Product name: Anti-Histone H3 (tri methyl K9) antibody - ChIP Grade

Description: Rabbit polyclonal to Histone H3 (tri methyl K9) - ChIP Grade

Host species: Rabbit

Specificity: Specific for Histone H3 tri methyl Lysine 9. Shows slight cross-reactivity with tri methyl K27, which shares a similar epitope (please see Western blot image). Does not react with mono or di methylated K9.

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

Species reactivity: Reacts with: Mouse, Rat, Chicken, Human, Saccharomyces cerevisiae, Xenopus laevis, Drosophila melanogaster, Indian muntjac, Xenopus tropicalis, Cyanidioschyzon merolae

Predicted to work with: Mammals

Immunogen: Synthetic peptide within Human Histone H3 aa 1-100 (N terminal) (tri methyl K9) conjugated to keyhole limpet haemocyanin. The exact sequence is proprietary.

(Peptide available as ab1773)

Positive control: WB: Calf thymus histone preparation and HeLa whole cell extract. IHC-P: Normal human colon tissue.

General notes: Every new batch of this antibody is tested in house in ChIP. Learn about ChIP assay kits, other ChIP antibodies, protocols and more in the ChIP assay guide.

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

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 ab8898 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>1/50.</td>
<td></td>
</tr>
<tr>
<td>IHC-P</td>
<td>1/400.</td>
<td>Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
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<tr>
<td>ICC/IF</td>
<td>1/500.</td>
<td></td>
</tr>
<tr>
<td>ChIP</td>
<td>Use 2-4 µg for 25 µg of chromatin.</td>
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<tr>
<td>WB</td>
<td>Use a concentration of 1 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 15.4 kDa). Can be blocked with Human Histone H3 (tri methyl K9) peptide (ab1773).</td>
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</tr>
<tr>
<td>ChIP/Chip</td>
<td>Use at an assay dependent concentration.</td>
<td></td>
</tr>
<tr>
<td>Flow Cyt</td>
<td>1/100. PubMed: 24713002 ab171870 - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody.</td>
<td></td>
</tr>
<tr>
<td>CHIPseq</td>
<td>Use at an assay dependent concentration. PubMed: 21795385</td>
<td></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 (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 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 ab8898 (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.

X-ChIP assay was performed using nuclear lysates prepared from mouse ES cells. Crosslinking was done for 15 minutes in 1% formaldehyde. Primary antibody was incubated first with peptides ab7228, ab1342, ab1782, ab1773, ab1772 and ab1771 in a chip competition assay and then used in chip at 0.0133 µg/µg chromatin (chip sonication buffer) and incubated with sample for 24 hours at 4°C.

Positive control: ChIP coupled with a peptide competition assay to validate the specificity of the antibody.

Negative control: Genomic region (chr10:79154149-79155200) with no evidence of H3K9me3.

RT-PCR detection method was used.

Polrmt: PCR primers situated in the coding regions of Polymerase (RNA) mitochondrial (DNA directed).

Agrn: PCR primers situated in the coding regions of Agrin

**All lanes**: Anti-Histone H3 (tri methyl K9) antibody - ChIP Grade (ab8898) 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 (ab7228) at 0.5 µg/ml
- **Lane 3**: Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (mono methyl K4) peptide (ab1340) at 0.5 µg/ml
- **Lane 4**: Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (di methyl K4) peptide (ab7768) at 0.5 µg/ml
- **Lane 5**: Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (tri methyl K4) peptide (ab1342) at 0.5 µg/ml
- **Lane 6**: Calf Thymus Histone Preparation Nuclear Lysate with...
Human Histone H3 (mono methyl K9) peptide (ab1771) at 0.5 µg/ml

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

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

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

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

**Lane 11**: Calf Thymus Histone Preparation Nuclear Lysate 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

Performed under reducing conditions.

**Predicted band size**: 15.4 kDa

**Observed band size**: 17 kDa

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

Lane 8 shows that Rabbit polyclonal to Histone H3 (tri methyl K9) is blocked by the addition of the immunizing peptide (ab1773). Cross-reactivity with Histone H3 peptide - tri methyl K27 (ab1782) is also shown in Lane 11.

ab8898 staining H3K9me3 in mouse 3T3MEF by ICC/IF

(Immunocytochemistry/immunofluorescence). Cells were fixed with PFA, permeabilized with CSK buffer and MeOH and blocked with 5% BSA for 30 minutes at 21°C. Samples were incubated with primary antibody (1/500 in PBS +5% BSA + 01% Tween20) for 2 hours at 21°C. An undiluted Alexa Fluor® 546-conjugated Goat anti-rabbit IgG was used as the secondary antibody.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (tri methyl K9) antibody - ChIP Grade (ab8898)

IHC image of ab8898 staining Histone H3 (tri methyl K9) in normal 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 ab8898, 1/400 dilution, 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.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K9) antibody - ChIP Grade (ab8898)

These images were kindly submitted by Prof Bryan Turner, University of Birmingham. Undifferentiated Mouse Embryonic Stem cells or cells differentiated for 7 days were incubated with ab8898. The staining is specific for centromeric heterochromatin on metaphase chromosomes.

Indian muntjac fibroblast cells stained with anti-Histone H3 tri methyl K9, ab8898, (green, left panel, deconvolution image; red, right panel, epifluorescence image).

The centromeres are enriched in Histone H3 tri methyl K9. There are also additional bands that occur throughout the chromosomes. Note that these images are taken in situ and are imaged under conditions where distinct cytogenetic-like banding patterns have not previously been possible to visualize (e.g., several acetylated antibodies have been reported to be associated with chromosome bands but, although not homogenously distributed along in situ chromosomes, they do not generate distinct banding patterns).
Immunocytochemistry/Immunofluorescence - Anti-Histone H3 (tri methyl K9) antibody - ChIP Grade (ab8898)

ICC/IF image of ab8898 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 (ab8898, 0.1µg/ml) overnight at +4°C. The secondary antibody (green) was ab96899, a goat anti-rabbit DyLight® 488 (IgG; H+L) used at a 1/250 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.

Western blot - Anti-Histone H3 (tri methyl K9) antibody - ChIP Grade (ab8898)

All lanes: Anti-Histone H3 (tri methyl K9) antibody - ChIP Grade (ab8898) at 1/1000 dilution

Lane 1: Fruit fly embryo tissue lysate - nuclear at 4.5 µg
Lane 2: Fruit fly embryo tissue lysate - nuclear at 1.5 µg
Lane 3: Fruit fly embryo tissue lysate - nuclear at 0.5 µg

Secondary

All lanes: HRP-conjugated Goat anti-rabbit IgG polyclonal at 1/2000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 15.4 kDa

Observed band size: 15 kDa why is the actual band size different from the predicted?

Additional bands at: 45 kDa (possible non-specific binding)

Exposure time: 1 minute

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