abcam

Product datasheet

Anti-Histone H3 antibody [EPR21228] ab213257

Recombinant

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Overview

Product name Anti-Histone H3 antibody [EPR21228]

Description Rabbit monoclonal [EPR21228] to Histone H3

Host species Rabbit

Tested applications Suitable for: ChIP, WB

Species reactivity Reacts with: Mouse, Cow, Human, Recombinant fragment

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

Positive control WB: HeLa and NIH3T3 histone preparation nuclear lysates, H3.1 recombinant protein

General notes This product was made using synthetic libraries and phage display technology.

This antibody is a recombinant chimeric antibody. Rabbit chimeric monoclonal antibody (Human

Fab/ Rabbit Fc).

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 pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 0.05% BSA, 40% Glycerol (glycerin, glycerine)

Clonality Monoclonal
Clone number EPR21228

Isotype IgG1

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab213257 in the following tested applications.

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

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Application	Abreviews	Notes
ChIP		Use 5 µg for 25 µg of chromatin.
WB		Use a concentration of 1 µg/ml. Predicted molecular weight: 17 kDa.

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

Developmental stage

Belongs to the histone H3 family.

Post-translational modifications

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

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

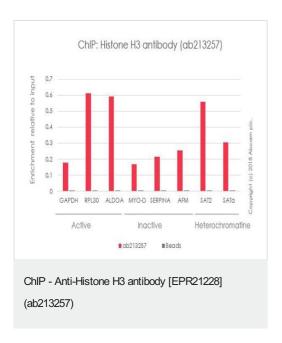
at Thr-7 (H3T6ph) by PRKCBB is a specific tag for epigenetic transcriptional activation that prevents demethylation of Lys-5 (H3K4me) by LSD1/KDM1A. At centromeres, specifically phosphorylated at Thr-12 (H3T11ph) from prophase to early anaphase, by DAPK3 and PKN1. Phosphorylation at Thr-12 (H3T11ph) 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.

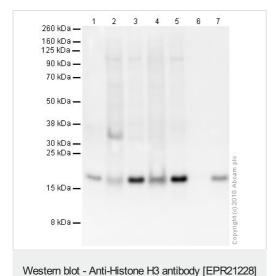
Cellular localization

Nucleus. Chromosome.

Images



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, 5µg of ab213257 (red), and 20 µl of Protein A/G sepharose beads. No antibody was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci).



(ab213257)

All lanes : Anti-Histone H3 antibody [EPR21228] (ab213257) at 1 µg/ml

Lane 1: Calf Thymus Histone lysate at 0.5 µg

Lane 2: Hela whole cell lysate at 20 µg

Lane 3: HeLa histone preparation nuclear lysate at 10 µg

Lane 4: NIH3T3 whole cell lysate at 10 µg

Lane 5: NIH3T3 histone preparation nuclear lysate at 10 µg

Lane 6 : Histone H2B recombinant protein at 0.1 μg **Lane 7 :** Histone H3.1 recombinant protein at 0.1 μg

Secondary

All lanes : HRP conjugated Goat Anti-Rabbit lgG (H+L) at 1/50000 dilution

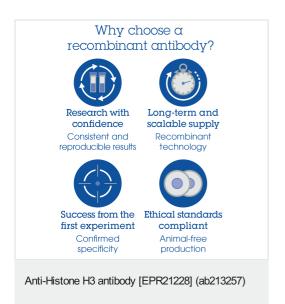
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 17 kDa

Exposure time: 12 minutes

This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 40 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% milk before being incubated with ab213257 overnight at 4°C. Antibody binding was detected using a goat anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution ab133406.



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