### Overview

<table>
<thead>
<tr>
<th>Product name</th>
<th>Anti-Histone H3 (acetyl K9) antibody - ChIP Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>Rabbit polyclonal to Histone H3 (acetyl K9) - ChIP Grade</td>
</tr>
<tr>
<td>Host species</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Specificity</td>
<td>Specific for acetyl K9, but cross-reacts slightly with K27.</td>
</tr>
<tr>
<td>Tested applications</td>
<td><strong>Suitable for:</strong> IHC-Fr, IHC-P, ChIP, IP, WB, CHIPseq, ICC/IF, Flow Cyt</td>
</tr>
</tbody>
</table>
| Species reactivity    | **Reacts with:** Mouse, Rat, Cow, Human, Saccharomyces cerevisiae, Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster, Zebrafish, Common marmoset, Rice  
  **Predicted to work with:** a wide range of other species |
| Immunogen             | Synthetic peptide corresponding to Human Histone H3 aa 1-100 (N terminal) (acetyl K9) conjugated to keyhole limpet haemocyanin.  
  (Peptide available as ab16635) |
| General notes         | Learn about ChIP assay kits, other ChIP antibodies, protocols and more in the ChIP assay guide. |

### Properties

<table>
<thead>
<tr>
<th>Form</th>
<th>Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage instructions</td>
<td>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.</td>
</tr>
</tbody>
</table>
| Storage buffer | Preservative: 0.02% Sodium Azide  
  Constituents: 1% BSA, PBS, pH 7.4 |
| Purity        | Immunogen affinity purified |
| Clonality     | Polyclonal |
| Isotype       | IgG |

### Applications
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.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC-Fr</td>
<td></td>
<td>1/500.</td>
</tr>
<tr>
<td>IHC-P</td>
<td></td>
<td>Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
</tr>
<tr>
<td>ChIP</td>
<td></td>
<td>Use 2-4 µg for 25 µg of chromatin.</td>
</tr>
<tr>
<td>IP</td>
<td></td>
<td>Use at 20 µg/mg of lysate.</td>
</tr>
<tr>
<td>WB</td>
<td></td>
<td>1/500. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).</td>
</tr>
<tr>
<td>CHIPseq</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 22196736</td>
</tr>
<tr>
<td>ICC/IF</td>
<td></td>
<td>Use at a concentration of 1 µg/ml.</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td></td>
<td>Use at an assay dependent concentration. ab171870 - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody.</td>
</tr>
</tbody>
</table>

**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 PADI4 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.
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**

Chromatin was prepared from Hela cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 min. The ChIP was performed with 25 µg of chromatin, 2 µg of ab10812 (blue), and 20 µl of Protein A/G sepharose beads. No antibody was added to the beads control (yellow). 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 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 µg/ml
- **Lane 5:** Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (acetyl K18) peptide (ab24003) at 0.5 µg/ml
- **Lane 6:** Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (acetyl K27) peptide (ab24404) at 0.5 µg/ml
- **Lane 7:** Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (acetyl K23) peptide (ab48359) at 0.5 µg/ml

Lysates/proteins at 0.5 µg per lane.

**Secondary**

- **All lanes:** Goat polyclonal to Rabbit IgG - 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

*why is the actual band size different from the predicted?*

**Exposure time:** 30 seconds
ICC/IF image of ab10812 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 (ab10812, 1µg/ml) 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. This antibody also gave a positive result in 100% Methanol fixed (5 min) Hek293, HepG2, and MCF-7 cells at 5µg/ml.

IHC image of Histone H3 (acetyl K9) staining in human breast carcinoma FFPE section, performed on a Bond™ 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.

**All lanes**: Anti-Histone H3 (acetyl K9) antibody - ChIP Grade (ab10812) at 1/1000 dilution

**Lanes 1-2**: Tissue lysate prepared from rat dorsal spinal cord at 5 µg
**Lanes 3-4**: Tissue lysate prepared from rat dorsal spinal cord at 8 µg
**Lane 5**: Tissue lysate prepared from rat dorsal spinal cord at 8 µg with Human Histone H3 (acetyl K9) peptide (ab16635)

**Secondary**
All lanes: HRP conjugated rabbit polyclonal

Developed using the ECL technique.

**Predicted band size:** 15 kDa

**Observed band size:** 17 kDa why is the actual band size different from the predicted?

**Exposure time:** 2 minutes

Each sample is from a different extract.

ab10812 staining Histone H3 (acetyl K9) in Mouse thyroid tissue sections by Immunohistochemistry (IHC-Fr - frozen sections). Tissue was fixed with paraformaldehyde, permeabilized with 0.1% Tween 20 and blocked with 10% serum for 30 minutes at 24°C. Samples were incubated with primary antibody (1/200 in 10% serum in PBS) for 1 hour at 24°C. An Alexa Fluor® 555-conjugated Goat anti-rabbit IgG polyclonal (1/500) was used as the secondary antibody.

**Immunohistochemistry (Frozen sections) - Anti-Histone H3 (acetyl K9) antibody - ChIP Grade (ab10812)**

This image is courtesy of an Abreview submitted by Annabelle Klein

ab10812 staining histone H3 (acetyl K9) in A549 cells treated with scriptaid (ab120883), 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 ab120883 (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 ab10812 (0.1 μg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A anti-rabbit DyLight 488 secondary antibody (ab96899) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with...
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 ab6639 (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 chromocenters. Middle image: Distribution of Histone H3 (acetyl K9) in the nucleus (arrows indicate absence of signal from chromocenters). Right image: Merged image.

Please note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"