abcam

Product datasheet

Anti-Histone H4 (acetyl K8) antibody [EP1002Y] - ChIP Grade ab45166

Recombinant RabMAb

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Overview

Product name	Anti-Histone H4 (acetyl K8) antibody [EP1002Y] - ChIP Grade
Description	Rabbit monoclonal [EP1002Y] to Histone H4 (acetyl K8) - ChIP Grade
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ChIP, ChIP-sequencing, WB, IHC-P, ICC/IF, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human Histone H4 aa 1-100 (N terminal) (acetyl K8). The exact sequence is proprietary. Database link: **P62805**
Positive control	WB: HeLa whole cell lysate +TSA, C6 cell lysate, C6 cell + TSA lysate, NIH/3T3 +TSA whole cell lysate. IHC-P: Human normal colon FFPE tissue sections, mouse kidney paraffin-embedded tissue sections, rat kidney paraffin-embedded tissue sections. ICC/IF: C6 + TSA lysates. ChIP: Chromatin prepared from HeLa cells ChiP-Seq: HeLa 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 (glycerin, glycerine), 0.17% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP1002Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab45166 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
Flow Cyt (Intra)		Use at an assay dependent concentration.
ChIP	★★★★★ (1)	Use 2 µg for 25 µg of chromatin.
ChIP-sequencing		Use 4µg for 10^7 cells.
WB		1/5000 - 1/10000. Predicted molecular weight: 11 kDa.
IHC-P		1/250 - 1/2500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
ICC/IF		1/150 - 1/500.
IP		1/20 - 1/50.

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.

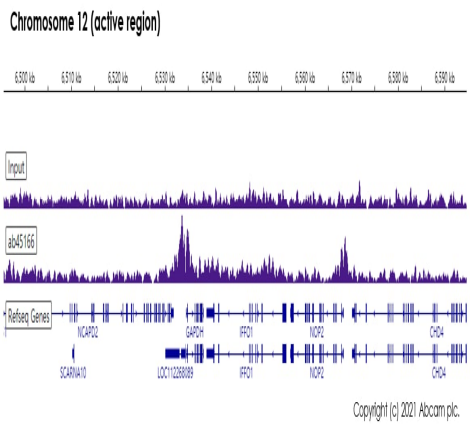
Sequence similarities Belongs to the histone H4 family.

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.

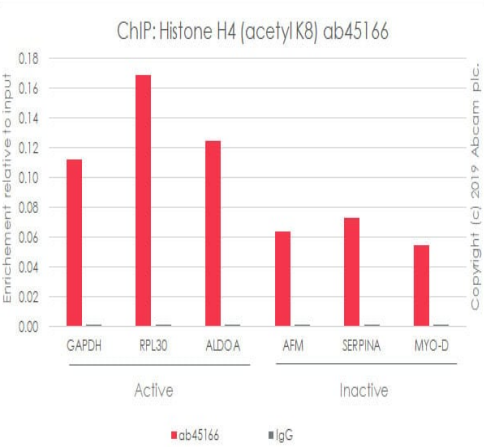
Images



ChIP-sequencing - Anti-Histone H4 (acetyl K8) antibody [EP1002Y] - ChIP Grade (ab45166)

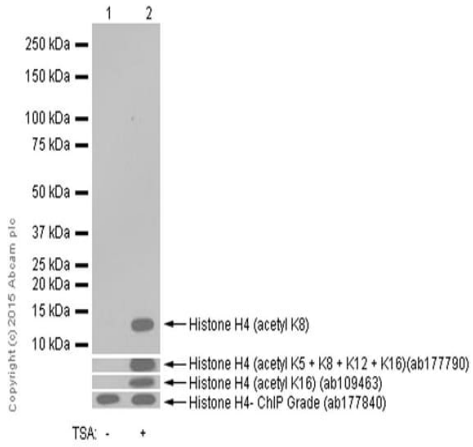
Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10^7 HeLa cells and 4 µg of ab45166 [EP1002Y]. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

Additional screenshots of mapped reads can be downloaded **here**.



ChIP - Anti-Histone H4 (acetyl K8) antibody [EP1002Y] - ChIP Grade (ab45166)

Chromatin was prepared from HeLa cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 2µg of ab45166 (red), and 20µl of Protein A/G sepharose beads. No antibody was added to the beads control (grey). The immunoprecipitated DNA was quantified by real time PCR (Taqman approach). Primers and probes are located in the first kb of the transcribed region.



