abcam

Product datasheet

Anti-Histone H4 (acetyl K5) antibody [EP1000Y] - ChIP Grade ab51997

Recombinant RabMAb

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Overview

Product name	Anti-Histone H4 (acetyl K5) antibody [EP1000Y] - ChIP Grade
Description	Rabbit monoclonal [EP1000Y] to Histone H4 (acetyl K5) - ChIP Grade
Host species	Rabbit
Specificity	In addition to H4K5Ac, this antibody also detects H4K8Ac (Histone H4 acetylated on Lysine 8) at high antigen coating concentration.
Tested applications	**Suitable for:** ChIP, ELISA, WB, IHC-P, ICC/IF, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human, Recombinant fragment **Predicted to work with:** Xenopus laevis, Rice
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	HeLa, NIH/3T3, C6 cells or human brain glioma, human cervical carcinoma, human normal colon FFPE, mouse liver and rat cerebral cortex tissue. ChIP: Chromatin was prepared from MEF cells.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP1000Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab51997 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ChIP	★★★★★ (3)	Use 5 µg for 25 µg of chromatin.
ELISA		Use at an assay dependent concentration.
WB	★★★★☆ (2)	1/500000. Detects a band of approximately 11 kDa (predicted molecular weight: 11 kDa). **For unpurified use at 1/10000- 1/50000.**
IHC-P		1/500. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
ICC/IF	★★★★★ (2)	1/5000. **For unpurified use at 1/250- 1/500.**
IP		1/30.

Target

Function 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.

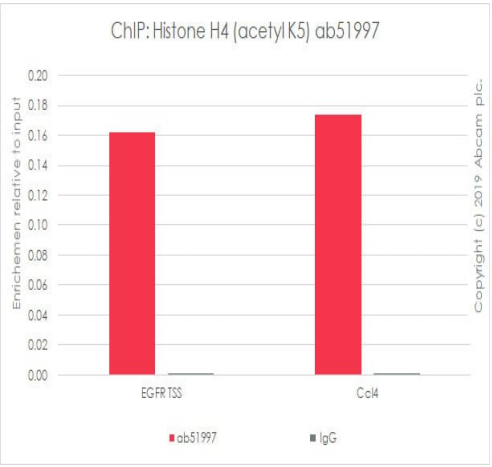
Sequence similarities Belongs to the histone H4 family.

Post-translational modifications Acetylation at Lys-6 (H4K5ac), Lys-9 (H4K8ac), Lys-13 (H4K12ac) and Lys-17 (H4K16ac) occurs in coding regions of the genome but not in heterochromatin.
Citrullination at Arg-4 (H4R3ci) by PADI4 impairs methylation.
Monomethylation and asymmetric dimethylation at Arg-4 (H4R3me1 and H4R3me2a, respectively) by PRMT1 favors acetylation at Lys-9 (H4K8ac) and Lys-13 (H4K12ac). Demethylation is performed by JMJD6. Symmetric dimethylation on Arg-4 (H4R3me2s) by the PRDM1/PRMT5 complex may play a crucial role in the germ-cell lineage.
Monomethylated, dimethylated or trimethylated at Lys-21 (H4K20me1, H4K20me2, H4K20me3). Monomethylation is performed by SET8. Trimethylation is performed by SUV420H1 and SUV420H2 and induces gene silencing.
Ubiquitinated by the CUL4-DDB-RBX1 complex in response to ultraviolet irradiation. This may weaken the interaction between histones and DNA and facilitate DNA accessibility to repair

proteins. Monoubiquitinated at Lys-92 of histone H4 (H4K91ub1) in response to DNA damage. The exact role of H4K91ub1 in DNA damage response is still unclear but it may function as a licensing signal for additional histone H4 post-translational modifications such as H4 Lys-21 methylation (H4K20me).
Sumoylated, which is associated with transcriptional repression.

