abcam

Product datasheet

Anti-Histone H3 antibody - ChIP Grade ab46765

12 References　5 图像

概述

产品名称	Anti-Histone H3抗体- ChIP Grade
描述	兔多克隆抗体to Histone H3 - ChIP Grade
经测试应用	**适用于:** IP, IHC-P, ChIP, ICC/IF, WB
种属反应性	**与反应:** Rat, Human, Saccharomyces cerevisiae
免疫原	Synthetic peptide corresponding to Saccharomyces cerevisiae Histone H3 aa 100 to the C-terminus conjugated to Keyhole Limpet Haemocyanin (KLH). Database link: P68431 (Peptide available as ab46990)
阳性对照	WB: Hela, PC12, S.cerevisiae and S.pombe whole cell lysates and calf thymus histone prep. nuclear lysate. ICC/IF: HeLa cells.

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	Preservative: 0.02% Sodium Azide Constituents: 1% BSA, PBS, pH 7.4
纯度	Immunogen affinity purified
克隆	**多克隆**
同种型	IgG

应用

Our Abpromise guarantee covers the use of **ab46765** in the following tested applications.

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

应用	Ab 评论	说明
IP		Use a concentration of 5 µg/ml.

IHC-P	Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ChIP	Use 2-25 µg for µg of chromatin. PubMed: 20007597 ChIP protcol helped a customer obtain positive results for S.cerevisiae with ab46765.
ICC/IF	Use a concentration of 1 µg/ml.
WB	Use a concentration of 0.1 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).Can be blocked with *S. cerevisiae* Histone H3 peptide (ab46990).

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.

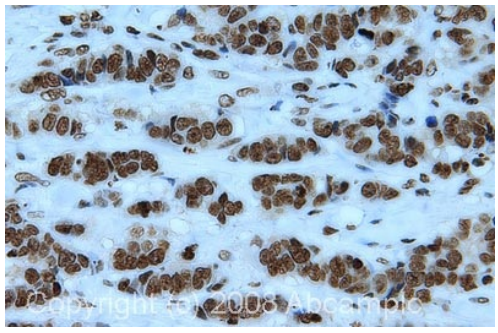
翻译后修饰

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 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.

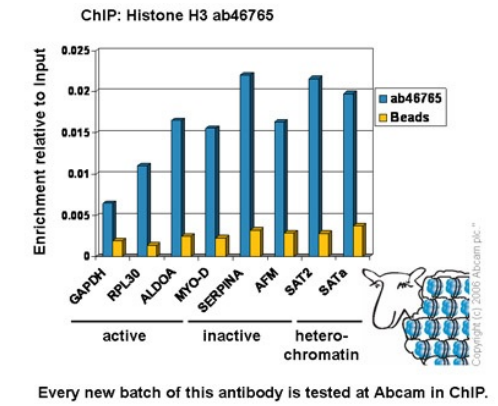
细胞定位 Nucleus. Chromosome.

Anti-Histone H3 antibody - ChIP Grade 图像



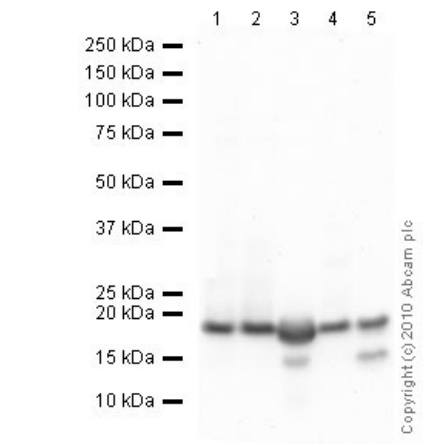
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Histone H3 antibody - ChIP Grade (ab46765)

IHC image of Histone H3 staining in human breast carcinoma FFPE section, performed on a Bond^{TM} system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab46765, 5µg/ml, for 8 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.



ChIP - Histone H3 antibody - ChIP Grade (ab46765)

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 ab46765 (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 - Histone H3 antibody - ChIP Grade (ab46765)

All lanes : Anti-Histone H3 antibody - ChIP Grade (ab46765) at 1 µg/ml

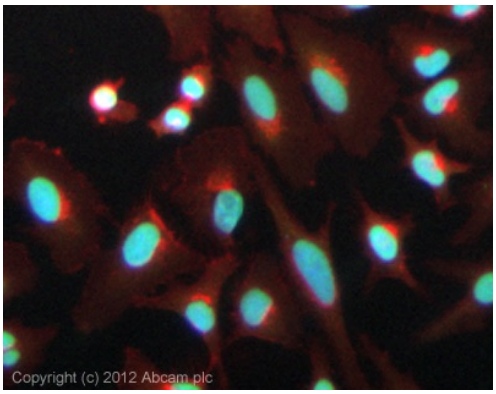
Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate at 10 µg
Lane 2 : PC12 (Rat adrenal pheochromocytoma cell line) Whole Cell Lysate at 10 µg
Lane 3 : S.cerevisiae (Y190) Whole Cell Lysate at 10 µg
Lane 4 : S.pombe Whole Cell Lysate at 10 µg
Lane 5 : Calf Thymus Histone Preparation Nuclear Lysate at 0.5 µg

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

Performed under reducing conditions.

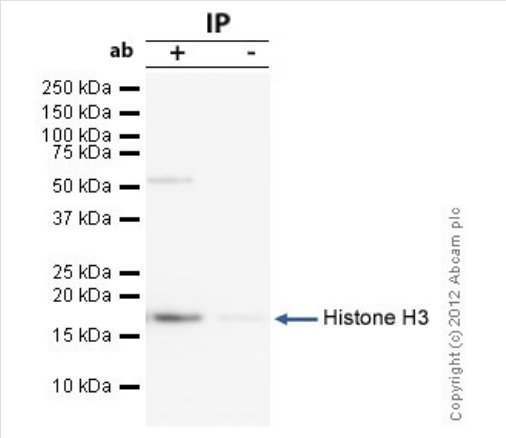
Predicted band size : 15 kDa
Observed band size : 17 kDa

Exposure time : 4 minutes



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 antibody - ChIP Grade (ab46765)

ICC/IF image of ab46765 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 (ab46765, 1µg/ml) overnight at +4°C. The secondary antibody (green) was ab96899, a goat anti-rabbit DyLight® 488 (IgG; H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Immunoprecipitation - Anti-Histone H3 antibody - ChIP Grade (ab46765)

Histone H3 was immunoprecipitated using 0.5mg Hela whole cell extract, 5µg of Rabbit polyclonal to Histone H3 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^{o}C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab46765.
Secondary: Clean-Blot IP Detection Reagent (HRP) at 1/500 dilution.
Band: 17kDa; Histone H3

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