Acetyl-Histone H4 Antibody Sampler Kit

HAK21107

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
Histone H4 (acetyl K5) [ET1602-40]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,IP,ChIP,CUT&Tag-seq	H,M,R	11 kDa
Histone H4 (acetyl K8) [HA721249]	20μl	WB,IF-Cell,IHC-P,ChIP	H,M,R	11 kDa
Histone H4 (acetyl K16) [ET7107-89]	20μl	WB,IHC-P,FC,IF-Cell,IF-Tissue,ChIP	H,M,R	11 kDa
Histone H4 [ET1612-43]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,ChIP	H,M,R	11 kDa
HRP-Goat Anti-Rabbit IgG (H+L) [HA1001]	100μl	WB,ELISA,IHC-P	Rab	

Description: The Acetyl-Histone H4 Antibody Sampler Kit provides an economical means of detecting total histone H4 as well as histone H4 acetylated at various residues including Lys12, Lys5, and Lys8. The kit contains enough primary and secondary antibody to perform two western blots with each antibody.

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

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

Background Modulation of chromatin structure plays an important role in the regulation of transcription in eukaryotes. The nucleosome, made up of DNA wound around eight core histone proteins (two each of H2A, H2B, H3, and H4), is the primary building block of chromatin. The amino-terminal tails of core histones undergo various posttranslational modifications, including acetylation, phosphorylation, methylation, and ubiquitination. These modifications occur in response to various stimuli and have a direct effect on the accessibility of chromatin to transcription factors and, therefore, gene expression. In most species, histone H2B is primarily acetylated at Lys5, 12, 15, and 20. Histone H3 is primarily acetylated at Lys9, 14, 18, 23, 27, and 56. Acetylation of H3 at Lys9 appears to have a dominant role in histone deposition and chromatin assembly in some organisms. Phosphorylation at Ser10, Ser28, and Thr11 of histone H3 is tightly correlated with chromosome condensation during both mitosis and meiosis. Phosphorylation at Thr3 of histone H3 is highly conserved among many species and is catalyzed by the kinase haspin. Immunostaining with phospho-specific antibodies in mammalian cells reveals mitotic phosphorylation at Thr3 of H3 in prophase and its dephosphorylation during anaphase.

Database links: UniProt ID: P62805, P62806, P62804, P62805, P62806, P62804, P62805, P62806, P62804, P62805, P62806, P62804

Images

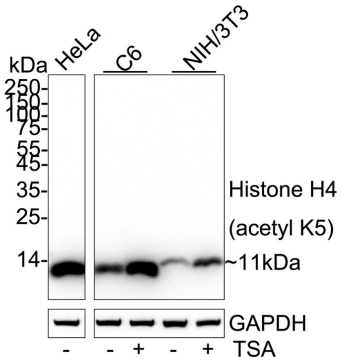

Fig1: Western blot analysis of Histone H4 (acetyl K5) on different lysates with Rabbit anti-Histone H4 (acetyl K5) antibody (ET1602-40) at 1/1,000 dilution.

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

Lysates/proteins at 10 μg/Lane.

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

Exposure time: 8 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 (ET1602-40) 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.

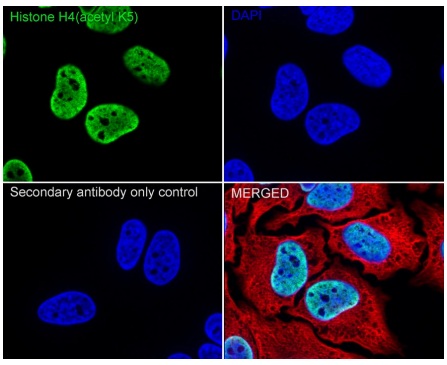

Fig2: Immunocytochemistry analysis of HeLa cells labeling Histone H4 (acetyl K5) with Rabbit anti-Histone H4 (acetyl K5) antibody (ET1602-40) at 1/500 dilution.

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 K5) antibody (ET1602-40) at 1/500 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.

