abcam

Product datasheet

Anti-Histone H3 (acetyl K9 + K14) antibody ab232952

6 Images

Overview

Product name	Anti-Histone H3 (acetyl K9 + K14) antibody
Description	Rabbit polyclonal to Histone H3 (acetyl K9 + K14)
Host species	Rabbit
Tested applications	**Suitable for:** WB, ChIP, Dot blot, ICC/IF, ChIP-sequencing
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Arabidopsis thaliana, Zebrafish, Aspergillus nidulans
Immunogen	Synthetic peptide corresponding to Human Histone H3 (acetyl K9 + K14) conjugated to keyhole limpet haemocyanin. Database link: **P68431**
Positive control	ChIP: Chromatin from HeLa cells. ChIPseq: Chromatin from HeLa S3 cells. WB: HeLa histone extract. ICC/IF: NIH/3T3 cells.
General notes	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 long term. Avoid freeze / thaw cycle.
Storage buffer	Preservatives: 0.05% Sodium azide, 0.05% Proclin 300 Constituent: PBS
Purity	Affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab232952 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
WB		1/1000. Predicted molecular weight: 15 kDa.
ChIP		Use at an assay dependent concentration. Use 1 - 2 µg for 1.5 x 10^6 cells.
Dot blot		1/20000.
ICC/IF		1/500.
ChIP-sequencing		Use 1µg for 10^6 cells.

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.

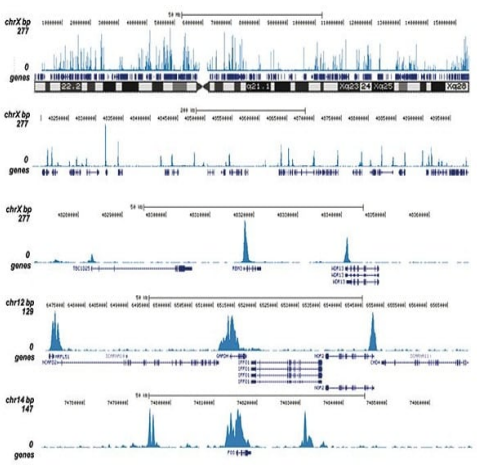
Developmental stage Expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation.

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.

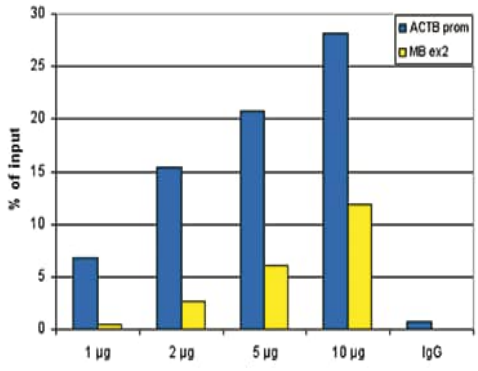
Cellular localization Nucleus. Chromosome.

Images



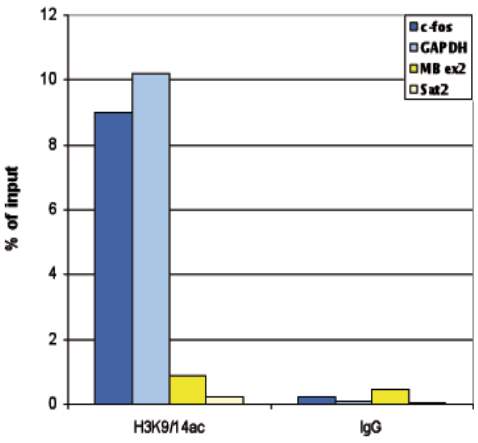
ChIP-sequencing - Anti-Histone H3 (acetyl K9 + K14) antibody (ab232952)

ChIP was performed with 1 µg ab232952 against Histone H3 (acetyl K9 + K14) on sheared chromatin from 1 million HeLa S3 cells. IgG (2 µg/IP) was used as a negative IP control. The IP'd DNA was analysed by QPCR with optimized PCR primer pairs for the promoters of the active GAPDH and c-fos genes, used as positive control targets, and the coding region of the inactive MB gene and the Sat2 satellite repeat, used as negative control targets. The IP'd DNA was subsequently analysed with an Illumina Genome Analyzer. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 36 bp tags were aligned to the human genome using the ELAND algorithm. The figure shows the peak distribution along the complete sequence and a 800 kb region of the X-chromosome (A and B) and in 100 kb regions surrounding the RBM3, GAPDH and c-fos genes (C, D and E). These results clearly show an enrichment of the H3K9/14 double acetylation at the promoters of active genes.



