Product datasheet

Anti-Histone H3 (mono methyl K9) antibody - ChIP Grade ab9045

17 Abreviews 62 References 7 Images

Overview

Product name Anti-Histone H3 (mono methyl K9) antibody - ChIP Grade
Description Rabbit polyclonal to Histone H3 (mono methyl K9) - ChIP Grade
Host species Rabbit
Specificity Weak cross reactivity is observed with mono methyl K27 Histone H3. No cross-reactivity is seen with di or tri methyl K27.

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

Species reactivity Reacts with: Mouse, Rat, Cow, Human, Xenopus laevis, Arabidopsis thaliana, Indian muntjac, Schizosaccharomyces pombe
Predicted to work with: Mammals

Immunogen Synthetic peptide within Human Histone H3 aa 1-100 (mono methyl K9) conjugated to Keyhole Limpet Haemocyanin (KLH). The exact sequence is proprietary.
(Peptide available as ab1771)

Positive control Calf Thymus Histone Preparation; Hela whole cell extract

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
Constituents: PBS, 1% BSA

Purity Immunogen affinity purified

Clonality Polyclonal

Isotype IgG

Applications

Our Abpromise guarantee covers the use of ab9045 in the following tested applications.

Our Abpromise guarantee is a commitment to the quality and performance of our antibodies. It ensures that our antibodies perform as expected in the tested applications, providing researchers with confidence in their results. For more information, please visit our website.
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 (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

Application Notes
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>IP</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>WB</td>
<td>1/1000</td>
<td>Detects a band of approximately 15 kDa (predicted molecular weight: 17 kDa). Can be blocked with Histone H3 peptide - mono methyl K9 (ab1771).</td>
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<tr>
<td>IHC-P</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td>1/100</td>
<td>ab171870 - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody.</td>
</tr>
<tr>
<td>ChIP</td>
<td></td>
<td>Use 4-5µg for 10⁶ cells.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
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Target
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 PRKCB 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 ab9045 (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 for active and inactive loci, Sybr green approach for heterochromatic loci). Primers and probes are located in the first kb of the transcribed region.
Anti-mono methyl lysine 9 of histone H3 (green) has a distribution often associated with euchromatic probes (small foci). Most of these foci localize to regions that contain obvious enrichments of DNA with DAPI staining (red). The perinucleolar chromatin is typically a site enriched in monomethylated lysine 9.

Top left: Mono-methyl Lys 9 (ab9045); Bottom left: DAPI; Top right: Merge of ab9045 (green) and DAPI (red).

ab9045 staining rat liver tissue sections by IHC-P. Sections were formaldehyde fixed and subjected to heat mediated antigen retrieval in citrate buffer pH 6.0 prior to blocking with 5% serum for 30 minutes at 20°C. The primary antibody was diluted 1/400 and incubated with the sample for 45 minutes at 20°C. A HRP-conjugated goat anti-rabbit antibody was used as the secondary.
Western blot - Anti-Histone H3 (mono methyl K9) antibody - ChIP Grade (ab9045)

All lanes: Anti-Histone H3 (mono methyl K9) antibody - ChIP Grade (ab9045) at 1 µg/ml

Lane 2: Human Histone H3 (unmodified) peptide (ab7228)

Lane 3: Human Histone H3 (mono methyl K27) peptide (ab1780)

Lane 4: Human Histone H3 (di methyl K27) peptide (ab1781)

Lane 5: Human Histone H3 (tri methyl K27) peptide (ab1782)

Lane 6: Human Histone H3 (mono methyl K4) peptide (ab1340)

Lane 7: Human Histone H3 (mono methyl K9) peptide (ab1771)

Predicted band size: 17 kDa

Rabbit polyclonal to Histone H3 K9 Methyl K9 (1/1000)

Peptides at 1 ug/ml

1XTBS, 5%BSA, 0.5% Tween

This antibody shows significantly greater reactivity with mono methyl K9. This can be seen in lane 7, as the addition of ab1771 (mono methyl K9) completely blocks the activity of ab9045. Weaker cross-reactivity is seen against mono methyl K27. This is shown in lane 3, as the addition of ab1780 only partially blocks the activity of ab9045.

All lanes: Anti-Histone H3 (mono methyl K9) antibody - ChIP Grade (ab9045) at 1 µg/ml

Lane 1: Calf Thymus Histone Preparation Nuclear Lysate (ab121)

Lane 2: Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone
Western blot - Anti-Histone H3 (mono methyl K9) antibody - ChIP Grade (ab9045)

Lane 3 : Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone H3 (mono methyl K4) peptide (ab1340) at 0.5 µg/ml

Lane 4 : Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone H3 (di methyl K4) peptide (ab7768) at 0.5 µg/ml

Lane 5 : Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone H3 (tri methyl K4) peptide (ab1342) at 0.5 µg/ml

Lane 6 : Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone H3 (mono methyl K9) peptide (ab1771) at 0.5 µg/ml

Lane 7 : Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone H3 (di methyl K9) peptide (ab1772) at 0.5 µg/ml

Lane 8 : Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone H3 (tri methyl K9) peptide (ab1773) at 0.5 µg/ml

Lane 9 : Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone H3 (mono methyl K27) peptide (ab1780) at 0.5 µg/ml

Lane 10 : Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone H3 (di methyl K27) peptide (ab1781) at 0.5 µg/ml

Lane 11 : Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone H3 (tri methyl K27) peptide (ab1782) at 0.5 µg/ml

Lysates/proteins at 0.5 µg per lane.

Secondary

All lanes : IRDye 680 Conjugated Goat Anti-Rabbit IgG (H+L) at 1/10000 dilution
Histone H3 (mono methyl K9) was immunoprecipitated using 0.5mg Hela whole cell extract, 5µg of Rabbit polyclonal to Histone H3 (mono methyl K9) and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10min, Hela whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab9045.

Secondary: Anti-rabbit IgG VeriBlot for IP secondary antibody (HRP) (ab131366) at 1/1000 dilution.

Band: 17kDa: Histone H3 (mono methyl K9).
Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (mono methyl K9) antibody - ChIP Grade (ab9045)

ICC/IF image of ab9045 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 (ab9045, 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) HepG2, Hek293 and MCF7 cells at 1µg/ml, and in 4% PFA fixed (10 min) HeLa, Hek293, HepG2 and MCF7 cells at 1µg/ml.

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