Anti-Histone H4 (acetyl K16) Antibody [JB21-44] ET7107-89

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, FC, IF-Cell, IF-Tissue, ChIP
Molecular Wt:	Predicted band size: 11 kDa
Clone number:	JB21-44

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 fiber. Two molecules of each of the four core histones (H2A, H2B, H3, and H4) form the octamer; formed of two H2A-H2B dimers and two H3-H4 dimers, forming two nearly symmetrical halves by tertiary structure. Over 80% of nucleosomes contain the linker Histone H1, derived from an intronless gene, that interacts with linker DNA between nucleosomes and mediates compaction into higher order chromatin. Histones are subject to posttranslational modification by enzymes primarily on their N-terminal tails, but also in their globular domains. Such modifications include methylation, citrullination, acetylation, phosphorylation, sumoylation, ubiquitination and ADP-ribosylation.

Immunogen: Recombinant protein within Human Histone H4 (acetyl K16) aa 1-50 / 103.

Positive control: HeLa cell lysate, HeLa treated with 1μM TSA for 18 hours cell lysate, C6 cell lysate, C6 treated with 1μM TSA for 18 hours cell lysate, NIH/3T3 cell lysate, NIH/3T3 treated with 400nM TSA for 18 hours cell lysate, HeLa, human colon tissue, mouse colon tissue.

Subcellular location: Chromosome, Nucleosome core, Nucleus.

Database links: SwissProt: P62805 Human | P62806 Mouse | P62804 Rat

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:200-1:1,000
FC	1:1,000
ChIP	Use 0.5~2 μg for 25 μg of chromatin.

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

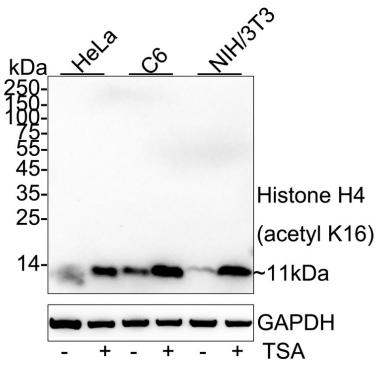

Fig1: Western blot analysis of Histone H4 (acetyl K16) on different lysates with Rabbit anti-Histone H4 (acetyl K16) antibody (ET7107-89) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 1μM TSA for 18 hours cell lysate
Lane 3: C6 cell lysate
Lane 4: C6 treated with 1μM TSA for 18 hours cell lysate
Lane 5: NIH/3T3 cell lysate
Lane 6: NIH/3T3 treated with 400nM TSA for 18 hours cell lysate

Lysates/proteins at 10 μg/Lane.

Predicted band size: 11 kDa
Observed band size: 11 kDa

Exposure time: 3 minutes; 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 (ET7107-89) 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.

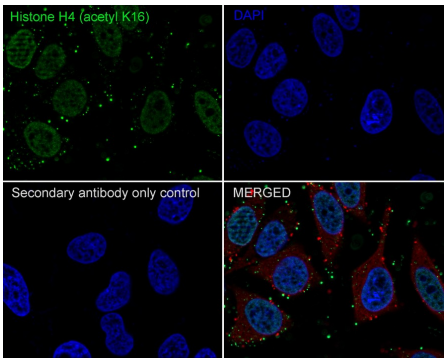

Fig2: Immunocytochemistry analysis of HeLa cells labeling Histone H4 (acetyl K16) with Rabbit anti-Histone H4 (acetyl K16) antibody (ET7107-89) at 1/100 dilution.

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 Rabbit anti-Histone H4 (acetyl K16) antibody (ET7107-89) 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 (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.

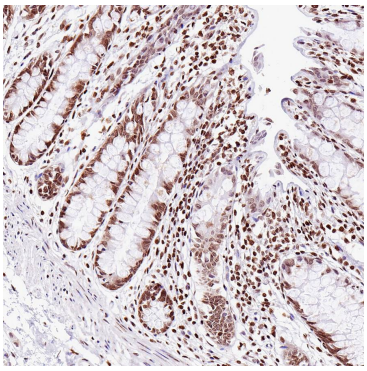

Fig3: Immunohistochemical analysis of paraffin-embedded human colon tissue with Rabbit anti-Histone H4 (acetyl K16) antibody (ET7107-89) at 1/200 dilution.

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 (ET7107-89) 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.

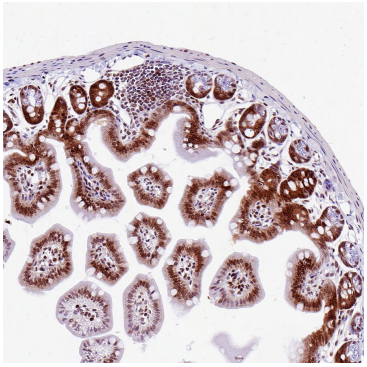

Fig4: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-Histone H4 (acetyl K16) antibody (ET7107-89) at 1/1,000 dilution.

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 (ET7107-89) 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.

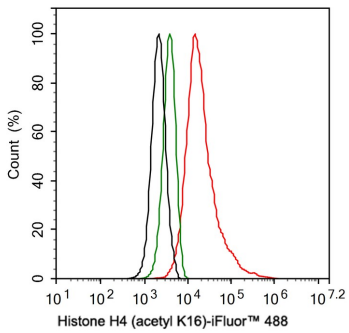

Fig5: Flow cytometric analysis of HeLa cells labeling Histone H4 (acetyl K16).

Cells were fixed and permeabilized. Then stained with the primary antibody (ET7107-89, 1ug/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).

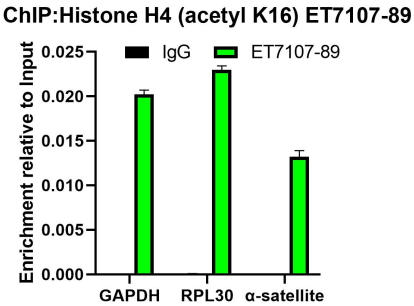

Fig6: Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells with Histone H4 (acetyl K16) (ET7107-89) or Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

Background References

1. Kurata M et al. Characterization of t(3;6)(q27;p21) breakpoints in B-cell non-Hodgkin's lymphoma and construction of the histone H4/BCL6 fusion gene, leading to altered expression of Bcl-6. Cancer Res 62:6224-6230 (2002).
2. Pesavento J J et al. Certain and progressive methylation of histone H4 at lysine 20 during the cell cycle. Mol Cell Biol 28:468-486 (2008).