated with 1μM TSA for 18 hours cell lysate

Lysates/proteins at 20 μg/Lane.

Predicted band size: 15 kDa
Observed band size: 15 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 (HA600048) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

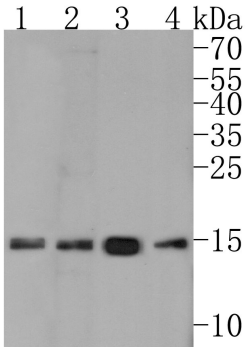

Fig2: Western blot analysis of Histone H3 (acetyl K27) on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (HA600048, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:20,000 dilution was used for 1 hour at room temperature.
Positive control:
Lane 1: PC-12 cell lysate
Lane 2: 293 cell lysate
Lane 3: MCF-7 cell lysate
Lane 4: NIH/3T3 cell lysate

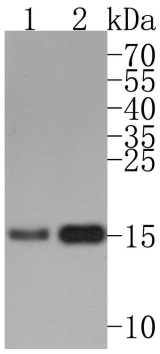

Fig3: Western blot analysis of Histone H3 (acetyl K27) on Hela cell lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (HA600048, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:20,000 dilution was used for 1 hour at room temperature.
Lane 1: Untreated Hela whole cell lysates
Lane 2: Hela treated with Trichostatin A whole cell lysates

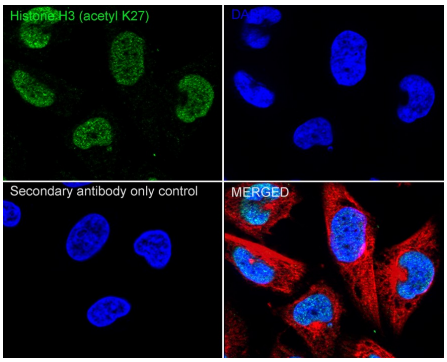

Fig4: Immunocytochemistry analysis of HUVEC cells labeling Histone H3 (acetyl K27) with Mouse anti-Histone H3 (acetyl K27) antibody (HA600048) at 1/100 dilution.

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 Mouse anti-Histone H3 (acetyl K27) antibody (HA600048) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) 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 (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) was used as the secondary antibody at 1/1,000 dilution.

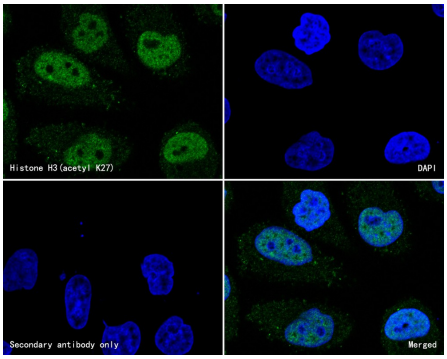

Fig5: ICC staining of Histone H3 (acetyl K27) in HeLa cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (HA600048, 1/100) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Mouse IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

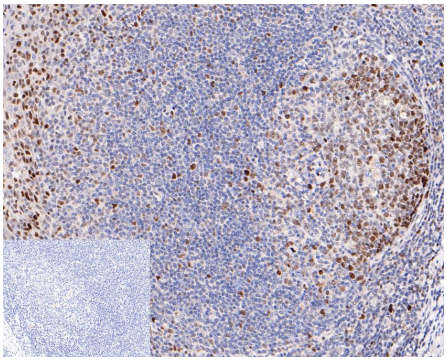

Fig6: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-Histone H3 (acetyl K27) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA600048, 1/600) for 30 minutes 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.

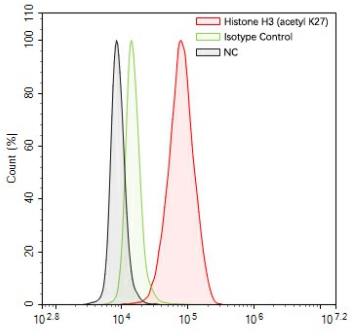

Fig7: Flow cytometric analysis of Histone H3 (acetyl K27) was done on HeLa cells. The cells were fixed, permeabilized and stained with the primary antibody (HA600048, 1ug/ml) (red) compared with Mouse IgG, monoclonal - Isotype Control (green). After incubation of the primary antibody at +4℃ for 1 hour, the cells were stained with a Alexa Fluor®488 conjugate-Goat anti-Mouse IgG Secondary antibody at 1/1000 dilution for 30 minutes at +4℃ (dark incubation).Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

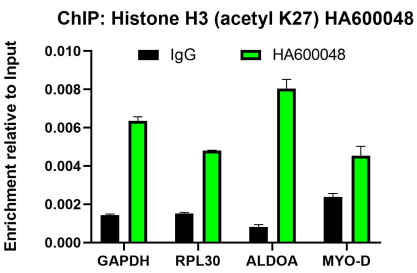

Fig8: Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells treated with 500ng/mL TSA for 4 hours with Histone H3 (acetyl K27) (HA600048) or Normal Mouse IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The 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. Ray-Gallet D. et. al. The Histone H3 Family and Its Deposition Pathways. Adv Exp Med Biol. 2021
2. Francis NJ. et. al. Inheritance of Histone (H3/H4): A Binary Choice? Trends Biochem Sci. 2021 Jan