abcam

Product datasheet

Anti-Histone H3 (mono methyl K9) antibody - ChIP Grade ab8896

11 Abreviews 59 References 8 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	Specific for mono-methyl lysine 9 of Histone H3. There is no cross-reactivity with lysine 27 of Histone H3.
Tested applications	**Suitable for:** WB, ChIP, ICC/IF
Species reactivity	**Reacts with:** Cow, Human **Predicted to work with:** Caenorhabditis elegans, a wide range of other species, Mammals
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab1771**)
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

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

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab8896 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★★ (5)	Use a concentration of 0.5 - 2 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).
ChIP	★★★★★ (2)	Use 2-25 µg for µg of chromatin.
ICC/IF	★★★★★ (3)	Use at an assay dependent concentration.

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.

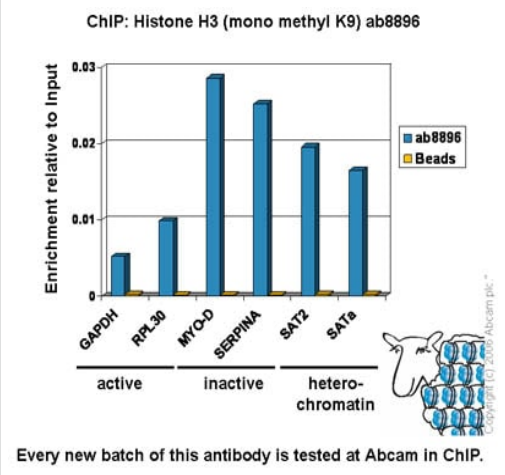
Developmental stage Expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation.

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.

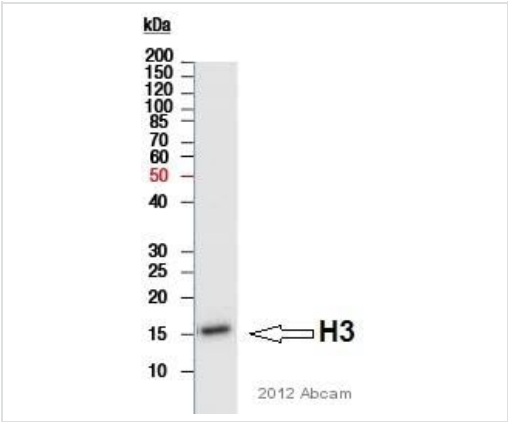
Cellular localization Nucleus. Chromosome.

Images



ChIP - Anti-Histone H3 (mono methyl K9) antibody - ChIP Grade (ab8896)

Chromatin was prepared from Hela cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10min. The ChIP was performed with 25µg of chromatin, 2µg of ab8896 (blue), and 20µl 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.



Western blot - Anti-Histone H3 (mono methyl K9) antibody - ChIP Grade (ab8896)

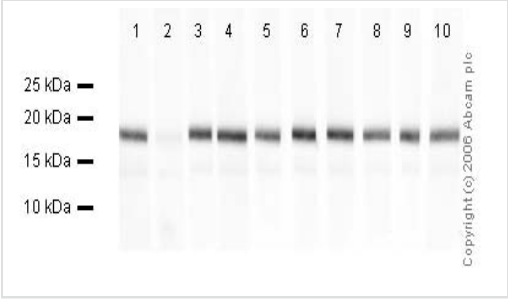
This image is courtesy of an anonymous Abreview

Anti-Histone H3 (mono methyl K9) antibody - ChIP Grade (ab8896) at 1/300 dilution (Incubated for 10 minutes at 20°C. Diluent buffer used was 0.5% milk in TBS, 0.1% Tween.) + HUH-7 cells at 15 µg

Secondary

Sheep anti-rabbit IgG (HRP) at 1/20000 dilution

Predicted band size: 15 kDa



Western blot - Anti-Histone H3 (mono methyl K9) antibody - ChIP Grade (ab8896)

All lanes : Anti-Histone H3 (mono methyl K9) antibody - ChIP Grade (ab8896) at 1 µg/ml