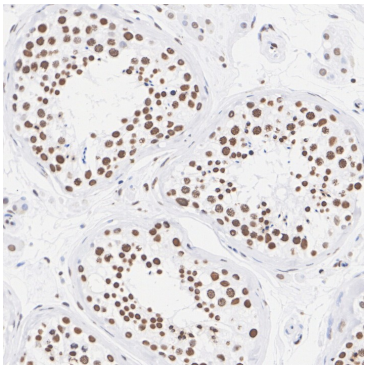

Fig3: Immunohistochemical analysis of paraffin-embedded human testis tissue with Rabbit anti-Histone H4 (acetyl K5) antibody (ET1602-40) at 1/1,000 dilution.

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 (ET1602-40) 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.

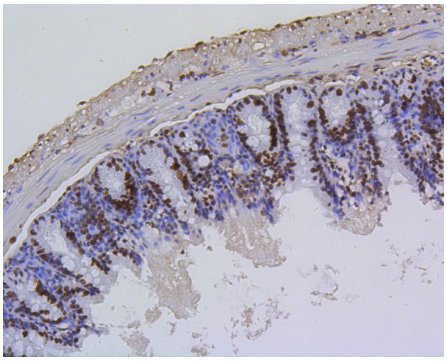

Fig4: Immunohistochemical analysis of paraffin-embedded mouse colon tissue using anti-Histone H4 (acetyl K5) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1602-40, 1/50) 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.

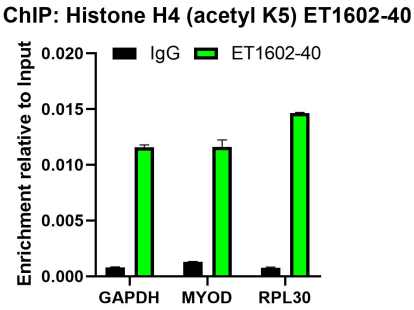

Fig5: Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells with Histone H4 (acetyl K5) (ET1602-40) or Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

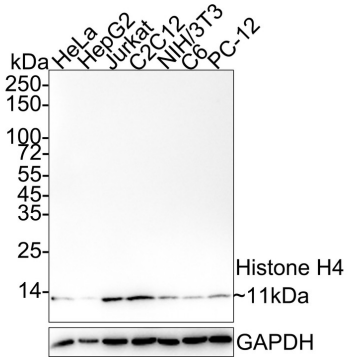

Fig6: Western blot analysis of Histone H4 on different lysates with Rabbit anti-Histone H4 antibody (ET1612-43) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HepG2 cell lysate
Lane 3: Jurkat cell lysate
Lane 4: C2C12 cell lysate
Lane 5: NIH/3T3 cell lysate
Lane 6: C6 cell lysate
Lane 7: PC-12 cell lysate

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 58 seconds;

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 (ET1612-43) at 1/2,000 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.

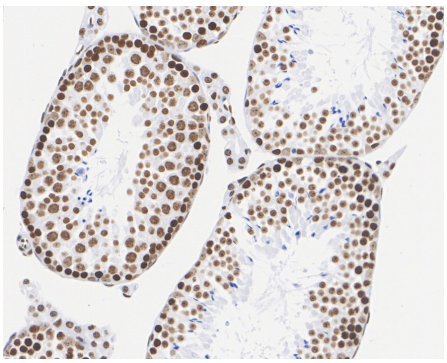

Fig7: Immunohistochemical analysis of paraffin-embedded mouse testis tissue with Rabbit anti-Histone H4 antibody (ET1612-43) at 1/20,000 dilution.

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 (ET1612-43) at 1/20,000 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.

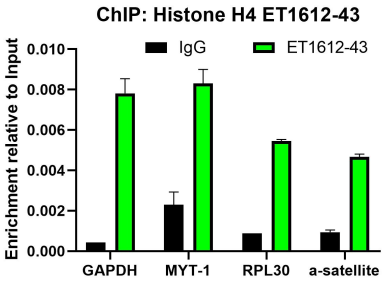

Fig8: Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells with Histone H4 (ET1612-43) or Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

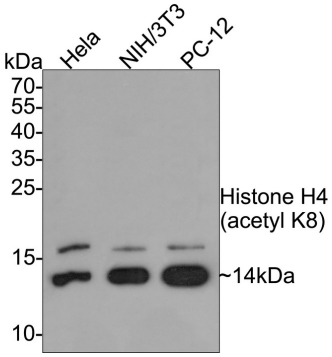

Fig9: Western blot analysis of Histone H4 (acetyl K8) on different lysates with Rabbit anti-Histone H4 (acetyl K8) antibody (HA721249) at 1/500 dilution.

