abcam

Product datasheet

Histone H3 (K9 methylation) Panel (mono methyl K9, di methyl K9, tri methyl K9) abl 13754

1 References 6 Images

Overview

Product name Histone H3 (K9 methylation) Panel (mono methyl K9, di methyl K9, tri methyl K9)

Product overview ab113754 is a Histone H3 (K9 methylation) Panel designed for the validation and

characterization of the methylation state of Histone H3 on K9. Methylation of the canonical core histones can contribute to the formation of transcriptionally active and inactive chromatin in response to various signalling pathways and is a central modification for regulating epigenetic transitions in chromatin. Histone H3 methylation on K9 is a repressive modification, and H3 K9 tri-

methylation has been shown to establish binding sites for heterochromatin protein 1.

Notes Explore our range of antibody sample panels designed to provide you with a variety of trial-

size antibodies in a convenient and cost-effective format.

Properties

Storage instructions Store at -20°C. Please refer to protocols.

Components	1 units
ab1220 - Anti-Histone H3 (di methyl K9) antibody [mAbcam 1220] - ChIP Grade	1 x 25µg
ab9045 - Anti-Histone H3 (mono methyl K9) antibody - ChIP Grade	1 x 25µg
ab8898 - Anti-Histone H3 (tri methyl K9) antibody - ChIP Grade	1 x 25µg
ab176842 - Rabbit monoclonal to Histone H3 [EPR16987]	1 x 10µl

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.

1

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 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.

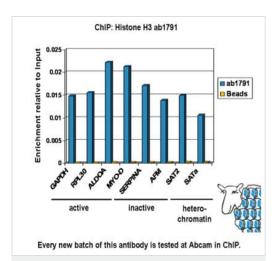
Phosphorylated at Thr-4 (H3T3ph) by GSG2/haspin during prophase and dephosphorylated during anaphase. Phosphorylation at Ser-11 (H3S10ph) by AURKB is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis. In addition phosphorylation at Ser-11 (H3S10ph) by RPS6KA4 and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

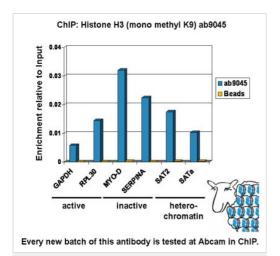
Nucleus. Chromosome.

Images



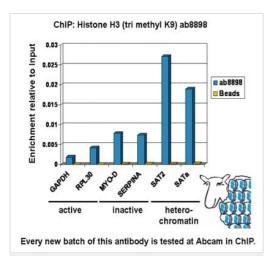
ChIP - Histone H3 (K9 methylation) Panel (H3, mono methyl K9, di methyl K9, tri methyl K9) (ab113754)

Chromatin was prepared from U2OS cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 min. The ChIP was performed with 25 μg of chromatin, 2 μg of $\underline{ab1791}$ (blue), and 20 μl 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). Primers and probes are located in the first kb of the transcribed region.



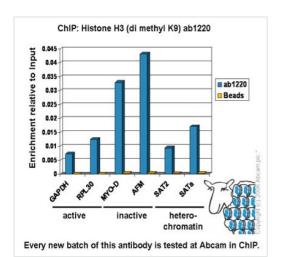
ChIP - Histone H3 (K9 methylation) Panel (H3, mono methyl K9, di methyl K9, tri methyl K9) (ab113754)

Chromatin was prepared from U2OS cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 min. The ChIP was performed with 25 μ g of chromatin, 2 μ g of ab9045 (blue), and 20 μ l 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.



ChIP - Histone H3 (K9 methylation) Panel (H3, mono methyl K9, di methyl K9, tri methyl K9) (ab113754)

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Western blot - Histone H3 (K9 methylation) Panel (H3, mono methyl K9, di methyl K9, tri methyl K9) (ab113754)

All lanes : Anti-Histone H3 (mono methyl K9) antibody - ChIP Grade (ab9045) at 1 µg/ml

Lane 2: Human Histone H3 (unmodified) peptide (ab7228)

Lane 3: Human Histone H3 (mono methyl K27) peptide (ab1780)

Lane 4: Human Histone H3 (di methyl K27) peptide (ab1781)

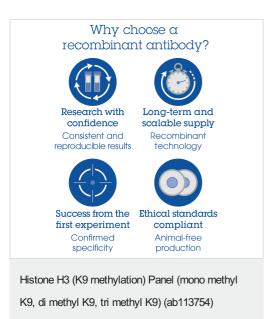
Lane 5: Human Histone H3 (tri methyl K27) peptide (ab1782)

Lane 6: Human Histone H3 (mono methyl K4) peptide (ab1340)

Lane 7: Human Histone H3 (mono methyl K9) peptide (ab1771)

<u>ab9045</u> shows significantly greater reactivity with mono methyl K9.
This can be seen in lane 7, as the addition of <u>ab1771</u> (mono methyl K9) completely blocks the activity of <u>ab9045</u>. Weaker cross-reactivity is seen against mono methyl K27. This is shown in lane 3,

as the addition of $\underline{ab1780}$ only partially blocks the activity of $\underline{ab9045}$.



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