# abcam

## Product datasheet

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

★★★★★ 64 Abreviews 904 References 5 图像

概述

产品名称 Anti-Histone H3 (tri methyl K36)抗体- ChIP Grade

描述 兔多克隆抗体to Histone H3 (tri methyl K36) - ChIP Grade

宿主 Rabbit

经测试应用 适用于: ICC/IF, WB, ChIP

种属反应性 与反应: Cow, Human

> 预测可用于: Mouse, Rat, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster, Plants, Schizosaccharomyces pombe,

Zebrafish, Silk worm, Rice, Xenopus tropicalis, Trypanosoma brucei

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

(Peptide available as ab1785)

阳性对照 ICC: HeLa and Saos-2 cells. WB: Calf Thymus Histone Preparation Nuclear Lysate ChIP:

Chromatin prepared from U-2 OS.

常规说明 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 guestions, 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

性能

形式 Liquid

存放说明 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.

存储溶液 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.

纯**度** Immunogen affinity purified

**Primary antibody**说明 For detection of methylated histone H3

应用

#### The Abpromise guarantee

Abpromise™承诺保证使用ab9050于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ICC/IF	<b>★★★★★ (11)</b>	Use a concentration of 0.1 - 1 µg/ml.  We recommend using <b>Goat Anti-Rabbit IgG H&amp;L (Alexa Fluor 9 488) preadsorbed (ab150081) secondary antibody</b> .
WB	**** (19)	Use a concentration of 1 µg/ml. Detects a band of approximately 15 kDa (predicted molecular weight: 15 kDa).
ChIP	<b>★★★★★ (17)</b>	Use at an assay dependent concentration.

#### 靶标

功能

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.

序列相似性

Belongs to the histone H3 family.

发展阶段

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

翻译后修饰

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.

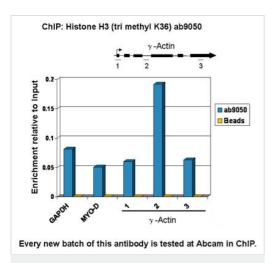
Phosphorylated at Thr-4 (H3T3ph) by GSG2/haspin during prophase and dephosphorylated during anaphase. Phosphorylation at Ser-11 (H3S10ph) by AURKB is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis. In addition phosphorylation at Ser-11 (H3S10ph) by RPS6KA4 and 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.

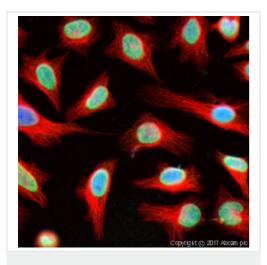
细胞定位

Nucleus. Chromosome.

图片



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



Immunocytochemistry/ Immunofluorescence - 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  $\mu$ g of chromatin, 2  $\mu$ g of ab9050 (blue), and 20  $\mu$ l 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  $\gamma$ -Actin gene (active).

Schematic diagram of the  $\gamma$ -Actin gene is shown on the top of the figure.

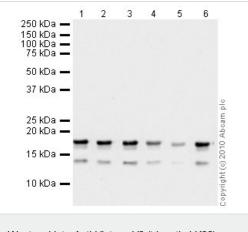
Black boxes represent exons and thin lines represent introns.

PCR products are depicted as bars under the gene.

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 lgG H&L (Alexa Fluor<sup>®</sup> 594) preadsorbed (ab150120) (pseudo-colored red) and Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 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  $1.43 \mu M$  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  $0.5 \ \mu g/ml$ 

**Lane 3 :** Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (mono methyl K36) peptide (ab1783) at 0.5  $\mu$ g/ml

**Lane 4**: Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (di methyl K36) peptide (<u>ab1784</u>) at 0.5 μg/ml **Lane 5**: Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (tri methyl K36) peptide (<u>ab1785</u>) at 0.5 μg/ml **Lane 6**: Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (tri methyl K37) peptide (<u>ab24417</u>) at 0.5 μg/ml

Lysates/proteins at 0.5 µg per lane.

#### Secondary

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

Performed under reducing conditions.

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

ab9050 DAPI

we control 1

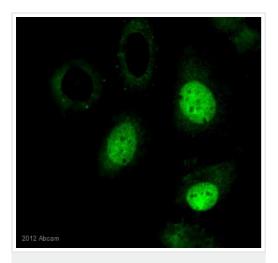
MERGED

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  $\mu$ g/ml and <u>ab7291</u> (anti beta-Tubulin) at 1  $\mu$ g/ml overnight at +4°C, followed by a further incubation at room temperature for 1 hour with a goat anti-rabbit AlexaFluor<sup>®</sup>488 secondary (<u>ab150081</u>) at 2  $\mu$ g/ml (shown in green) and a goat anti-mouse AlexaFluor<sup>®</sup>594 (<u>ab150120</u>) at 2  $\mu$ g/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<sup>®</sup>488-conjugated Goat anti-rabbit IgG polyclonal (1/250) was used as the secondary antibody.

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