# Anti-Histone H3 (acetyl K27) Antibody [PSH13-93] HA723586

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell
Molecular Wt:	Predicted band size: 15 kDa
Clone number:	PSH13-93
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:	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 1 $\mu$ M TSA for 18 hours cell lysate, mouse colon tissue, mouse skin tissue, rat skin tissue, HeLa treated with 500ng/mL TSA for 4 hours, NIH/3T3 treated with 500ng/mL TSA for 4 hours, C6 treated with 500ng/mL TSA for 4 hours.
Subcellular location:	Chromosome, Nucleosome core, Nucleus.
Database links:	SwissProt: P68431 Human   P84243 Human   Q16695 Human   Q71DI3 Human   Q6NXT2 Human
Recommended Dilutions: WB IHC-P IF-Cell	1:5,000 1:200 1:200
Storage Buffer:	PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Store at +4 $^\circ\!\!\mathbb{C}$ after thawing. Aliquot store at -20 $^\circ\!\!\mathbb{C}$ . Avoid repeated freeze / thaw cycles.
Purity:	Protein A affinity purified.

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Orders:0086-571-88062880

Technical:0086-571-89986345

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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

#### Images



**Fig1:** Western blot analysis of Histone H3 (acetyl K27) on different lysates with Rabbit anti-Histone H3 (acetyl K27) antibody (HA723586) at 1/5,000 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  $1\mu M$  TSA for 18 hours cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 15 kDa Observed band size: 15 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 (HA723586) at 1/5,000 dilution was used in primary antibody dilution (K1803) at  $4^{\circ}$ C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.



**Fig2:** Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-Histone H3 (acetyl K27) antibody (HA723586) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA723586) 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.

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**Fig3:** Immunohistochemical analysis of paraffin-embedded mouse skin tissue with Rabbit anti-Histone H3 (acetyl K27) antibody (HA723586) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA723586) 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.

**Fig4:** Immunohistochemical analysis of paraffin-embedded rat skin tissue with Rabbit anti-Histone H3 (acetyl K27) antibody (HA723586) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA723586) 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:** Immunocytochemistry analysis of HeLa cells untreated / treated with 500ng/mL TSA for 4 hours labeling Histone H3 (acetyl K27) with Rabbit anti-Histone H3 (acetyl K27) antibody (HA723586) at 1/200 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-Histone H3 (acetyl K27) antibody (HA723586) at 1/200 dilution in 1% BSA in PBST overnight at 4 °C. 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  $+4^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluor 1594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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**Fig6:** Immunocytochemistry analysis of NIH/3T3 cells untreated / treated with 500ng/mL TSA for 4 hours labeling Histone H3 (acetyl K27) with Rabbit anti-Histone H3 (acetyl K27) antibody (HA723586) at 1/200 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-Histone H3 (acetyl K27) antibody (HA723586) at 1/200 dilution in 1% BSA in PBST overnight at 4  $^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluor <sup>TM</sup> 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 $^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluor <sup>TM</sup> 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.



**Fig7:** Immunocytochemistry analysis of C6 cells untreated / treated with 500ng/mL TSA for 4 hours labeling Histone H3 (acetyl K27) with Rabbit anti-Histone H3 (acetyl K27) antibody (HA723586) at 1/200 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-Histone H3 (acetyl K27) antibody (HA723586) at 1/200 dilution in 1% BSA in PBST overnight at 4 °C. 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°C. Goat Anti-Mouse IgG H&L (iFluor  $^{\text{TM}}$  594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

#### **Background References**

1. Wilson J.P. et. al. Proteomic analysis of fatty-acylated proteins in mammalian cells with chemical reporters reveals Sacylation of histone H3 variants. Mol. Cell. Proteomics 10:M110.001198-M110.001198(2011).

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