Anti-Histone H4 (acetyl K12) Antibody [PSH25-67] - BSA and Azide free

HA751986

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, IP, ChIP
Molecular Wt:	Predicted band size: 11 kDa
Clone number:	PSH25-67

Description: Core component of nucleosome. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA accessibility is regulated via a complex set of post-translational modifications of histones, also called histone code, and nucleosome remodeling. H4K8ac is part of 17 modifications of a group of active promoters. H4K8ac is found more often in active promoters and transcribed regions than other marks. H4K8ac is modified by a different group of enzymes than other H4 lysines.

Immunogen: Synthetic peptide within Human Histone H4 aa 1-50 (acetyl K12).

Positive control: HeLa (Human cervical adenocarcinoma cell) cell lysate, HeLa treated with 1μM TSA for 24 hours cell lysate, NIH/3T3 (Mouse fibroblast) cell lysate, NIH/3T3 treated with 400nM TSA for 18 hours cell lysate, C6 (Rat glioma cell) cell lysate, C6 treated with 1μM TSA for 18 hours cell lysate.

Subcellular location: Nucleus. Chromosome.

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

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:2,000
IHC-P	1:500-1:1,000
IP	1-2μg/sample
ChIP	Use 0.5~2 μg for 25 μg of chromatin.

Storage Buffer: PBS (pH7.4).

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

Purity: Protein A affinity purified.

Images

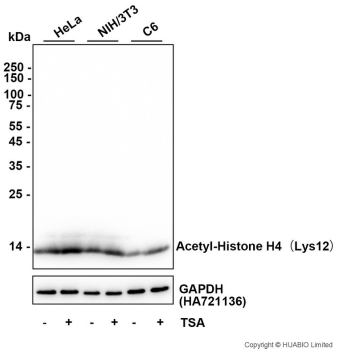

Fig1: Western blot analysis of Histone H4 (acetyl K12) on different lysates with Rabbit anti-Histone H4 (acetyl K12) antibody (HA751986) at 1/50,000 dilution.

Lane 1: HeLa (Human cervical adenocarcinoma cell) cell lysate
Lane 2: HeLa treated with 1μM TSA for 24 hours cell lysate
Lane 3: NIH/3T3 (Mouse fibroblast) cell lysate
Lane 4: NIH/3T3 treated with 400nM TSA for 18 hours cell lysate
Lane 5: C6 (Rat glioma cell) cell lysate
Lane 6: C6 treated with 1μM TSA for 18 hours cell lysate

Lysates/proteins at 10 μg/Lane.
Exposure time: 2 seconds; ECL: K1801

Blocking: 5% NFDM/TBST, 1 hour at room temperature
Primary antibody: HA751986, 1/50,000 in primary antibody dilution buffer (K1803), overnight at 4 °C
Secondary antibody: Goat anti-Rabbit IgG-HRP (HA1001), 1/50,000 in 5% NFDM/TBST, 1 hour at room temperature

Predicted band size: 11 kDa
Observed band size: 15 kDa

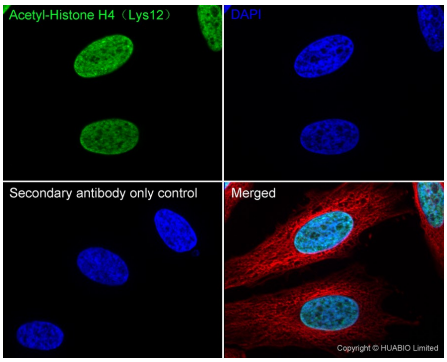

Fig2: Application: Immunocytochemistry (IF-cell)

Species: Human
Sample: HeLa (Human cervical adenocarcinoma cell)

Fixation: 4% Paraformaldehyde, 15 minutes at room temperature.
Permeabilization: 0.1% Triton X-100, 15 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 1 hour at room temperature.
Antibody dilution buffer: 1% BSA in PBST.

Primary antibody: HA751986, 1/2,000, overnight at 4°C.
Secondary antibody: Goat Anti-Rabbit IgG (iFluor™ 488, HA1121), 45 minutes at room temperature.
Counterstain: Beta tubulin (HA601187, Red), 1/100, overnight at 4℃. The nuclear counterstain was DAPI (Blue).

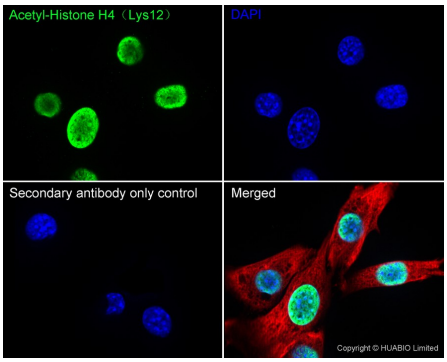

Fig3: Application: Immunocytochemistry (IF-cell)

Species: Mouse
Sample: NIH/3T3 (Mouse fibroblast)

Fixation: 4% Paraformaldehyde, 15 minutes at room temperature.
Permeabilization: 0.1% Triton X-100, 15 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 1 hour at room temperature.
Antibody dilution buffer: 1% BSA in PBST.

Primary antibody: HA751986, 1/2,000, overnight at 4°C.
Secondary antibody: Goat Anti-Rabbit IgG (iFluor™ 488, HA1121), 45 minutes at room temperature.
Counterstain: Beta tubulin (HA601187, Red), 1/100, overnight at 4℃. The nuclear counterstain was DAPI (Blue).

