Product name: Anti-Histone H3 (mono methyl K4) antibody - ChIP Grade

Description: Rabbit polyclonal to Histone H3 (mono methyl K4) - ChIP Grade

Host species: Rabbit

Specificity: Specific for mono-methylated Lysine 4 of histone H3. Does not recognise di- or tri-methyl Lysine 4 nor methylation at Lysine 9.

Tested applications: Suitable for: IP, Flow Cyt, Electron Microscopy, ICC/IF, Dot blot, ICC, ChIP, WB, ChIPseq, IHC-P, ChIP/Chip

Species reactivity: Reacts with: Mouse, Human, Pig, Saccharomyces cerevisiae, Tetrahymena, Xenopus laevis, Drosophila melanogaster, Plasmodium falciparum, Xenopus tropicalis, Candida albicans

Predicted to work with: Cow, Indian muntjac, Plants, Mammals

Immunogen: Synthetic peptide within Human Histone H3 aa 1-100 (mono methyl K4) conjugated to keyhole limpet haemocyanin. The exact sequence is proprietary. (Peptide available as ab1340)


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

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

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

Our Abpromise guarantee covers the use of ab8895 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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<td>Use at an assay dependent concentration.</td>
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<tr>
<td>Flow Cyt</td>
<td></td>
<td>1/100. ab171870 - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody.</td>
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<td>Electron Microscopy</td>
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<td>Use at an assay dependent concentration. PubMed: 20543957</td>
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<td>ICC/IF</td>
<td></td>
<td>Use a concentration of 1 µg/ml. Works better if cells are fixed with methanol.</td>
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<tr>
<td>Dot blot</td>
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<td>1/1000.</td>
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<td>ICC</td>
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<td>Use at an assay dependent concentration.</td>
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<tr>
<td>ChIP</td>
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<td>Use 2 µg for 25 µg of chromatin.</td>
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<tr>
<td>WB</td>
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<td>1/500. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).</td>
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<td>ChIPseq</td>
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<td>Use at an assay dependent concentration. PubMed: 22196736</td>
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<td>IHC-P</td>
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<td>Use a concentration of 0.5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.</td>
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<td>ChIP/Chip</td>
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<td>Use at an assay dependent concentration.</td>
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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 (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**
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 ab8895 (blue), and 20 µl of Protein A/G sepharose beads. No antibody was added to the beads control (yellow). The immunoprecipitated DNA was quantified on the GAPDH and ALDOA (active) and MYO-D (inactive) promoters and over the γ-Actin gene (active). Schematic diagram of the γ-Actin gene is shown on the top of the figure. Black boxes represent exons and thin lines represent introns. PCR products are depicted as bars under the gene.

All lanes: Anti-Histone H3 (mono methyl K4) antibody - ChIP Grade (ab8895) at 1/500 dilution

Lane 1: Calf thymus histone lysate
Lane 2: Calf thymus histone lysate with Human Histone H3 (mono methyl K4) peptide (ab1340) at 1 µg/ml
Lane 3: Calf thymus histone lysate with Human Histone H3 (di methyl K4) peptide (ab7768) at 1 µg/ml
Lane 4: Calf thymus histone lysate with Human Histone H3 (tri methyl K4) peptide (ab1342) at 1 µg/ml
Lane 5: Calf thymus histone lysate with Human Histone H3 (mono methyl K9) peptide (ab1771) at 1 µg/ml
Lane 6: Calf thymus histone lysate with Human Histone H3 (mono methyl K27) peptide (ab1780) at 1 µg/ml
Lane 7: Calf thymus histone lysate with Human Histone H3 (unmodified) peptide (ab2903) at 1 µg/ml

Secondary
All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab6721) at 1/5000 dilution

Performed under reducing conditions.

Predicted band size: 15 kDa
ab8895 is specific for mono-methylated Lysine 4 of histone H3 and does not recognize di- or tri-methyl Lysine 4 nor methylation at Lysine 9. This is shown in lane 2 where the activity of the antibody is specifically blocked by the addition of the immunizing peptide (ab1340).

ab8895 stained Histone H3 (mono methyl K4) in HeLa cells. The cells were fixed with 100% methanol (5min) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab8895 at 1µg/ml and ab7291 (anti beta Tubulin) at 1µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat anti-rabbit AlexaFluor®488 secondary (ab150081) at 2 μg/ml (shown in green) and a goat anti-mouse AlexaFluor®594 secondary (ab150120) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.

Negative controls: 1– Rabbit primary antibody and anti-mouse secondary antibody; 2 – Mouse primary antibody and anti-rabbit secondary antibody. Controls 1 and 2 indicate that there is no unspecific reaction between primary and secondary antibodies used.

IHC image of ab8895 staining Histone H3 (mono methyl K4) in human colon formalin fixed paraffin embedded tissue sections, performed on a Leica Bond. 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 ab8895, 0.5µg/ml, for 15 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. No primary antibody was used in the negative control (shown on the inset).

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.
Electron Microscopy - Anti-Histone H3 (mono methyl K4) antibody - ChIP Grade (ab8895)


A: Enlarged region showing the distribution of the H3K4me1 labelling around and within the EC cavities of a murine rod photoreceptor.

B: Schematic representation of the labelling for the H3K4me1 mark. The bar represents 260 nm in A and 1 µm in B.

Indian muntjac fibroblast cells - interphase (top left) and prophase (top right and below), stained with:

Mono Methyl K4 antibody, ab8895, (green)
DAPI: red, top left and right; blue, below
Phospho Ser 10 antibody: red (below) and blue (top right)

The perinuclear and perinucleolar heterochromatin domains do not contain Mono Methyl K4. The Mono Methyl K4, rather, is distributed as small nuclear foci primarily found between DAPI-intense regions of the nucleus.

ab8895 used in Dot Blot at a 1/1000 dilution, 18 hours at 4°C.
The dot blot was done using 0.1 μg of peptide and screened multiple histone tail modifications to ensure no cross reactivity.
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