Lane 1: Hela cell lysate, 10 µg/Lane
Lane 2: NIH/3T3 cell lysate, 10 µg/Lane
Lane 3: PC-12 cell lysate, 10 µg/Lane

Predicted band size: 11 kDa
Observed band size: 14/16 kDa

Exposure time: 2 minutes;

15% 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 (HA721249) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

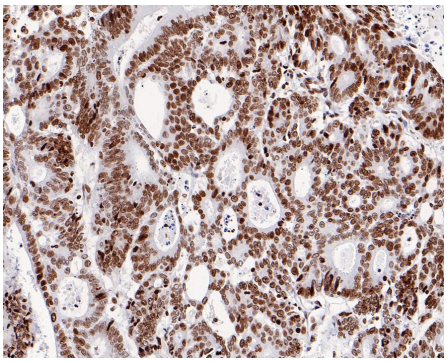

Fig10: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Rabbit anti-Histone H4 (acetyl K8) antibody (HA721249) at 1/5,000 dilution.

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 (HA721249) at 1/5,000 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.

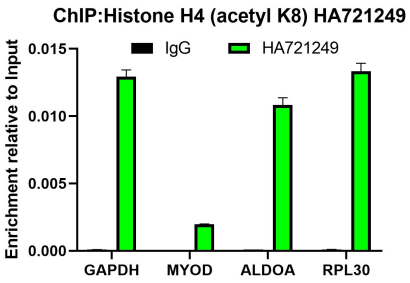

Fig11: Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells with Histone H4 (acetyl K8) (HA721249) or Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

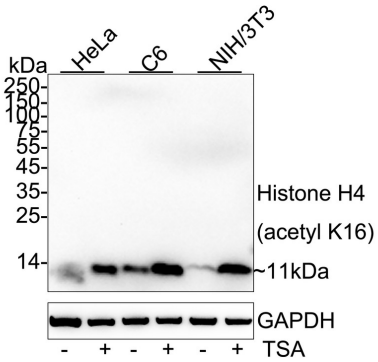

Fig12: Western blot analysis of Histone H4 (acetyl K16) on different lysates with Rabbit anti-Histone H4 (acetyl K16) antibody (ET7107-89) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 1μM TSA for 18 hours cell lysate
Lane 3: 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 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.

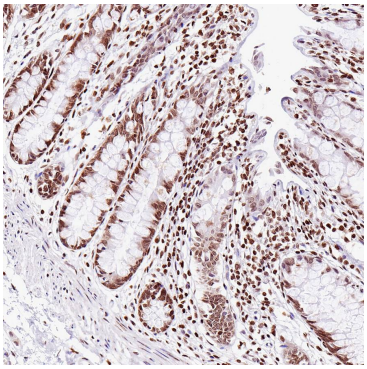

Fig13: Immunohistochemical analysis of paraffin-embedded human colon tissue with Rabbit anti-Histone H4 (acetyl K16) antibody (ET7107-89) at 1/200 dilution.

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.

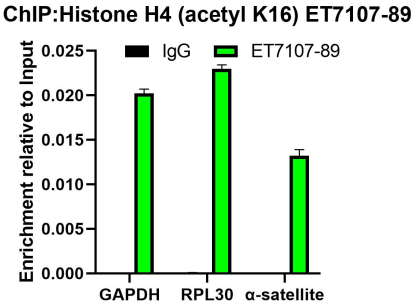

Fig14: Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells with Histone H4 (acetyl K16) (ET7107-89) or Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

Background References

1. Workman JL, Kingston RE. Alteration of nucleosome structure as a mechanism of transcriptional regulation. Annu Rev Biochem. 1998;67:545-79.
2. Hansen JC, Tse C, Wolffe AP. Structure and function of the core histone N-termini: more than meets the eye. Biochemistry. 1998 Dec 22;37(51):17637-41.
3. Strahl BD, Allis CD. The language of covalent histone modifications. Nature. 2000 Jan 6;403(6765):41-5.
4. Dai J, Sultan S, Taylor SS, Higgins JM. The kinase haspin is required for mitotic histone H3 Thr 3 phosphorylation and normal metaphase chromosome alignment. Genes Dev. 2005 Feb 15;19(4):472-88.