ChIP - Anti-Histone H3 (acetyl K9 + K14) antibody (ab232952)

ChIP assays were performed using HeLa (human epithelial cell line from cervix adenocarcinoma) cells, ab232952 against Histone H3 (acetyl K9 + K14) and optimized primer pairs for qPCR. ChIP was performed using sheared chromatin from 1.5 million cells. A titration of ab232952 consisting of 1, 2, 5 and 10 µg per ChIP experiment was analysed. IgG (5 µg/IP) was used as negative IP control. QPCR was performed using primers specific for the promoter of the ACTB gene as a positive control target and for exon 2 of the MB gene as a negative control target. The figure shows the recovery (the relative amount of immunoprecipitated DNA compared to input DNA). These results confirm the observation that acetylation of H3K9/14 is present at active promoters.



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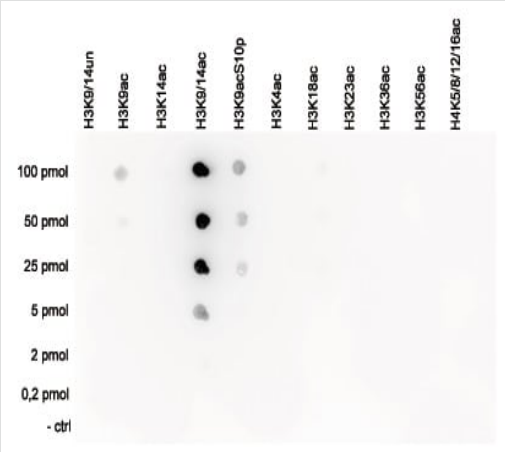
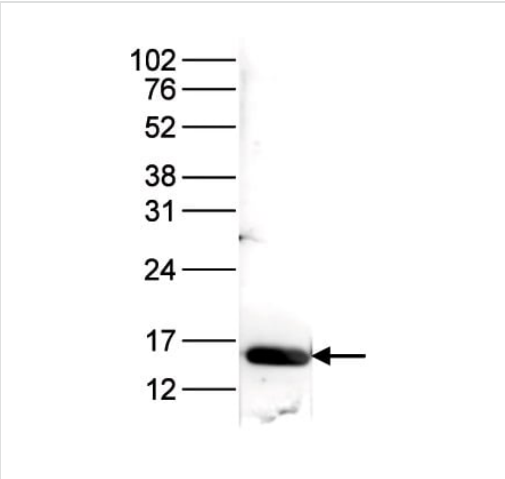


Dot Blot - Anti-Histone H3 (acetyl K9 + K14) antibody (ab232952)

Dot Blot analysis was performed to test the cross reactivity of ab232952 against Histone H3 (acetyl K9 + K14) with peptides containing other histone modifications and the unmodified H3K9/14 sequence. One hundred to 0.2 pmol of the respective peptides were spotted on a membrane. ab232952 was used at 1/20000 dilution. ab232952 shows a high specificity for the modification of interest.



Western blot - Anti-Histone H3 (acetyl K9 + K14) antibody (ab232952)

Anti-Histone H3 (acetyl K9 + K14) antibody (ab232952) at 1/1000 dilution + HeLa (human epithelial cell line from cervix adenocarcinoma) histone extract at 15 µg

Predicted band size: 15 kDa

Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (acetyl K9 + K14) antibody (ab232952)

NIH/3T3 (mouse embryo fibroblast cell line) cells stained for Histone H3 (acetyl K9 + K14) (green) using **ab232931** at 1/200 dilution in ICC/IF. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labeled with ab232952 (left) at 1/500 dilution in blocking solution followed by an anti-rabbit antibody conjugated to Alexa488. The middle panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right.

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