Anti-Histone H3 (acetyl K9) Antibody [PSH04-47] - ChIP Grade

HA722132

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, IF-Tissue, FC, ChIP, Dot Blot, IP
Molecular Wt:	Predicted band size: 15 kDa
Clone number:	PSH04-47

Description: In eukaryotes, DNA is wrapped around histone octamers to form the basic unit of chromatin structure. The octamer is composed of histones H2A, H2B, H3 and H4, and it associates with approximately 200 base pairs of DNA to form the nucleosome. The association of DNA with histones results in dense packing of chromatin, which restricts proteins involved in gene transcription from binding to DNA. p300 preferentially acetylates Histone H3 at lysines 14 and 18 and Histone H4 at lysines 5 and 8. PCAF in its native form, primarily acetylates Histone H3 at lysine 14 to a monoacetylated form, and less efficiently acetylates Histone H4 at lysine 8. Histone H4 may also be acetylated at lysines 12 and 16, and the involvement of acetylated H4 with Histones H2A, H2B and H3 suggests that acetylated histones may be involved in dynamic chromatin remodeling.

Immunogen: Synthetic peptide within human Histone H3 (acetyl K9) aa 1-50.

Positive control: HeLa cell lysate, HeLa treated with 500ng/mL TSA for 4 hours cell lysate, NIH/3T3 cell lysate, NIH/3T3 treated with 400nM TSA for 18 hours cell lysate, C6 cell lysate, C6 treated with 1μM TSA for 18 hours cell lysate, C6, HeLa cells treated with 500ng/mL TSA for 4 hours, human stomach tissue, mouse colon tissue, rat colon tissue, HeLa.

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	1:5,000-1:15,000
IHC-P	1:2,000
IF-Tissue	1:200
FC	1:1,000
ChIP	Use 0.5~2 μg for 25 μg of chromatin.
Dot Blot	1:1,000
IP	1-2μg/sample

Storage Buffer: PBS (pH7.4), 0.1% 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 A affinity purified.

Images

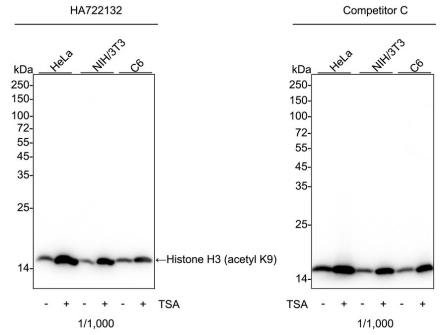

Fig1: Western blot analysis of Histone H3 (acetyl K9) on different lysates with Rabbit anti-Histone H3 (acetyl K9) antibody (HA722132) at 1/1,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 500ng/mL TSA for 4 hours cell lysate
Lane 3: NIH/3T3 cell lysate
Lane 4: NIH/3T3 treated with 400nM TSA for 18 hours cell lysate
Lane 5: C6 cell lysate
Lane 6: C6 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: 6 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 (HA722132) at 1/1,000 dilution and competitor's antibody at 1/1,000 dilution were 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.

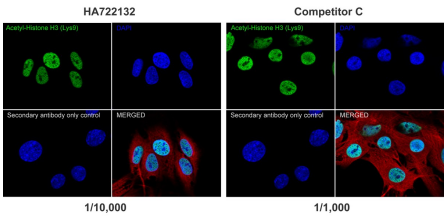

Fig2: Immunocytochemistry analysis of C6 cells labeling Histone H3 (acetyl K9) with Rabbit anti-Histone H3 (acetyl K9) antibody (HA722132) at 1/10,000 dilution and competitor's antibody at 1/1,000 dilution.

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-Histone H3 (acetyl K9) antibody (HA722132) at 1/10,000 dilution and competitor's antibody at 1/1,000 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.

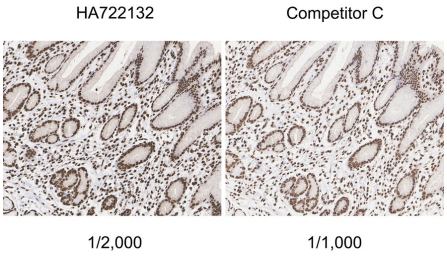

Fig3: Immunohistochemical analysis of paraffin-embedded human stomach tissue with Rabbit anti-Histone H3 (acetyl K9) antibody (HA722132) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) 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 (HA722132) at 1/2,000 dilution and competitor's antibody 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.

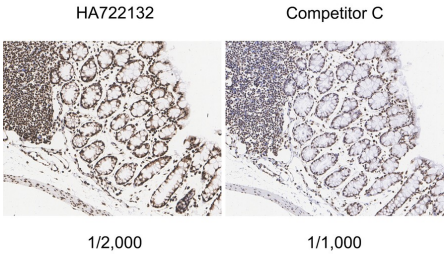

Fig4: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-Histone H3 (acetyl K9) antibody (HA722132) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) 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 (HA722132) at 1/2,000 dilution and competitor's antibody 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.