Western blot - Anti-Histone H4 (acetyl K8) antibody [EP1002Y] - ChIP Grade (ab45166)

All lanes : Anti-Histone H4 (acetyl K8) antibody [EP1002Y] - ChIP Grade (ab45166) at 1/5000 dilution

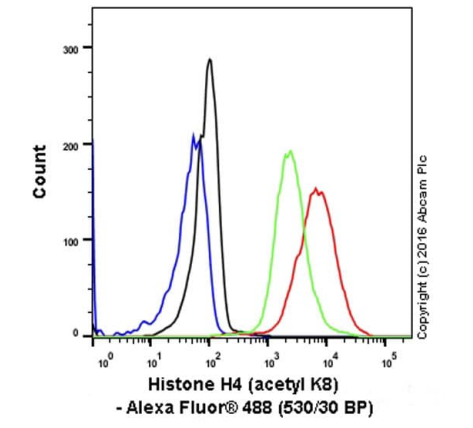
Lane 1 : Untreated HeLa (human cervix adenocarcinoma) whole cell lysate
Lane 2 : HeLa (human cervix adenocarcinoma) treated with Trichostatin A whole cell lysate

Lysates/proteins at 10 µg per lane.

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

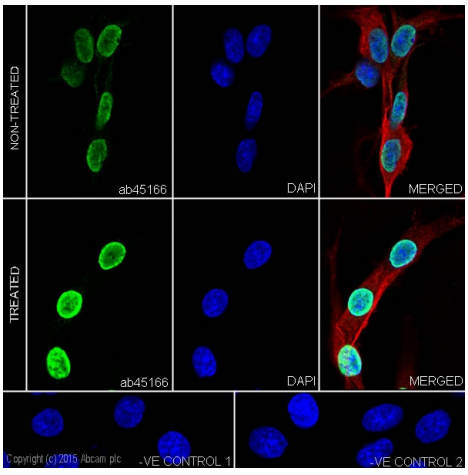
Predicted band size: 11 kDa
Observed band size: 11 kDa

Blocking and diluting buffer 5% NFDM/TBST



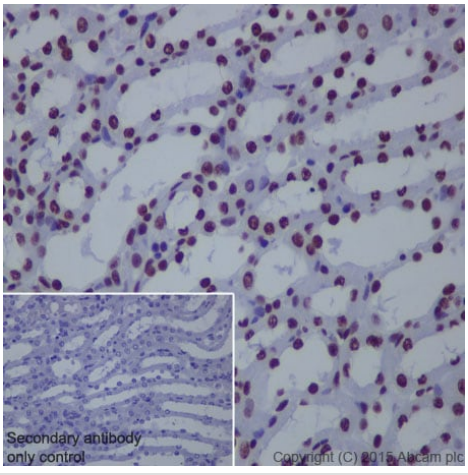
Flow Cytometry (Intracellular) - Anti-Histone H4 (acetyl K8) antibody [EP1002Y] - ChIP Grade (ab45166)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) treated (Red)/untreated (Green) with 500ng/ml Trichostatin A for 4 hours with purified ab45166 at 1/20 dilution. The secondary antibody was Goat anti rabbit IgG (Alexa Fluorr® 488) at 1/2000 dilution. A Rabbit monoclonal IgG (Black) was used as the isotype control and cells without incubation with primary antibody and secondary antibody (Blue) were used as unlabeled control.



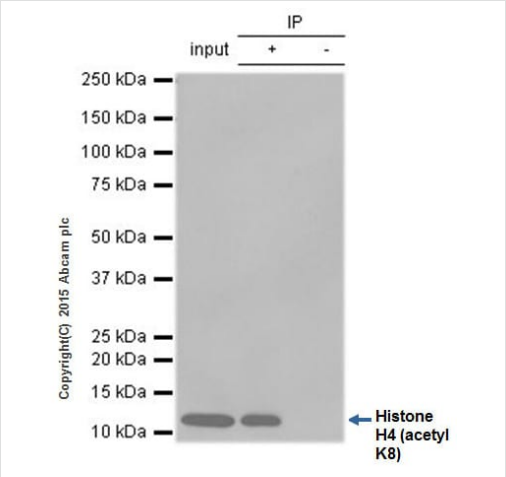
Immunocytochemistry/ Immunofluorescence - Anti-Histone H4 (acetyl K8) antibody [EP1002Y] - ChIP Grade (ab45166)