Lane 1 : Calf Thymus Histone Preparation
Lane 2 : Calf Thymus Histone Preparation with Human Histone H3 (mono methyl K9) peptide (**ab1771**) at 0.5 µg/ml
Lane 3 : Calf Thymus Histone Preparation with Human Histone H3 (di methyl K9) peptide (**ab1772**) at 0.5 µg/ml
Lane 4 : Calf Thymus Histone Preparation with Human Histone H3 (tri methyl K9) peptide (**ab1773**) at 0.5 µg/ml
Lane 5 : Calf Thymus Histone Preparation with Human Histone H3 (mono methyl K27) peptide (**ab1780**) at 0.5 µg/ml
Lane 6 : Calf Thymus Histone Preparation with Human Histone H3 (di methyl K27) peptide (**ab1781**) at 0.5 µg/ml
Lane 7 : Calf Thymus Histone Preparation with Human Histone H3 (tri methyl K27) peptide (**ab1782**) at 0.5 µg/ml
Lane 8 : Calf Thymus Histone Preparation with Human Histone H3 (mono methyl K4) peptide (**ab1340**) at 0.5 µg/ml
Lane 9 : Calf Thymus Histone Preparation with Human Histone H3 (unmodified) peptide (**ab7228**) at 0.5 µg/ml
Lane 10 : Calf Thymus Histone Preparation with Human Histone H3 (unmodified) peptide (**ab2623**) at 0.5 µg/ml

Lysates/proteins at 0.5 µg per lane.

Secondary

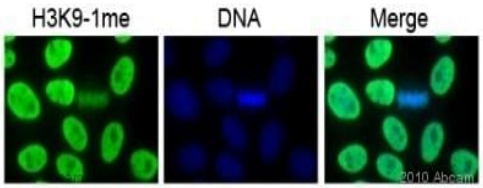
All lanes : Goat polyclonal to Rabbit IgG H&L (HRP) Pre-Adsorbed

at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 15 kDa
Observed band size: 17 kDa

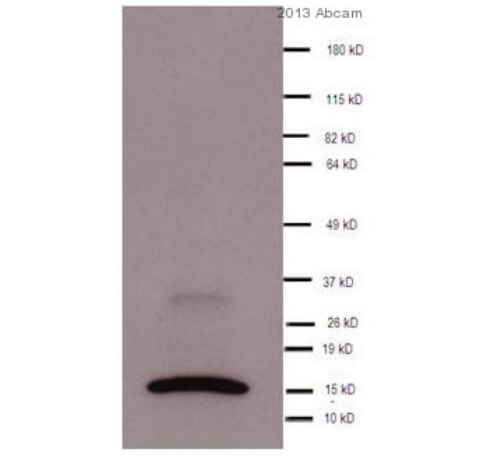
ab8896 specifically recognises mono-methyl K9 (lane 1) in calf thymus lysate at 17kDa. ab8896 is successfully blocked using the immunizing peptide (lane 2 **ab1771**), but not the di-methyl K9 (lane 3 **ab1772**), the tri-methyl K9 (lane 4 **ab1773**), the mono-methyl K27 (lane 5 **ab1780**), the di-methyl K27 (lane 6 **ab1781**), the tri-methyl K27 (lane 7 **ab1782**), the mono-methyl K4 (lane 8 **ab1340**) nor the corresponding unmodified peptides (lane 9 **ab7228**, lane 10 **ab2623**). This implies that ab8896 is specific to mono-methyl K9.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (mono methyl K9) antibody - ChIP Grade (ab8896)

This image is courtesy of an anonymous Abreview

Ab8896 staining Histone H3 (mono methyl K9) in HeLa (Cervical cancer cell line) cells by ICC/IF (Immunocytochemistry/Immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized with 0.3% Triton/TBS and blocked with 2% serum for 30 minutes at 25°C. Samples were incubated with primary antibody at 1/100 dilution for 1 hour at 25°C. An Alexa Fluor® 488-conjugated Goat anti-rabbit was used as a secondary antibody at a 1/1000 dilution.



