# abcam

# Product datasheet

# Anti-Histone H3 (acetyl K9) antibody - ChIP Grade ab10812

\*\*\* \* \* \* 25 Abreviews 263 References 6 Images

#### Overview

Product name Anti-Histone H3 (acetyl K9) antibody - ChIP Grade

**Description** Rabbit polyclonal to Histone H3 (acetyl K9) - ChIP Grade

Host species Rabbit

**Specificity** Specific for acetyl K9, but cross-reacts slightly with K27.

Tested applications Suitable for: IHC-P, ChIP, WB, ICC/IF

Species reactivity Reacts with: Cow, Human, Arabidopsis thaliana

**Predicted to work with:** Mouse, Rat, Saccharomyces cerevisiae, Caenorhabditis elegans, Drosophila melanogaster, Zebrafish, a wide range of other species, Common marmoset, Rice

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

(Peptide available as ab16635)

General notes Learn about ChIP assay kits, other ChIP antibodies, protocols and more in the ChIP assay

quide.

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

#### **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 or -

80°C. Avoid freeze / thaw cycle.

Storage buffer pH: 7.40

Preservative: 0.02% Sodium azide

Constituent: PBS

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Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

**Purity** Immunogen affinity purified

**Clonality** Polyclonal

**Isotype** IgG

# **Applications**

#### The Abpromise guarantee

Our Abpromise guarantee covers the use of ab10812 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
IHC-P	****(2)	Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ChIP	* * * <del>*</del> <del>*</del> <del>*</del> <u>*</u> (5)	Use 2-4 µg for 25 µg of chromatin. Use GAPDH ChIP primer pair <u>ab267832</u> as positive control.
WB	<b>★★★★</b> (11)	1/500. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).
ICC/IF	<b>★★★★★(2)</b>	Use a concentration of 1 µg/ml.

# **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 H3 family.

#### **Developmental stage**

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

# Post-translational modifications

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.

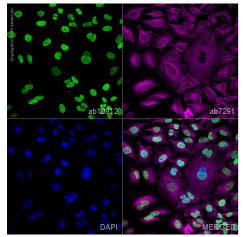
Phosphorylated at Thr-4 (H3T3ph) by GSG2/haspin during prophase and dephosphorylated during anaphase. Phosphorylation at Ser-11 (H3S10ph) by AURKB is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis. In addition phosphorylation at Ser-11 (H3S10ph) by RPS6KA4 and 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 Lys-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.

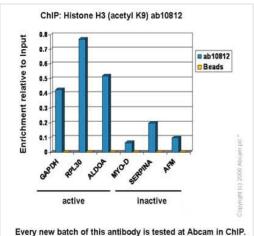
# **Cellular localization**

Nucleus. Chromosome.

#### **Images**



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (acetyl K9) antibody - ChIP Grade (ab10812)



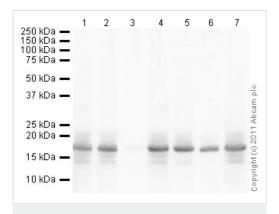
ChIP - Anti-Histone H3 (acetyl K9) antibody - ChIP Grade (ab10812)

ab10812 staining Histone H3 (acetyl K9) in HeLa cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab10812 at 1µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin -Loading Control. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit lgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse IgG -H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour magenta). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 100% methanol (5 min).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.

Chromatin was prepared from Hela cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10min. The ChIP was performed with 25µg of chromatin, 2µg of ab10812 (blue), and 20µl of Protein A/G sepharose beads. No antibody was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Tagman approach). Primers and probes are located in the first kb of the transcribed region.



