


Product datasheet

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

★★★★★ [18 Abreviews](#) [108 References](#) [6 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, ChIP, ICC/IF
Species reactivity	Reacts with: Cow, Human, Arabidopsis thaliana Predicted to work with: Mouse, Rat, Saccharomyces cerevisiae, Xenopus laevis, Indian muntjac, Schizosaccharomyces pombe, Mammals 
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as ab1771)
General notes	<p>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.</p> <p>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</p>

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	<p>pH: 7.40</p> <p>Preservative: 0.02% Sodium azide</p> <p>Constituent: PBS</p> <p>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</p>

	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 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	★★★★★ (7)	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).
ChIP	★★★★★ (1)	Use 4-5µg for 10 ⁶ cells.
ICC/IF	★★★★★ (4)	Use at an assay dependent concentration.

Target

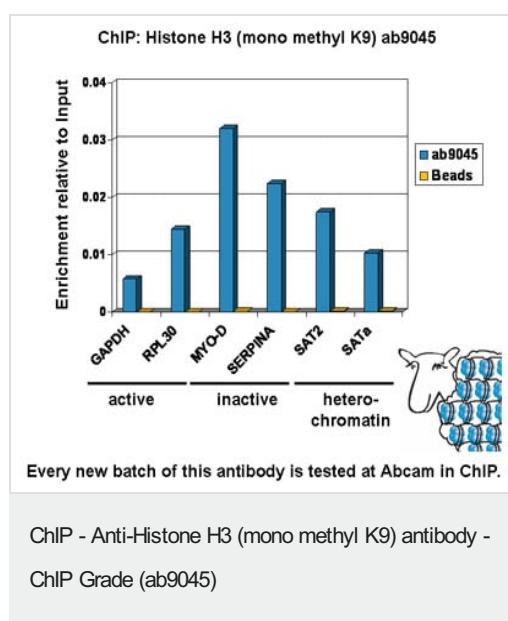
Function	Core component of nucleosome. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA 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	<p>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).</p> <p>Citrullination at Arg-9 (H3R8ci) and/or Arg-18 (H3R17ci) by PAD4 impairs methylation and represses transcription.</p> <p>Asymmetric dimethylation at Arg-18 (H3R17me2a) by CARM1 is linked to gene activation.</p> <p>Symmetric dimethylation at Arg-9 (H3R8me2s) by PRMT5 is linked to gene repression.</p> <p>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.</p> <p>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.</p> <p>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</p>

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.

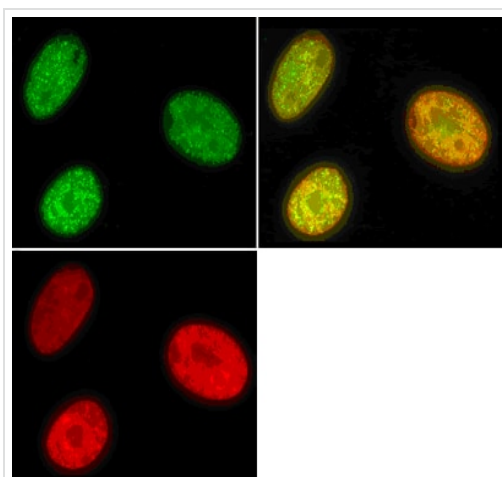
Cellular localization

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.

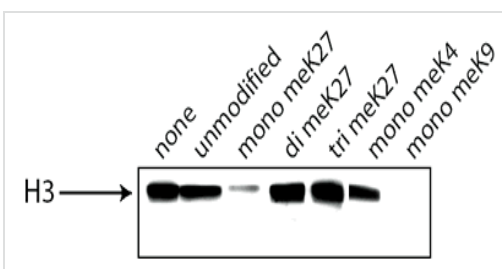


Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (mono methyl K9) antibody - ChIP Grade (ab9045)

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).



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](#))

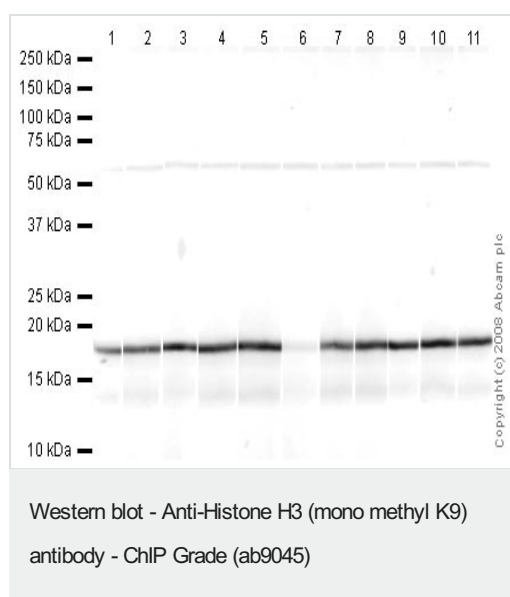
Predicted band size: 17 kDa

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

Peptides at 1 µg/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

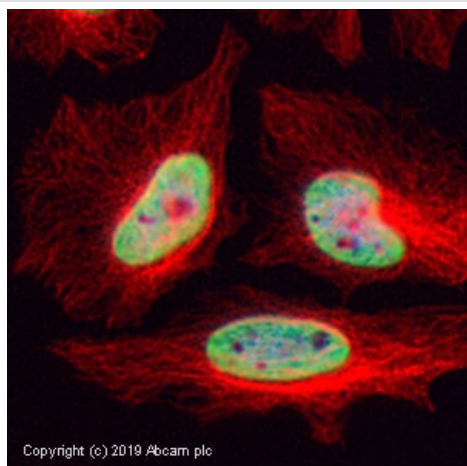
Lysates/proteins at 0.5 µg per lane.

Secondary

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

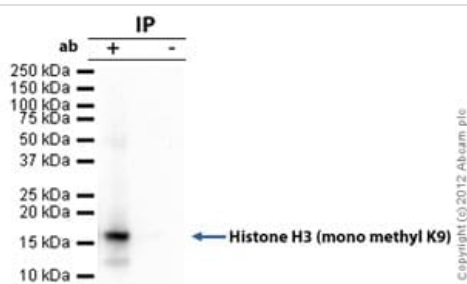
Performed under reducing conditions.

Predicted band size: 17 kDa



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

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-Triton for 1h. The cells were then incubated with the antibody ab9045 at 0.1 µg/ml and **ab7291** (Mouse monoclonal to alpha Tubulin - Loading Control) used at a 1/1000 dilution overnight at +4°C. The secondary antibodies were **ab150081**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed, (pseudo-colored green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594) preadsorbed, (colored red), both used at a 1/1000 dilution for 1 hour at room temperature. DAPI was used to stain the cell nuclei (colored blue) at a concentration of 1.43 µM for 1 hour at room temperature.



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, 5 µg of Rabbit polyclonal to Histone H3 (mono methyl K9) and 50 µl 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 40 µl SDS loading buffer and incubated for 10min at 70°C; 10 µl 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 Detection Reagent (HRP) (ab131366)** at 1/1000 dilution.

Band: 17kDa: Histone H3 (mono methyl K9).

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