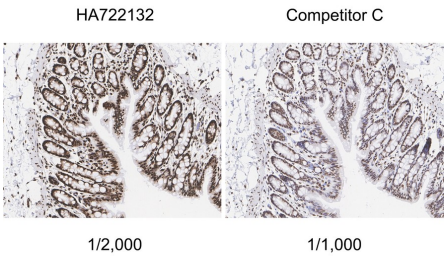

Fig5: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-Histone H3 (acetyl K9) antibody (HA722132) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) 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 (HA722132) at 1/2,000 dilution and competitor's antibody 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.

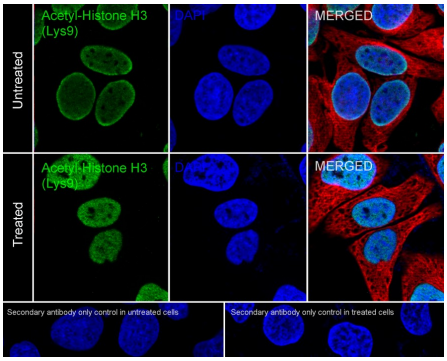

Fig6: Immunocytochemistry analysis of HeLa cells treated with or without 500ng/mL TSA for 4 hours labeling Histone H3 (acetyl K9) with Rabbit anti-Histone H3 (acetyl K9) antibody (HA722132) at 1/15,000 dilution.

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-Histone H3 (acetyl K9) antibody (HA722132) at 1/15,000 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.

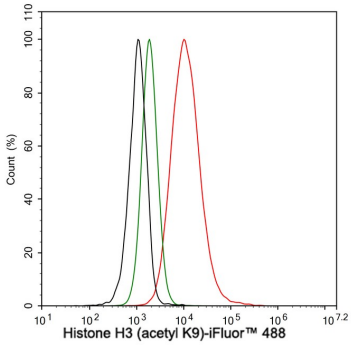

Fig7: Flow cytometric analysis of HeLa cells labeling Histone H3 (acetyl K9).

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722132, 1μg/mL) (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).

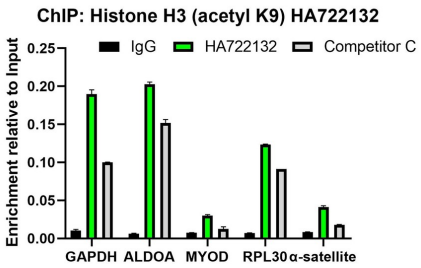

Fig8: Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells with Histone H3 (acetyl K9) (HA722132) / Competitor's antibody / Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

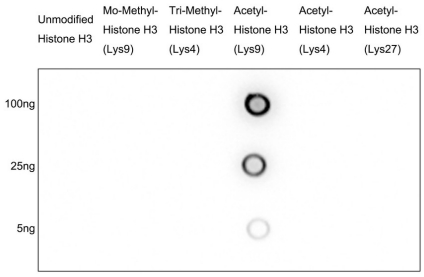

Fig9: Dot blot analysis of Histone H3 (acetyl K9) on different proteins with Rabbit anti-Histone H3 (acetyl K9) antibody (HA722132) at 1/1,000 dilution. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution for 1 hour at room temperature.

Lane 1: Unmodified Histone H3 (negative)
Lane 2: Mono-Methyl-Histone H3 (Lys9) (negative)
Lane 3: Tri-Methyl-Histone H3 (Lys9) (negative)
Lane 4: Acetyl-Histone H3 (Lys9) (positive)
Lane 5: Acetyl-Histone H3 (Lys4) (negative)
Lane 6: Acetyl-Histone H3 (Lys27) (negative)

Proteins loading: 100ng, 25ng, 5ng;

Blocking and dilution buffer: 5% NFDM/TBST;

Exposure time: 30 seconds; ECL: K1801.

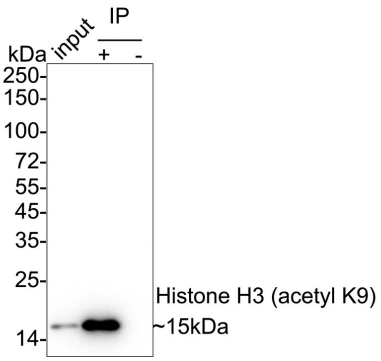

Fig10: Histone H3 (acetyl K9) was immunoprecipitated from 0.2 mg HeLa cell lysate with HA722132 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using HA722132 at 1/2,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: HA722132 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of HA722132 in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 18 seconds; ECL: K1801

Background References

1. Wani S et al. Human SCP4 is a chromatin-associated CTD phosphatase and exhibits the dynamic translocation during erythroid differentiation. J Biochem 160:111-20 (2016).
2. Ni JZ et al. A transgenerational role of the germline nuclear RNAi pathway in repressing heat stress-induced transcriptional activation in C. elegans. Epigenetics Chromatin 9:3 (2016).