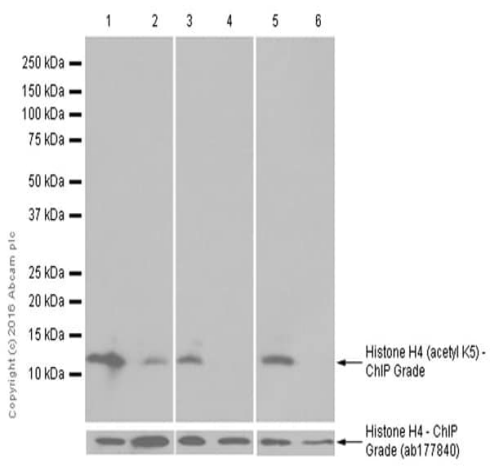
Cellular localization Nucleus. Chromosome.

Images



ChIP - Anti-Histone H4 (acetyl K5) antibody [EP1000Y] - ChIP Grade (ab51997)

Chromatin was prepared from MEF (Mouse embryonic fibroblast cell line) cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 5µg of ab51997 (red), and 20 µl protein A/G sepharose beads. 2µg of rabbit normal IgG was added to the beads control (grey). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).



Western blot - Anti-Histone H4 (acetyl K5) antibody [EP1000Y] - ChIP Grade (ab51997)

All lanes : Anti-Histone H4 (acetyl K5) antibody [EP1000Y] - ChIP Grade (ab51997) at 1/500000 dilution (purified)

Lane 1 : Nuclear extract of HeLa (Human epithelial cell line from cervix adenocarcinoma) treated with 7mM Sodium Butyrate for 24 hours
Lane 2 : Untreated nuclear extract of HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate
Lane 3 : NIH/3T3 (Mouse embryonic fibroblast cell line) treated with 500ng/ml Trichostatin A for 4 hours whole cell lysates
Lane 4 : Untreated NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysates
Lane 5 : C6 (Rat glial tumor cell line) treated with 500ng/ml Trichostatin A for 4 hours whole cell lysates
Lane 6 : Untreated C6 (Rat glial tumor cell line) whole cell lysates

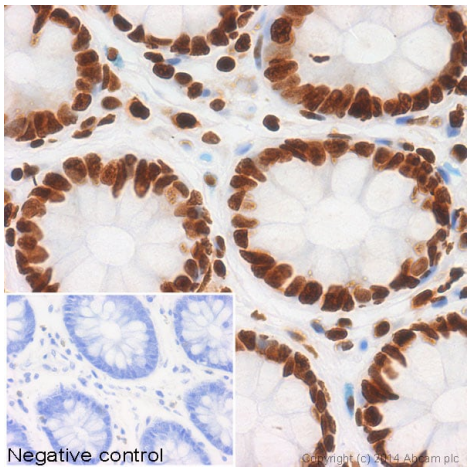
Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000

dilution

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

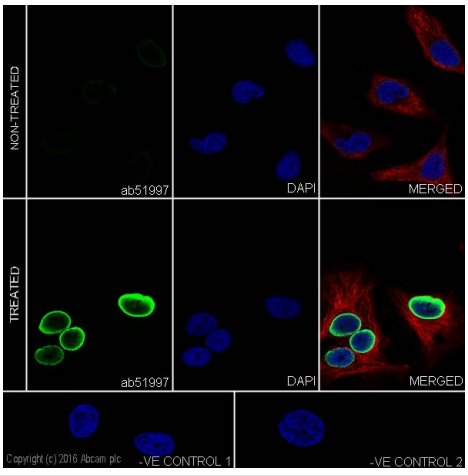
Blocking and diluting buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H4 (acetyl K5) antibody [EP1000Y] - ChIP Grade (ab51997)

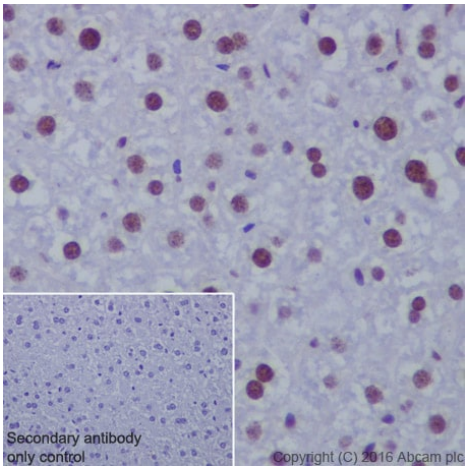
IHC image of unpurified ab51997 staining Histone H4 (acetyl K5) in human colon formalin fixed paraffin embedded tissue sections, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab51997, 1/200 dilution, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. No primary antibody was used in the negative control (shown on the inset).

