

Product datasheet

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

★★★★☆ 17 Abreviews 71 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	WB: Calf thymus histone preparation and Hela whole cell extract. ICC/IF: HeLa cell line

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.

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

Application	Abreviews	Notes
IP		Use at an assay dependent concentration.
WB	★★★★☆	1/1000. Detects a band of approximately 15 kDa (predicted molecular weight: 17 kDa). Can be blocked with Histone H3 peptide - mono methyl K9 (ab1771).
IHC-P	★★★★★	Use at an assay dependent concentration.
Flow Cyt		1/100. ab171870 - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody.
ChIP	★★★★★	Use 4-5µg for 10 ⁶ cells.
ICC/IF	★★★★☆	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 PAD4 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

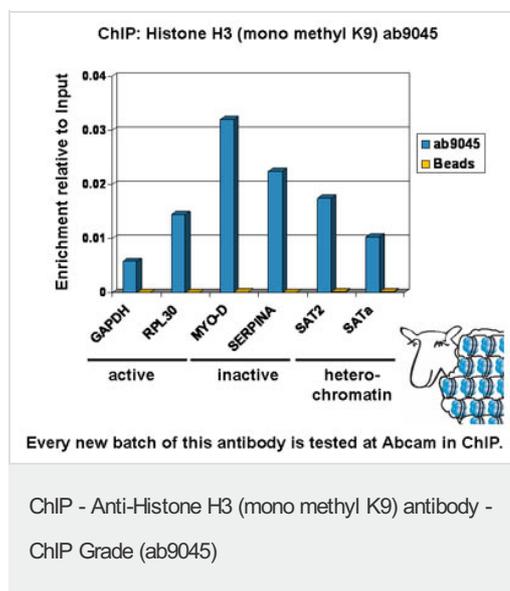
condensation and cell-cycle progression during mitosis and meiosis. In addition phosphorylation at Ser-11 (H3S10ph) by RPS6KA4 and RPS6KA5 is important during interphase because it enables the transcription of genes following external stimulation, like mitogens, stress, growth factors or UV irradiation 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.

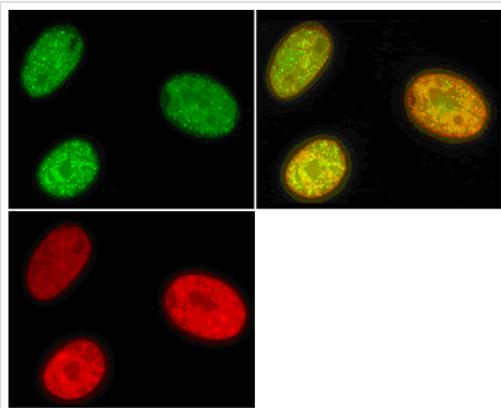
Cellular localization

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 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.



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

This image is courtesy of Kirk McManus in the lab of Michael Hendzel, University of Alberta

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.

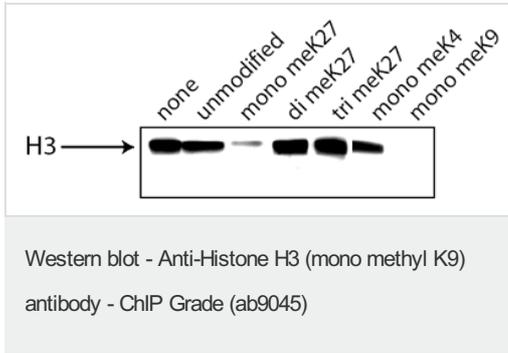
Top left: Mono-methyl Lys 9 (ab9045); Bottom left: DAPI; Top right: Merge of ab9045 (green) and DAPI (red).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (mono methyl K9) antibody - ChIP Grade (ab9045)

This image is courtesy of an anonymous Abreview

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.



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](#))

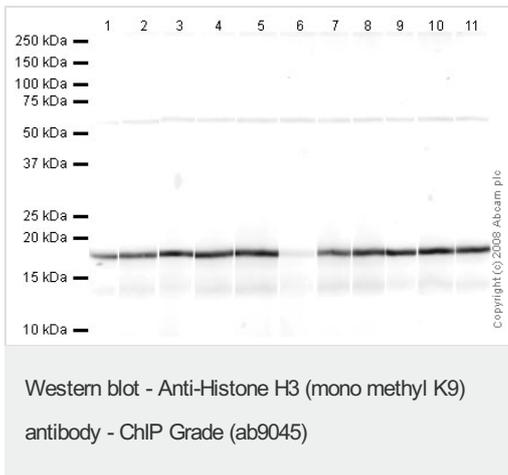
Predicted band size: 17 kDa

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 H3 (unmodified) peptide ([ab7228](#)) at 0.5 µg/ml

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

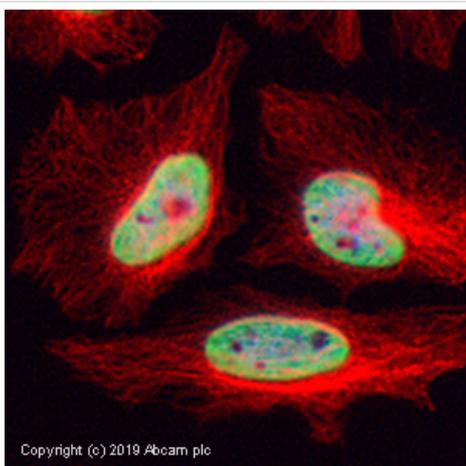
Lysates/proteins at 0.5 µg per lane.

Secondary

All lanes : IRDye 680 Conjugated Goat Anti-Rabbit IgG (H+L) at 1/10000 dilution

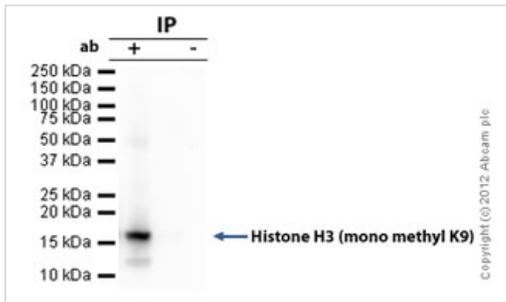
Performed under reducing conditions.

Predicted band size: 17 kDa



ab9045 staining Histone H3 (mono methyl K9) in HeLa cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab9045 at 1 µg/ml then detected with an Alexa Fluor[®] 488 goat anti-rabbit secondary antibody ([ab150081](#)) at a 1/1000 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue), and [ab195889](#), Mouse monoclonal to alpha Tubulin (Alexa Fluor[®] 594), at a 1/250 dilution (shown in red).

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



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

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).

Please note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <https://www.abcam.com/abpromise> or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors