

Product datasheet

Histone H3 (di-methyl K9) Quantification Kit (Colorimetric, Circulating) ab233499

[2 Images](#)

Overview

Product name	Histone H3 (di-methyl K9) Quantification Kit (Colorimetric, Circulating)
Detection method	Colorimetric
Sample type	Serum, Plasma
Assay type	Quantitative
Sensitivity	0.5 ng/well
Assay time	2h 30m
Species reactivity	Reacts with: Mouse, Rat, Human
Product overview	Histone H3 (di-methyl K9) Quantification Kit (Colorimetric, Circulating) (ab233499) is designed to specifically measure circulating dimethyl histone H3K9 (H3K9me2) in biological fluid samples such as plasma and serum from human, mouse or rat.

Histone H3 proteins dimethylated at K9 in the sample are captured on the strip wells coated with anti-H3K9me2 antibody. The captured H3K9me2 proteins can be then recognized with detection antibody followed by a color development reagent. The ratio of H3K9me2 is proportional to the intensity of absorbance. The absolute amount of H3K9me2 can be quantitated by comparing to the standard control.

This kit only recognizes H3K9me2. There is no cross-reactivity with unmodified H3 or other modifications at the same lysine site.

The detection limit is as low as 0.5 ng/well with dynamic range of 1-20 ng/well within the indicated amount range of the plasma/serum.

Notes

Epigenetic activation or inactivation of genes plays a critical role in many important human diseases, especially in cancer. A major mechanism for epigenetic gene inactivation is methylation of CpG islands in genomic DNA caused by DNA methyltransferases. Histone methyltransferases (HMTs) control or regulate DNA methylation through chromatin-dependent transcriptional repression or activation. HMTs transfer 1-3 methyl groups from S-adenosyl-L-methionine to the lysine and arginine residues of histone proteins. G9a is the major histone methyltransferase that catalyzes dimethylation of histone H3 at lysine 9 (H3K9) in mammalian cells. JHDMs and JMJDs the major histone demethylases that demethylates H3K9. H3K9me2 has been viewed as a signature mark of transcription repression genes, which is placed

exclusively in the 5'- region downstream of the promoter. The H3K9me2 can also be changed by inhibition or activation of HMTs. Circulating histone H3K9me2 in plasma or serum has been observed and demonstrated as the marker for many different diseases or pathological changes such as cancer progression. Therefore, detection of circulating H3K9me2 would provide useful information for a better understanding of epigenetic regulation of gene activation and silencing, histone modification-associated pathological processes, screening of disease-related biomarkers, as well as for developing histone modification-targeted drugs.

Platform Microplate reader

Properties

Storage instructions Please refer to protocols.

Components	1 x 48 tests	1 x 96 tests
10X Wash Buffer	1 x 14ml	1 x 28ml
8-Well Assay Strips	4 units	10 units
Adhesive Covering Film	1 unit	1 unit
Control Assay Strips	2 units	2 units
Detection Antibody	1 x 6µl	1 x 12µl
Developer Solution	1 x 5ml	1 x 10ml
Histone Assay Buffer	1 x 4ml	1 x 8ml
Standard Control	1 x 10µl	1 x 20µl
Stop Solution	1 x 5ml	1 x 10ml

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.

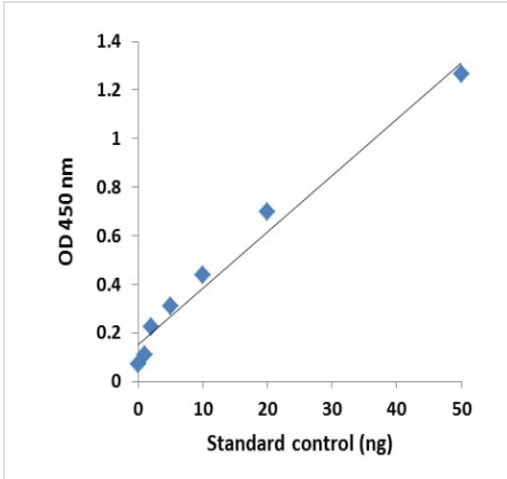
Phosphorylation at Ser-11 (H3S10ph), which is linked to gene activation, prevents methylation at Lys-10 (H3K9me) but facilitates acetylation of H3 and H4. Phosphorylation at Ser-11 (H3S10ph) by AURKB mediates the dissociation of HP1 proteins (CBX1, CBX3 and CBX5) from heterochromatin. Phosphorylation 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.

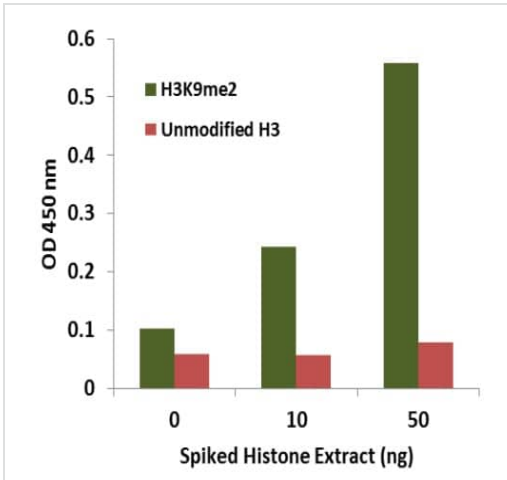
Cellular localization

Nucleus. Chromosome.

Images



Example Standard Curve.



Histone extracts were prepared from HL-60 cells and spiked into bovine plasma at different concentrations.

Histone extracts were prepared from HL-60 (human promyelocytic leukemia cell line) cells and spiked into bovine plasma at different concentrations. The amount of H3K9me2 was measured using Histone H3 (di-methyl K9) Quantification Kit (Colorimetric, Circulating) (ab233499).

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