**Product datasheet**

**Anti-Histone H3 (acetyl K9) antibody - ChIP Grade ab4441**

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**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**
In Dot blot detects 50ng of mono-acetylated peptide corresponding to position Lys9 in the N-terminal sequence of Histone H3. Does not detect the mono-acetylated peptide corresponding to acetyl-lysine at position 14 or unacetylated Histone H3.

**Tested applications**
Suitable for: ICC/IF, Dot blot, WB, ChIP, Flow Cyt

**Species reactivity**
Reacts with: Mouse, Rat, Human, Tetrahymena, Xenopus laevis, Caenorhabditis elegans, Drosophila melanogaster, Schizosaccharomyces pombe, Toxoplasma gondii

Predicted to work with: Saccharomyces cerevisiae

**Immunogen**
Synthetic peptide corresponding to Human Histone H3 aa 1-12 (acetyl K9) conjugated to Keyhole Limpet Haemocyanin (KLH).

**Sequence:**
ARTKQTAR(Ac)KSTG-C

**Positive control**
WB: TSA-treated and acid extracted HeLa and NIH/3T3 cells. ICC: polytene chromosomes of Drosophila melanogaster

**Properties**

**Form**
Liquid

**Storage instructions**

**Storage buffer**
pH: 7.40
Preservative: 0.05% Sodium azide
 Constituents: 0.184% Tris glycine, 30% Glycerol, 0.87% Sodium chloride

**Purity**
Protein A purified

**Clonality**
Polyclonal

**Isotype**
IgG
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 (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

Applications
Our Abpromise guarantee covers the use of ab4441 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>
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<tbody>
<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>Dot blot</td>
<td>1/1000.</td>
<td>Detects 50ng of mono-acetylated peptide corresponding to position Lys9 in the N-terminal sequence of Histone H3. Does not detect the mono-acetylated peptide corresponding to acetyl-lysine at position 14 or unacetylated Histone H3.</td>
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<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/10000. Detects a band of approximately 17 kDa.</td>
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<tr>
<td>ChIP</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use 2µg for 10^6 cells.</td>
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<tr>
<td>Flow Cyt</td>
<td>Use at an assay dependent concentration. ab171870 - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody.</td>
<td></td>
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</tbody>
</table>
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 PRKCB2 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 U2OS 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 ab4441 (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.
ChIP analysis using unpurified ab4441 binding Histone H3 in human primary CD34+ cell lysate. Cells were cross-linked for 15 minutes with 1% formaldehyde. Samples were incubated with primary antibody (0.2 µg/µg of chromatin) for 16 hours at 4°C. Protein binding was detected using real-time PCR.

**Positive controls:**
Region upstream of the transcription start site of the ACTB gene.
Region upstream of the transcription start site of the EGR1 gene.

**Negative Controls:**
Region on chromosome 12 (Untr12) that is far from any known gene annotation and not expected to be bound by Histone H3 (acetyl K9).

**All lanes**: Anti-Histone H3 (acetyl K9) antibody - ChIP Grade (ab4441) at 1/2500 dilution

**Lane 1**: Mouse MEL cell nuclear lysate

**Lane 2**: Mouse MEL cell nuclear lysate treated with 0.4 µM Trichostatin A treatment for 18 hours.

Lysates/proteins at 9 µg per lane.

**Secondary**

**All lanes**: Donkey Anti-Rabbit IgG H&L (HRP) (ab6802) at 1/20000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

**Exposure time**: 1 minute
Fruit fly (Drosophila melanogaster) Cell (polytene chromosomes) were fixed in formaldehyde, blocked in 1% BSA for 30 minutes and incubated with ab4441 (1/100) for 12 hours.

**Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (acetyl K9) antibody - ChIP Grade (ab4441)**

This image was submitted as part of a review by Anita Ciurciu.

**Western blot - Anti-Histone H3 (acetyl K9) antibody - ChIP Grade (ab4441)**

**All lanes**: Anti-Histone H3 (acetyl K9) antibody - ChIP Grade (ab4441) at 0.5 µg/ml

- **Lane 1**: Untreated HeLa cell acid-extract
- **Lane 2**: HeLa cell acid-extract treated with sodium butyrate

ab4441, Histone H3, acetylated (Lys9) Pab Rabbit x Human

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