abcam

Product datasheet

Anti-Histone H3 (tri methyl K36) antibody - ChIP Grade ab9050

64 Abreviews 904 References 5 Images

Overview

Product name	Anti-Histone H3 (tri methyl K36) antibody - ChIP Grade
Description	Rabbit polyclonal to Histone H3 (tri methyl K36) - ChIP Grade
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, WB, ChIP
Species reactivity	**Reacts with:** Cow, Human **Predicted to work with:** Mouse, Rat, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster, Plants, Schizosaccharomyces pombe, Zebrafish, Silk worm, Rice, Xenopus tropicalis, Trypanosoma brucei
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab1785**)
Positive control	ICC: HeLa and Saos-2 cells. WB: Calf Thymus Histone Preparation Nuclear Lysate ChIP: Chromatin prepared from U-2 OS.
General notes	For detection of methylated histone H3. Learn about ChIP assay kits, other ChIP antibodies, protocols and more in the **ChIP assay guide**. 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

The concentration of this product may vary between lots.
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

Primary antibody notes For detection of methylated histone H3

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab9050 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
ICC/IF	★★★★★ **(11)**	Use a concentration of 0.1 - 1 µg/ml. We recommend using **Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) secondary antibody**.
WB	★★★★★ **(19)**	Use a concentration of 1 µg/ml. Detects a band of approximately 15 kDa (predicted molecular weight: 15 kDa).
ChIP	★★★★★ **(17)**	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.

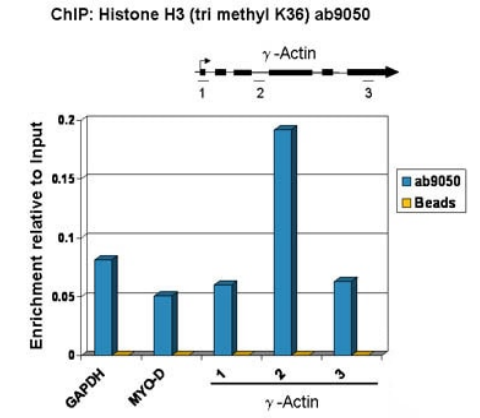
Developmental stage Expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation.

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 (tri methyl K36) antibody - ChIP Grade (ab9050)

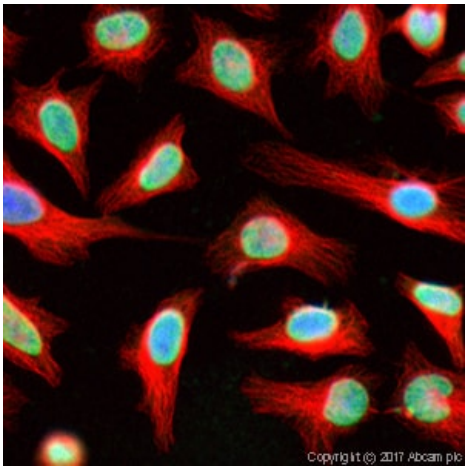
Chromatin was prepared from U-2 OS (Human bone osteosarcoma epithelial cell line) cells according to the Abcam X-ChIP protocol.

Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25 µg of chromatin, 2 µg of ab9050 (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 (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.

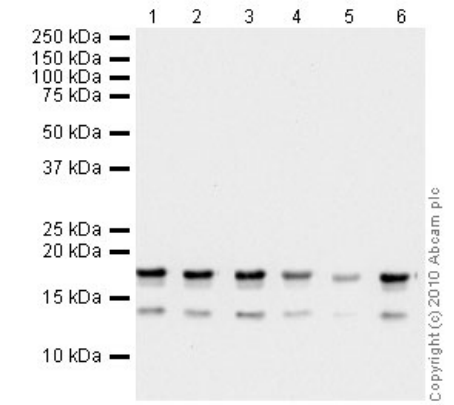


Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K36) antibody - ChIP Grade (ab9050)

ab9050 stained in HeLa (Human epithelial cell line from cervix adenocarcinoma) cells.

