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Product datasheet

Histone H3 Modification Multiplex Assay Kit (Colorimetric, Circulating) ab233495

1 Image

Overview

Product name	Histone H3 Modification Multiplex Assay Kit (Colorimetric, Circulating)
Detection method	Colorimetric
Sample type	Serum, Plasma
Assay time	2h 30m
Species reactivity	**Reacts with:** Mouse, Rat, Human
Product overview	The Histone H3 Modification Multiplex Assay Kit (Colorimetric, Circulating)(ab233495) is designed for measuring multiple Histone H3 modifications simultaneously from plasma and serum. In an assay with this kit, each Histone H3 modified at specific sites will be captured by an antibody that is coated on the strip wells and specifically targets the appropriate histone modification pattern. The captured histone modified at specific sites will be detected with a detection antibody, followed by a color development reagent. The ratio of modified histone is proportional to the intensity of absorbance measured by a microplate reader at a wavelength of 450 nm. Up to 22 modified Histone H3 patterns can be measured simultaneously.
Notes	Histone modifications have been defined as epigenetic modifiers. Post-translational modifications of histones include the acetylation of specific lysine residues by histone acetyltransferases (HATs), deacetylation by histone deacetylases (HDACs), methylation of lysine and arginine residues by histone methytransferases (HMTs), the demethylation of lysine residues by histone demethylases (HDMTs), and the phosphorylation of specific serine groups by histone kinases (HKs). Additional histone modifications include the attachment of ubiquitin (Ub), small ubiquitin-like modifiers (SUMOs), and poly ADP-ribose (PAR) units. Next to DNA methylation, histone acetylation and histone methylation are the most well characterized epigenetic marks. Generally, tri-methylation at H3-K4, H3-K36, or H3-K79 results in an open chromatin configuration and is therefore characteristic of euchromatin. Euchromatin is also characterized by a high level of histone acetylation, which is mediated by histone acetyltransferases. Conversely, histone deacetylases have the ability to remove this epigenetic mark, which leads to transcriptional repression. Condensed heterochromatin is enriched in tri-methylation of H3-K9 and H3-K27, and silencing of euchromatin loci caused by histone deacetylation involves the recruitment of specific K9 histone methyltransferases. Methylated H3-K9 provides a binding site for the chromodomain-containing heterochromatin protein 1 (HP1), which induces transcriptional repression and heterochromatinization. At euchromatic loci, this process is mediated by co-repressors, such as

retinoblastoma protein pRb or KAP1. Histone demethylases have the opposite effect on transcription. For example, the histone demethylase LSD1 is responsible for H3K4 demethylation, which leads to transcriptional inactivation. Other histone demethylases, such as jumonji (JHDM2A), are responsible for H3K9 demethylation, whereas JHDM1 has the ability to convert active chromatin marks such as H3-K36me2, to an unmodified state. Lysine residues can be mono-, di-, or trimethylated, each of which can differentially regulate chromatin structure and transcription. Along with other histone modifications such as phosphorylation, this enormous variation leads to a multiplicity of possible combinations of different modifications. This may constitute a “histone code”, which can be read and interpreted by different cellular factors.

Abnormal histone modification patterns have been associated with many different diseases such as cancer, autoimmune disorders, and inflammatory and neurological diseases. Circulating modified histones in the plasma or serum are demonstrated to be and considered as the markers for many different diseases or pathological change. Therefore, detection of circulating histone H3 modifications would provide useful information for a better understanding of epigenetic regulation of gene activation and silencing, histone modification-associated pathological disease process, screening of diseaserelated biomarkers, as well as for developing histone modification-targeted drugs.

Platform Microplate reader

Properties

Storage instructions Please refer to protocols.

Components	1 x 96 tests
10X Wash Buffer	1 x 28ml
96-Well Strip Plate (With Frame)	1 unit
Adhesive Covering Film	1 unit
Detection Antibody, 1000X	1 x 12µl
Developer Solution	1 x 12ml
Extra 8-Well Strips	2 units
Histone Assay Buffer	1 x 8ml
Standard Control, 100 µg/ml	1 x 20µl
Stop Solution	1 x 12ml

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.

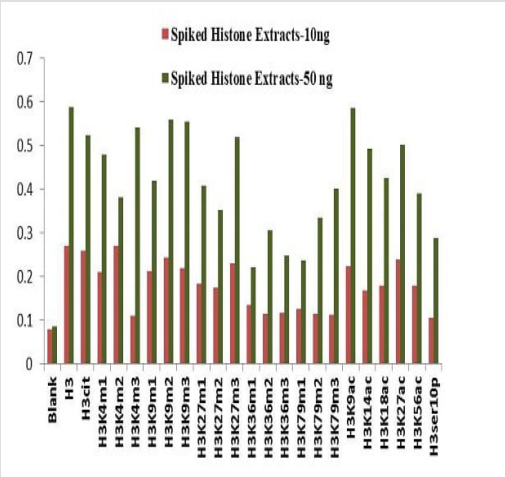
Post-translational modifications

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



Histone extracts were prepared from HL-60 cells using a total Histone extraction kit and spiked into bovine plasma at different concentrations.

The signal intensity of each H3 modification was measured using the Histone H3 Modification Multiplex Assay Kit (Colorimetric, Circulating)(ab233495).

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