Immunocytochemistry/immunofluorescence staining of 4% paraformaldehyde fixed; 0.1% triton X 100 permeabilized C6 (rat glioma) cells (non-treated-top panels) and (C6 + TSA(500ng/ml, 4hr)-middle panels) with purified ab45166 at dilution of 1/150. The secondary antibody used was Alexa Fluor® 488; goat anti-rabbit IgG (**ab150077**) at a dilution of 1/1000. Nucleus was counter-stained with DAPI (blue). **ab7291**, a mouse anti-tubulin antibody (1/1000) was used to stain tubulin along with **ab150120** (AlexaFluor®594 goat anti-mouse secondary, 1/1000) shown in the top right and middle right hand panels. The negative controls are shown in the bottom two panels- for negative control 1 rabbit primary antibody and anti-mouse secondary antibody (**ab150120**) was used. For negative control 2 mouse primary antibody (**ab7291**) and anti-rabbit secondary antibody (**ab150077**) was used.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H4 (acetyl K8) antibody [EP1002Y] - ChIP Grade (ab45166)

Immunohistochemical staining of paraffin-embedded mouse kidney sections labelling Histone H4 (acetyl K8) with purified ab45166 at dilution of 1:2500. The secondary antibody used was **ab97051**; a goat anti-rabbit IgG H&L (HRP) at dilution of 1/500. The sample was counter-stained with hematoxylin. Antigen retrieval was performed using EDTA Buffer; pH 9.0. PBS was used instead of the primary antibody as the negative control and is shown in the inset.



Immunoprecipitation - Anti-Histone H4 (acetyl K8) antibody [EP1002Y] - ChIP Grade (ab45166)

ab45166 (purified) at 1/20 immunoprecipitating Histone H4 (acetyl K8) in HeLa treated with Trichostatin A whole cell lysate.

Lane 1 (input): HeLa treated with Trichostatin A whole cell lysate (10µg)

Lane 2 (+): ab45166 + HeLa treated with Trichostatin A whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab45166 in HeLa treated with Trichostatin A whole cell lysate.

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

Blocking buffer and concentration: 5% NFDM/TBST.

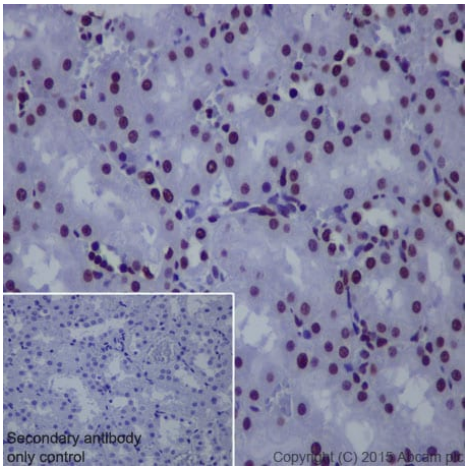
Diluting buffer and concentration: 5% NFDM /TBST.

Lanes 1-2 : Anti-Histone H4 (acetyl K8) antibody [EP1002Y] - ChIP Grade (ab45166) at 1/20 dilution
Lane 3 : Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) at 1/20 dilution

Lane 1 : HeLa (human cervix adenocarcinoma) treated with Trichostatin A whole cell lysate at 10 µg
Lanes 2-3 : HeLa (human cervix adenocarcinoma) treated with Trichostatin A whole cell lysate

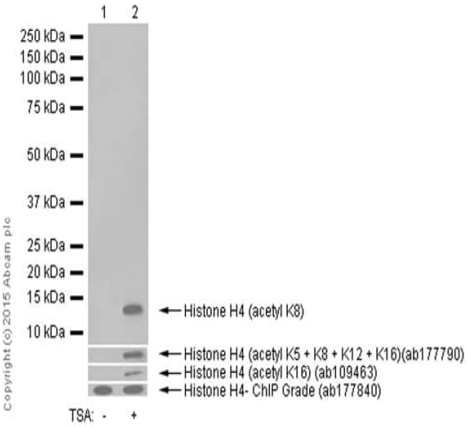
Secondary
All lanes : VeriBlot for IP Detection Reagent (HRP) (**ab131366**) at 1/10000 dilution

Observed band size: 11 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H4 (acetyl K8) antibody [EP1002Y] - ChIP Grade (ab45166)

Immunohistochemical staining of paraffin-embedded rat kidney sections labelling Histone H4 (acetyl K8) with purified ab45166 at dilution of 1:2500. The secondary antibody used was **ab97051**; a goat anti-rabbit IgG H&L (HRP) at dilution of 1/500. The sample was counter-stained with hematoxylin. Antigen retrieval was performed using EDTA Buffer; pH 9.0. PBS was used instead of the primary antibody as the negative control and is shown in the inset.