Western blot - Anti-Histone H3 (acetyl K9) antibody - ChIP Grade (ab10812)

**All lanes :** Anti-Histone H3 (acetyl K9) antibody - ChIP Grade (ab10812) at 1 μg/ml

Lane 1: Calf Thymus Histone Preparation Nuclear Lysate

**Lane 2 :** Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (unmodified ) peptide (ab2903) at 0.5 µg/ml

**Lane 3**: Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (acetyl K9) peptide (ab16635) at 0.5 μg/ml

**Lane 4 :** Calf Thymus Histone Preparation Nuclear Lysate with Histone H3 peptide - acetyl K14 at  $0.5~\mu g/ml$ 

**Lane 5 :** Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (acetyl K18) peptide (<u>ab24003</u>) at 0.5 µg/ml

 $\textbf{Lane 6:} \ \text{Calf Thymus Histone Preparation Nuclear Lysate with } \\ \text{Human Histone H3 (acetyl K27) peptide } (\underline{\textbf{ab24404}}) \ \text{at 0.5 } \mu\text{g/ml}$ 

**Lane 7 :** Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (acetyl K23) peptide (ab48359) at 0.5  $\mu$ g/ml

Lysates/proteins at 0.5 µg per lane.

# Secondary

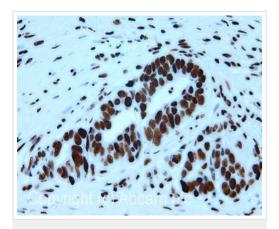
**All lanes :** Goat polyclonal to Rabbit lgG - H&L - Pre-Adsorbed (HRP) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

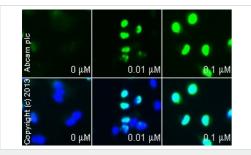
Predicted band size: 15 kDa Observed band size: 17 kDa

Exposure time: 30 seconds



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H3 (acetyl K9) antibody - ChIP Grade (ab10812)

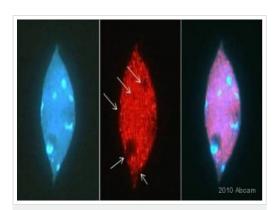
IHC image of Histone H3 (acetyl K9) staining in human breast carcinoma FFPE section, performed on a Bond TM system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab10812, 1µg/ml, for 8 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.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (acetyl K9) antibody - ChIP Grade (ab10812)

<u>ab10812</u> staining histone H3 (acetyl K9) in A549 cells treated with scriptaid (<u>ab120883</u>), by ICC/IF. Increase in histone H3 (acetyl K9) expression correlates with increased concentration of scriptaid, as described in literature.

The cells were incubated at 37°C for 24 hour in media containing different concentrations of <a href="mailto:ab12083">ab12083</a> (scriptaid) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with <a href="mailto:ab10812">ab10812</a> (0.1 µg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A <a href="mailto:anti-rabbit">anti-rabbit</a> <a href="mailto:DyLight 488">DyLight 488</a> secondary antibody (<a href="mailto:ab96899">ab96899</a>) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (acetyl K9) antibody - ChIP Grade (ab10812)

This image was kindly supplied by Ms Vedrana Vicic by Abreview  $\,$ 

ab10812 staining Histone H3 (acetyl K9) in the root tips of *Arabidopsis thaliana* by Immunocytochemistry/
Immunofluorescence. Cells were fixed in 2% paraformaldehyde for 30 minutes at room temperature. Blocking and permeabilization was carried with 4% BSA solution containing 0,5% Triton X-100 in PBS at room temperature for 45 minutes. Slides were washed in PBS and incubated with primary antibody at a 1/200 dilution in 1% PBS for 1 hour at 37°C. Slides were washed in PBS and incubated with the secondary antibody <u>ab6639</u> (Goat anti-rabbit Cy3 ® (H&L)) at a 1/500 dilution in 1% BSA in PBS for 1 hour at 37°C. Slides were counterstained with DAPI (2µg/mL) for 10 minutes at room temperature.Left image: DAPI stained interphase nucleus with prominent chromocentersMiddle image: Distribution of Histone H3 (acetyl K9) in the nucleus (arrows indicate absence of signal from chromocenters)Right image: Merged image

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