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



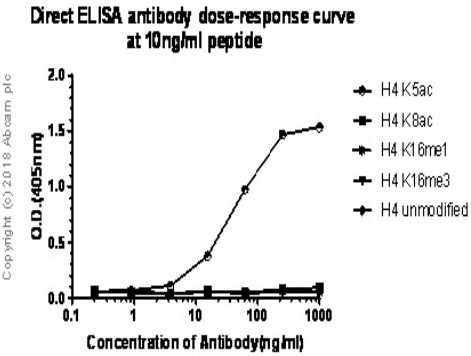
Immunocytochemistry/ Immunofluorescence - Anti-Histone H4 (acetyl K5) antibody [EP1000Y] - ChIP Grade (ab51997)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma)treated with 500ng/m Trichostatin A for 4 hours labeling Histone H4 (acetyl K5) with purified ab51997 at 1/5000 dilution (0.1µg/ml). Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% tritonX-100. **ab195889**, an Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) was used to counterstain at 1/200 (2.5 µg/ml). **ab150077**, a Goat anti rabbit IgG(Alexa Fluor® 488) secondary antibody was used at 1/1000 dilution. PBS instead of the primary antibody was used as a control. DAPI nuclear staining.



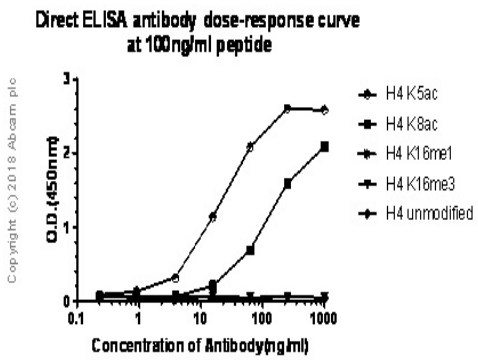
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H4 (acetyl K5) antibody [EP1000Y] - ChIP Grade (ab51997)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse liver tissue sections labeling Histone H4 (acetyl K5) with purified ab51997 at 1/500 dilution (1 µg/ml). Heat mediated antigen retrieval was performed using EDTA Buffer, PH9. Hematoxylin was used to counter stain. **ab97051**, a Goat Anti-Rabbit IgG H&L (HRP) secondary antibody was used at 1/500 dilution. PBS instead of the primary antibody was used as the negative control.



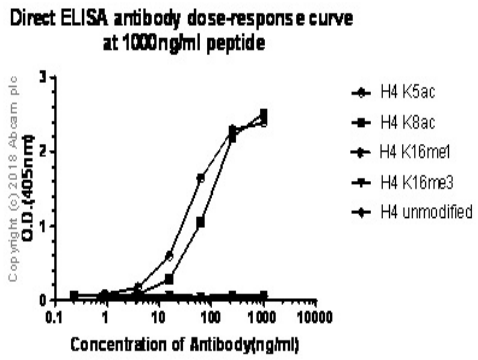
ELISA - Anti-Histone H4 (acetyl K5) antibody [EP1000Y] - ChIP Grade (ab51997)

Direct ELISA with Histone H4 K5ac peptide, Histone H4 K8ac peptide, Histone H4 K16me1 peptide, Histone H4 K16me3 peptide, and Histone H4 unmodified peptide, all at 10ng/ml. ab51997 used as the primary antibody at a range of 0~1000ng/ml. Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG(H+L) used as the secondary antibody at 1:2500 dilution.



