abcam

Product datasheet

Anti-Histone H3 (acetyl K23) antibody [EPR17712] - ChIP Grade ab177275



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Overview

Product name Anti-Histone H3 (acetyl K23) antibody [EPR17712] - ChIP Grade

Description Rabbit monoclonal [EPR17712] to Histone H3 (acetyl K23) - ChIP Grade

Host species Rabbit

Tested applications Suitable for: WB, ICC/IF, ChIP, PepArr, ChIP-sequencing

Species reactivity Reacts with: Mouse, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HeLa and NIH/3T3 treated with 500 ng/ml Trichostatin A for 4 hours whole cell lysates.

 $\label{localized} \mbox{ICC/IF: HeLa cells. ChIP and ChIP-seq: Chromatin prepared from HeLa cells.}$

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 long

term. Avoid freeze / thaw cycle.

Storage buffer Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

ClonalityMonoclonalClone numberEPR17712

Isotype IgG

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Applications

The Abpromise quarantee

Our **Abpromise guarantee** covers the use of ab177275 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
WB		1/1000. Detects a band of approximately 15 kDa (predicted molecular weight: 15 kDa).
ICC/IF		1/2000.
ChIP		Use 2 µg for 25 µg of chromatin.
PepArr		Use at an assay dependent concentration.
ChIP-sequencing		Use 4µg for 10 ⁷ cells.

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

Developmental stage

Post-translational modifications

Belongs to the histone H3 family.

Expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation.

Acetylation is generally linked to gene activation. Acetylation on Lys-10 (H3K9ac) impairs methylation at Arg-9 (H3R8me2s). Acetylation on Lys-19 (H3K18ac) and Lys-24 (H3K24ac) favors methylation at Arg-18 (H3R17me).

Citrullination at Arg-9 (H3R8ci) and/or Arg-18 (H3R17ci) by PAD4 impairs methylation and represses transcription.

Asymmetric dimethylation at Arg-18 (H3R17me2a) by CARM1 is linked to gene activation. Symmetric dimethylation at Arg-9 (H3R8me2s) by PRMT5 is linked to gene repression.

Asymmetric dimethylation at Arg-3 (H3R2me2a) by PRMT6 is linked to gene repression and is mutually exclusive with H3 Lys-5 methylation (H3K4me2 and H3K4me3). H3R2me2a is present at the 3' of genes regardless of their transcription state and is enriched on inactive promoters, while it is absent on active promoters.

Methylation at Lys-5 (H3K4me), Lys-37 (H3K36me) and Lys-80 (H3K79me) are linked to gene activation. Methylation at Lys-5 (H3K4me) facilitates subsequent acetylation of H3 and H4. Methylation at Lys-80 (H3K79me) is associated with DNA double-strand break (DSB) responses and is a specific target for TP53BP1. Methylation at Lys-10 (H3K9me) and Lys-28 (H3K27me) are linked to gene repression. Methylation at Lys-10 (H3K9me) is a specific target for HP1 proteins (CBX1, CBX3 and CBX5) and prevents subsequent phosphorylation at Ser-11 (H3S10ph) and acetylation of H3 and H4. Methylation at Lys-5 (H3K4me) and Lys-80 (H3K79me) require preliminary monoubiquitination of H2B at 'Lys-120'. Methylation at Lys-10 (H3K9me) and

Lys-28 (H3K27me) are enriched in inactive X chromosome chromatin.

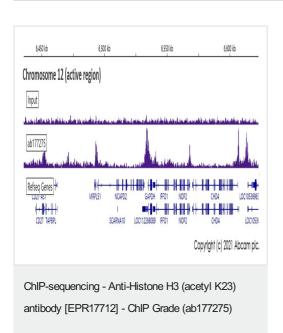
Phosphorylated at Thr-4 (H3T3ph) by GSG2/haspin during prophase and dephosphorylated during anaphase. Phosphorylation at Ser-11 (H3S10ph) by AURKB is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis. In addition phosphorylation at Ser-11 (H3S10ph) by RPS6KA4 and RPS6KA5 is important during interphase because it enables the transcription of genes following external stimulation, like mitogens, stress, growth factors or UV irradiation and result in the activation of genes, such as c-fos and c-jun. Phosphorylation at Ser-11 (H3S10ph), which is linked to gene activation, prevents methylation at Lvs-10 (H3K9me) but facilitates acetylation of H3 and H4. Phosphorylation at Ser-11 (H3S10ph) by AURKB mediates the dissociation of HP1 proteins (CBX1, CBX3 and CBX5) from heterochromatin. Phosphorylation at Ser-11 (H3S10ph) is also an essential regulatory mechanism for neoplastic cell transformation. Phosphorylated at Ser-29 (H3S28ph) by MLTK isoform 1, RPS6KA5 or AURKB during mitosis or upon ultraviolet B irradiation. Phosphorylation at Thr-7 (H3T6ph) by PRKCBB is a specific tag for epigenetic transcriptional activation that prevents demethylation of Lys-5 (H3K4me) by LSD1/KDM1A. At centromeres, specifically phosphorylated at Thr-12 (H3T11ph) from prophase to early anaphase, by DAPK3 and PKN1. Phosphorylation at Thr-12 (H3T11ph) by PKN1 is a specific tag for epigenetic transcriptional activation that promotes demethylation of Lys-10 (H3K9me) by KDM4C/JMJD2C. Phosphorylation at Tyr-42 (H3Y41ph) by JAK2 promotes exclusion of CBX5 (HP1 alpha) from chromatin.