Western blot - Anti-Histone H3 (mono methyl K9) antibody - ChIP Grade (ab8896)

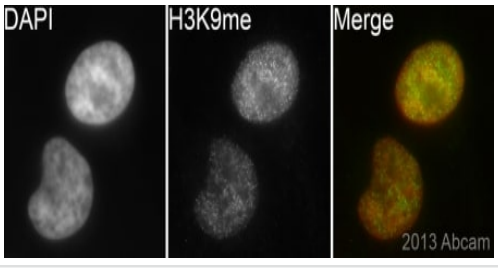
This image is courtesy of an anonymous Abreview

Diluent: TBS + 0.1% Tween 20 + 5% BSA. Incubated for 12 hours at 4°C + HeLa whole cell lysates at 20 µg

Secondary
Anti-rabbit IgG, HRP-linked Antibody at 1/2000 dilution

Predicted band size: 15 kDa

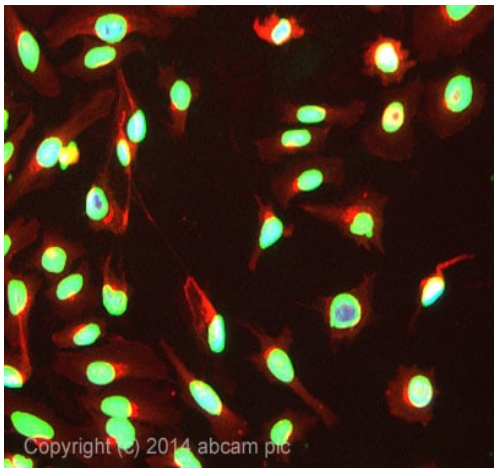
Exposure time: 1 minute



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (mono methyl K9) antibody - ChIP Grade (ab8896)

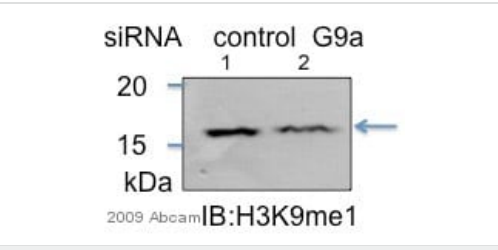
This image is courtesy of an Abreview submitted by Kirk Mcmanus.

Ab8896 staining Histone H3 (mono methyl K9) in HeLa cells by ICC/IF (Immunocytochemistry/Immunofluorescence). Cells were fixed with paraformaldehyde and permeabilized with 0.05% Triton X100 in PBS. Samples were incubated with primary antibody at 1/200 dilution in PBS for 1 hour at 22°C. An Alexa Fluor® 488-conjugated Goat anti-rabbit was used as the secondary antibody at a 1/200 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (mono methyl K9) antibody - ChIP Grade (ab8896)

ICC/IF image of ab8896 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 ab8896 at 1µg/ml overnight at +4°C. The secondary antibody (pseudo-colored green) was a goat **anti-rabbit Alexa Fluor® 488** (**ab150081**) IgG (H+L) preadsorbed, used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (pseudo-colored red) at a 1/200 dilution for 1h at room temperature. DAPI was used to stain the cell nuclei (pseudo-colored blue) at a concentration of 1.43µM for 1hour at room temperature.



Western blot - Anti-Histone H3 (mono methyl K9) antibody - ChIP Grade (ab8896)

This image is courtesy of an anonymous abreview.

All lanes : Anti-Histone H3 (mono methyl K9) antibody - ChIP Grade (ab8896) at 1/1000 dilution

Lane 1 : Nuclear lysates prepared from Hela cell: SiRNA untreated
Lane 2 : Nuclear lysates prepared from Hela cells treated with G9a SiRNA

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Alkaline Phosphatase conjugated rabbit polyclonal rabbit IgG (Fc) at 1/5000 dilution

Predicted band size: 15 kDa
Observed band size: 16 kDa

Exposure time: 10 minutes

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