Cells were fixed with 100% methanol for 5 minutes at room temperature and incubated with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% triton for 1 hour at room temperature to permeabilize the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab9050 at 0.1 µg/ml and **ab7291** (Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control) at 1/1000 dilution overnight at +4°C. The secondary antibodies were Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (**ab150120**) (pseudo-colored red) and Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (**ab150081**) secondary antibody (colored green) used at 1 µg/ml 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.



Western blot - Anti-Histone H3 (tri methyl K36) antibody - ChIP Grade (ab9050)

All lanes : Anti-Histone H3 (tri methyl K36) antibody - ChIP Grade (ab9050) at 1 µg/ml

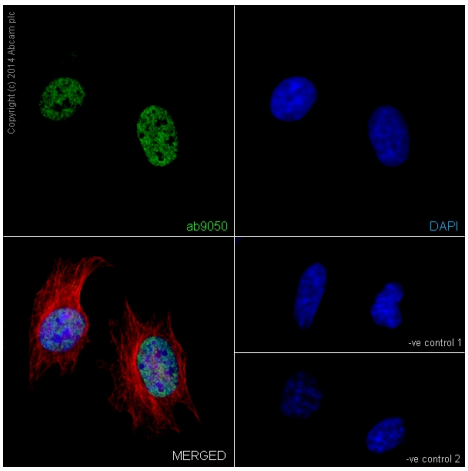
Lane 1 : Calf Thymus Histone Preparation Nuclear Lysate
Lane 2 : Calf Thymus Histone Preparation Nuclear Lysate with Histone H3 peptide - unmodified K36 at 0.5 µg/ml
Lane 3 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (mono methyl K36) peptide (**ab1783**) at 0.5 µg/ml
Lane 4 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (di methyl K36) peptide (**ab1784**) at 0.5 µg/ml
Lane 5 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (tri methyl K36) peptide (**ab1785**) at 0.5 µg/ml
Lane 6 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (tri methyl K37) peptide (**ab24417**) at 0.5 µg/ml

Lysates/proteins at 0.5 µg per lane.

Secondary
All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Performed under reducing conditions.

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



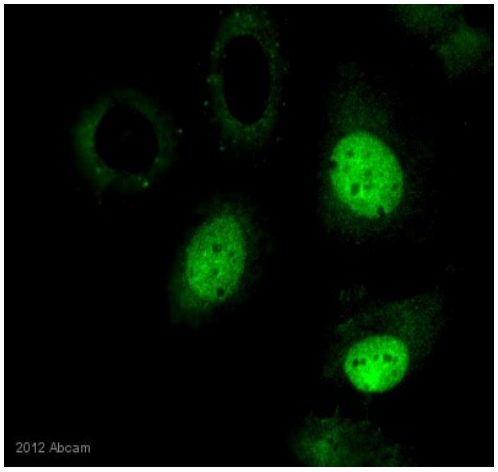
Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K36) antibody - ChIP Grade (ab9050)

ab9050 staining Histone H3 (tri-methyl K36) in HeLa (Human epithelial cell line from cervix adenocarcinoma) cells.

The cells were fixed with 100% methanol for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1 hour. The cells were then incubated with ab9050 at 0.1 µg/ml and **ab7291** (anti beta-Tubulin) at 1 µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1 hour with a goat anti-rabbit AlexaFluor®488 secondary (**ab150081**) at 2 µg/ml (shown in green) and a goat anti-mouse AlexaFluor®594 (**ab150120**) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labeled 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.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K36) antibody - ChIP Grade (ab9050)

This image is courtesy of an anonymous Abreview

ab9050 staining Histone H3 (tri methyl K36) in Saos-2 (Human osteosarcoma cell line) cells by ICC/IF (Immunocytochemistry/immunofluorescence).

Cells were fixed with paraformaldehyde, permeabilized with 0.25% Triton in PBS and blocked with 1% BSA for 1 hour at room temperature. Samples were incubated with primary antibody (1/1000) for 1 hour. An Alexa Fluor®488-conjugated Goat anti-rabbit IgG polyclonal (1/250) was used as the secondary antibody.

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