Western blot - Anti-Histone H4 (acetyl K8) antibody [EP1002Y] - ChIP Grade (ab45166)

All lanes : Anti-Histone H4 (acetyl K8) antibody [EP1002Y] - ChIP Grade (ab45166) at 1/5000 dilution

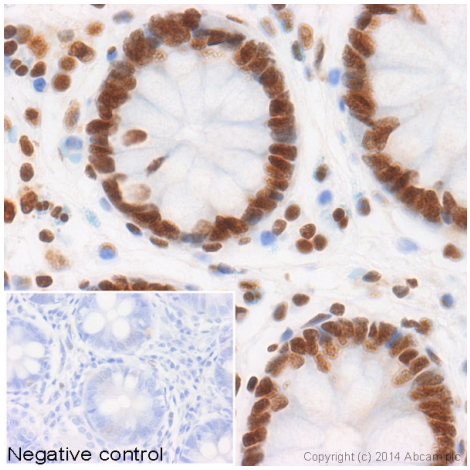
Lane 1 : Untreated C6 (rat glioma) whole cell lysate
Lane 2 : C6 (rat glioma) treated with Trichostatin A whole cell lysate

Lysates/proteins at 10 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

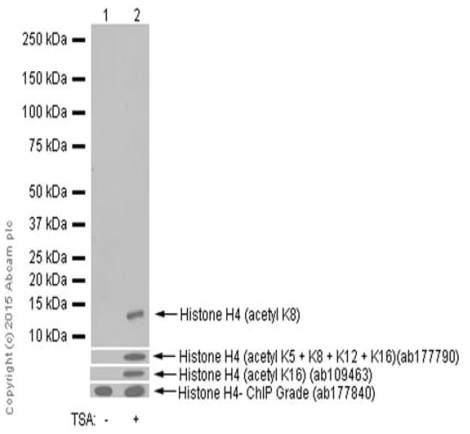
Blocking and diluting buffer 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H4 (acetyl K8) antibody [EP1002Y] - ChIP Grade (ab45166)

Immunohistochemical analysis of formalin fixed paraffin embedded human colon tissue sections labelling Histone H4 (acetyl K8) with unpurified ab45166 at dilution of 1/200.

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.



Western blot - Anti-Histone H4 (acetyl K8) antibody [EP1002Y] - ChIP Grade (ab45166)

All lanes : Anti-Histone H4 (acetyl K8) antibody [EP1002Y] - ChIP Grade (ab45166) at 1/5000 dilution

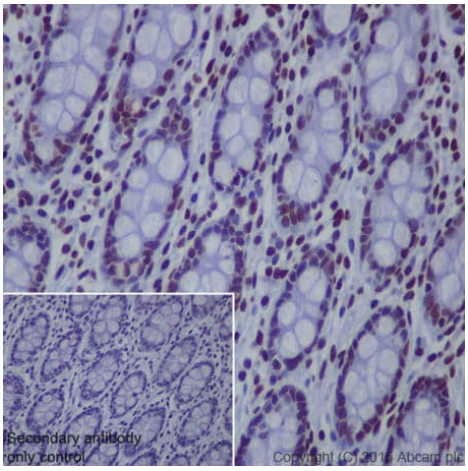
Lane 1 : Untreated NIH/3T3 (mouse embryo) whole cell lysate
Lane 2 : NIH/3T3 (mouse embryo) treated with Trichostatin A whole cell lysate

Lysates/proteins at 10 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 K8) antibody [EP1002Y] - ChIP Grade (ab45166)

Immunohistochemical staining of paraffin-embedded human colon sections labelling Histone H4 (acetyl K8) with purified ab45166 at dilution of 1:2500. The secondary antibody used was **ab97051**; a goat anti-rabbit IgG H&L (HRP) at dilution of 1/500. The sample was counter-stained with hematoxylin. Antigen retrieval was performed using EDTA Buffer; pH 9.0. PBS was used instead of the primary antibody as the negative control and is shown in the inset.

Anti-Histone H4 (acetyl K8) antibody [EP1002Y] - ChIP Grade (ab45166)

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