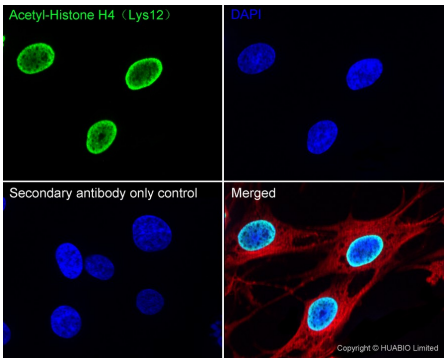

Fig4: Application: Immunocytochemistry (IF-cell)

Species: Rat
Sample: C6 (Rat glioma cell)

Fixation: 4% Paraformaldehyde, 15 minutes at room temperature.
Permeabilization: 0.1% Triton X-100, 15 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 1 hour at room temperature.
Antibody dilution buffer: 1% BSA in PBST.

Primary antibody: HA751986, 1/2,000, overnight at 4°C.
Secondary antibody: Goat Anti-Rabbit IgG (iFluor™ 488, HA1121), 45 minutes at room temperature.
Counterstain: Beta tubulin (HA601187, Red), 1/100, overnight at 4℃. The nuclear counterstain was DAPI (Blue).

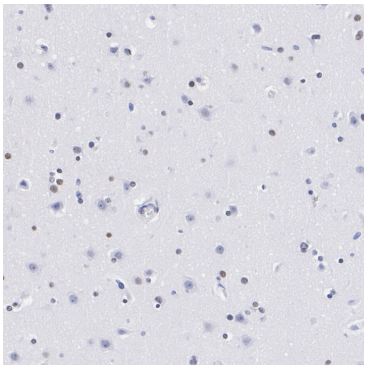

Fig5: Application: Immunohistochemistry (IHC-P)

Species: Human
Tissue: Brain
Sample: Paraffin-embedded section

Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Wash buffer: 1× TBST
Endogenous peroxidase blocking: 3% H_2O_2, 10 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.
Primary antibody: HA751986, 1/500, 1 hour at room temperature.
Secondary antibody: HA1119, 20 minutes at room temperature.

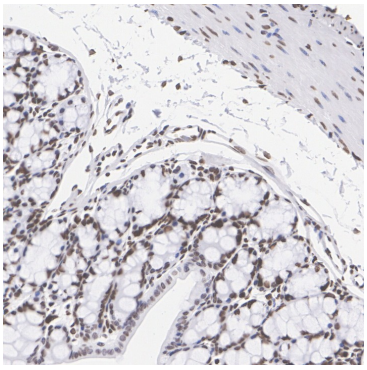

Fig6: Application: Immunohistochemistry (IHC-P)

Species: Mouse
Tissue: Colon
Sample: Paraffin-embedded section

Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Wash buffer: 1× TBST
Endogenous peroxidase blocking: 3% H_2O_2, 10 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.
Primary antibody: HA751986, 1/1,000, 1 hour at room temperature.
Secondary antibody: HA1119, 20 minutes at room temperature.

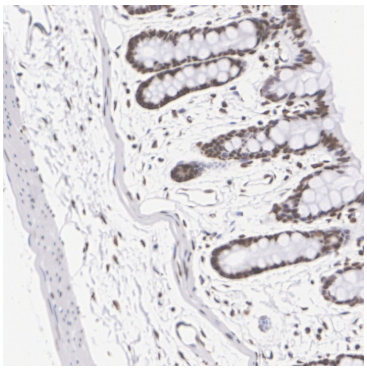

Fig7: Application: Immunohistochemistry (IHC-P)

Species: Rat
Tissue: Colon
Sample: Paraffin-embedded section

Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Wash buffer: 1× TBST
Endogenous peroxidase blocking: 3% H_2O_2, 10 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.
Primary antibody: HA751986, 1/1,000, 1 hour at room temperature.
Secondary antibody: HA1119, 20 minutes at room temperature.

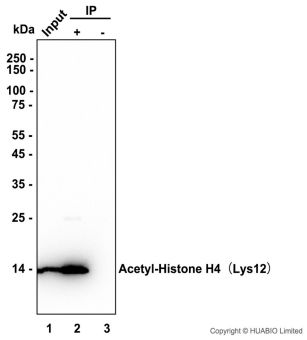

Fig8: Immunoprecipitation (IP)

Histone H4 (acetyl K12) was immunoprecipitated in 0.2 mg HeLa (Human cervix adenocarcinoma epithelial cell) cell lysate with HA751986 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA751986 at 1/5,000 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: HA751986 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of HA751986 in HeLa cell lysate

Exposure time: 2 seconds
Blocking: 5% NFDM/TBST, 1 hour at room temperature

Primary dilution: HA751986, 1/5,000 in primary antibody dilution buffer (K1803), 2 hours at room temperature

Predicted band size: 11 kDa
Observed band size: 15 kDa

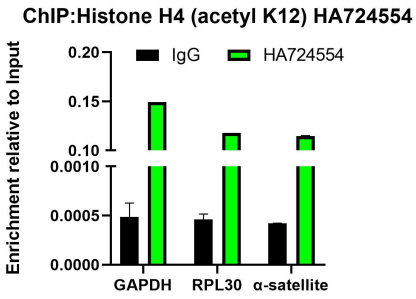

Fig9: Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells with Histone H4 (acetyl K12) (HA751986) / Competitor's antibody / Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

Background References

1. Huang, Suming; Litt, Michael D.; Ann Blakey, C. (2015-11-30). Epigenetic Gene Expression and Regulation. pp. 21–38.
2. Sadoul K, Boyault C, Pabion M, Khochbin S (2008). "Regulation of protein turnover by acetyltransferases and deacetylases". Biochimie. 90 (2): 306–12.