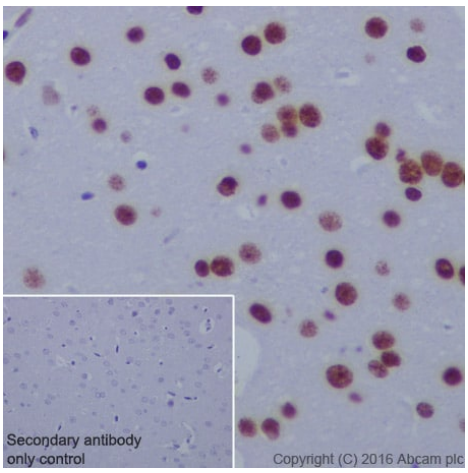
ELISA - Anti-Histone H4 (acetyl K5) antibody [EP1000Y] - ChIP Grade (ab51997)

Direct ELISA with Histone H4 K5ac peptide, Histone H4 K8ac peptide, Histone H4 K16me1 peptide, Histone H4 K16me3 peptide, and Histone H4 unmodified peptide, all at 100ng/ml. ab51997 used as the primary antibody at a range of 0~1000ng/ml. Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG(H+L) used as the secondary antibody at 1:2500 dilution.



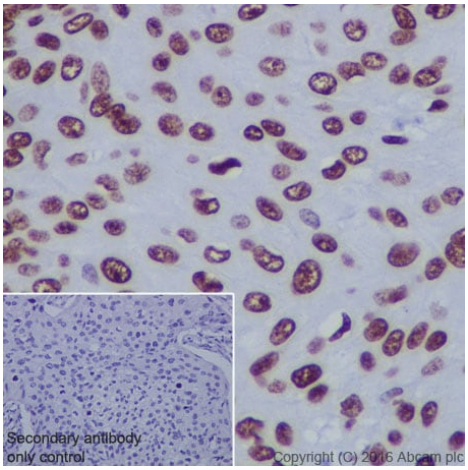
ELISA - Anti-Histone H4 (acetyl K5) antibody [EP1000Y] - ChIP Grade (ab51997)

Direct ELISA with Histone H4 K5ac peptide, Histone H4 K8ac peptide, Histone H4 K16me1 peptide, Histone H4 K16me3 peptide, and Histone H4 unmodified peptide, all at 1000ng/ml. ab51997 used as the primary antibody at a range of 0~1000ng/ml. Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG(H+L) used as the secondary antibody at 1:2500 dilution.



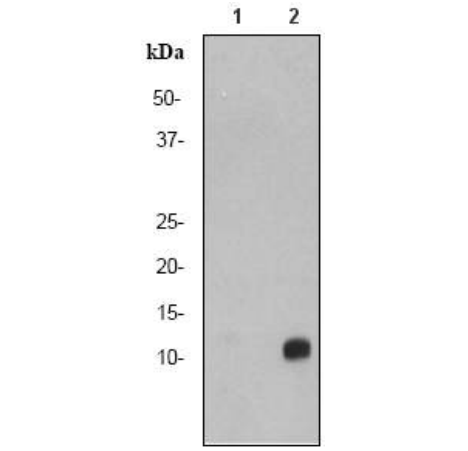
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H4 (acetyl K5) antibody [EP1000Y] - ChIP Grade (ab51997)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat cerebral cortex tissue sections labeling Histone H4 (acetyl K5) with purified ab51997 at 1/500 dilution (1 µg/ml). Heat mediated antigen retrieval was performed using EDTA Buffer, PH9. Hematoxylin was used to counter stain. **ab97051**, a Goat Anti-Rabbit IgG H&L (HRP) secondary antibody was used at 1/500 dilution. PBS instead of the primary antibody was used as the negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H4 (acetyl K5) antibody [EP1000Y] - ChIP Grade (ab51997)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervical carcinoma tissue sections labeling Histone H4 (acetyl K5) with purified ab51997 at 1/500 dilution (1 µg/ml). Heat mediated antigen retrieval was performed using EDTA Buffer, PH9. Hematoxylin was used to counter stain. **ab97051**, a Goat Anti-Rabbit IgG H&L (HRP) secondary antibody was used at 1/500 dilution. PBS instead of the primary antibody was used as the negative control.