Monoubiquitinated by RAG1 in lymphoid cells, monoubiquitination is required for V(D)J recombination (By similarity). 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.

Cellular localization

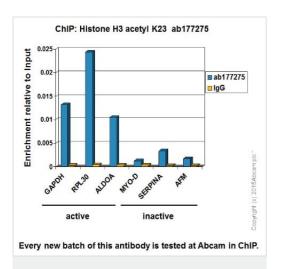
Nucleus. Chromosome.

Images



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 μ g of ab177275 [EPR17712]. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

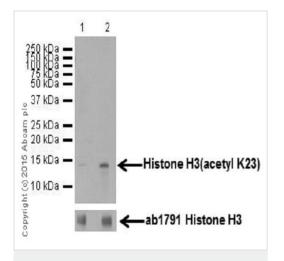
Additional screenshots of mapped reads can be downloaded **here**.



ChIP - Anti-Histone H3 (acetyl K23) antibody

[EPR17712] - ChIP Grade (ab177275)

Chromatin was prepared from HeLa (Human epithelial cells from cervix adenocarcinoma) 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 ab177275 (blue), and 20µl of Anti Rabbit IgG sepharose beads. 2µg of rabbit normal IgG was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).



Western blot - Anti-Histone H3 (acetyl K23) antibody [EPR17712] - ChIP Grade (ab177275) **All lanes :** Anti-Histone H3 (acetyl K23) antibody [EPR17712] - ChIP Grade (ab177275) at 1/1000 dilution

Lane 1 : Untreated HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate

Lane 2: HeLa (Human epithelial cells from cervix adenocarcinoma) treated with 500 ng/ml Trichostatin A for 4 hours whole cell lysate

Lysates/proteins at 10 µg per lane.

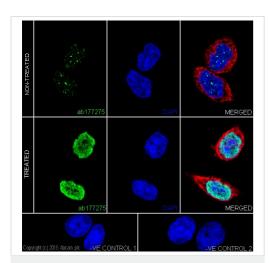
Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 15 kDa **Observed band size:** 15 kDa

Exposure time: 5 seconds

Blocking/Dilution buffer: 5% BSA/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (acetyl K23) antibody [EPR17712] -ChIP Grade (ab177275)

Western blot - Anti-Histone H3 (acetyl K23) antibody [EPR17712] - ChIP Grade (ab177275)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling Histone H3 (acetyl K23) with ab177275 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on HeLa cell line. Acetylation level increased after treatment with Trichostatin A (500 ng/ml) for 4 hours. The nuclear counterstain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (<u>ab7291</u>) at 1/1000 dilution followed by Goat Anti-Mouse IgG H&L (Alexa Fluor[®] 594) (<u>ab150120</u>) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab177275 at 1/2000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary antibody at 1/1000 dilution.

-ve control 2: Anti-alpha Tubulin mouse MAb (<u>ab7291</u>) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (<u>ab150077</u>) secondary antibody at 1/1000 dilution.

All lanes : Anti-Histone H3 (acetyl K23) antibody [EPR17712] - ChIP Grade (ab177275) at 1/1000 dilution

Lane 1 : Untreated NIH/3T3 (Mouse embryonic fibroblast cells) whole cell lysate

Lane 2: NIH/3T3 (Mouse embyronic fibroblast cells) treated with 500 ng/ml Trichostatin A for 4 hours whole cell lysate

Lysates/proteins at 10 µg per lane.

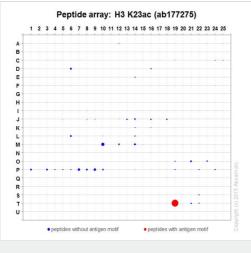
Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

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

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Peptide Array - Anti-Histone H3 (acetyl K23) antibody [EPR17712] - ChIP Grade (ab177275) ab177275 was tested in Peptide array against 501 different modified and unmodified histone peptides; each peptide is printed on the array at six concentrations (each in triplicate).

Circle area represents affinity between the antibody and a peptide: all antigen-containing peptides are displayed as red circles, all other peptides as blue circles. The affinity is calculated as area under curve when antibody binding values are plotted against the corresponding peptide concentration. Each circle area is normalized to the peptide with the strongest affinity.

The complete dataset, including full list of all peptides and information on the position of each peptide in the diagram, can be downloaded here.



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