Western blot - Anti-Histone H4 (acetyl K5) antibody [EP1000Y] - ChIP Grade (ab51997)

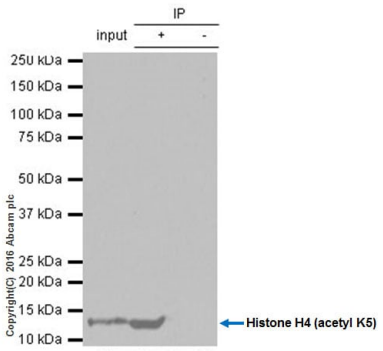
All lanes : Anti-Histone H4 (acetyl K5) antibody [EP1000Y] - ChIP Grade (ab51997) at 1/1000000 dilution (unpurified)

Lane 1 : Untreated HeLa cells
Lane 2 : TSA treated HeLa calls

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat anti-rabbit HRP labelled (1:2000)

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



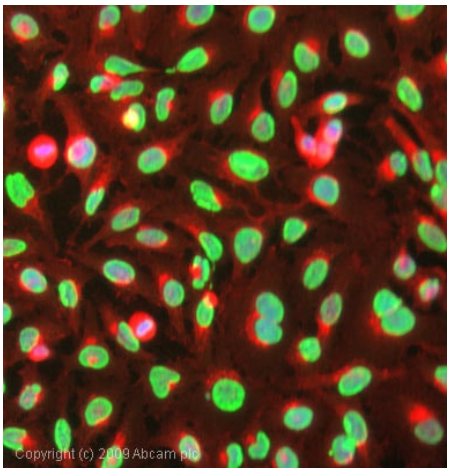
Immunoprecipitation - Anti-Histone H4 (acetyl K5) antibody [EP1000Y] - ChIP Grade (ab51997)

ab51997 (purified) at 1/30 dilution (2µg) immunoprecipitating Histone H4 (acetyl K5) in HeLa (Human epithelial cell line from cervix adenocarcinoma) treated with TSA whole cell lysate.

Lane 1 (input): HeLa (Human epithelial cell line from cervix adenocarcinoma) treated with TSA whole cell lysate 10ug
Lane 2 (+): ab51997+ HeLa (Human epithelial cell line from cervix adenocarcinoma) treated with TSA whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab51997 in HeLa (Human epithelial cell line from cervix adenocarcinoma) treated with TSA whole cell lysate

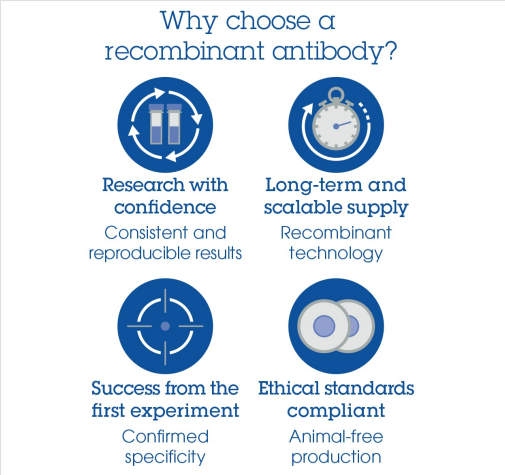
For western blotting, **ab131366** VeriBlot for IP (HRP) was used for detection (1/10000).

Blocking and diluting buffer and concentration: 5% NFDM/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H4 (acetyl K5) antibody [EP1000Y] - ChIP Grade (ab51997)

ICC/IF image of unpurtified ab51997 stained HeLa cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab51997, 1/1000 dilution) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Anti-Histone H4 (acetyl K5) antibody [EP1000Y] - ChIP Grade (ab51997)

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