abcam

Product datasheet

Anti-Histone H3 (mono methyl K56) antibody ab66857

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Overview

Product name	Anti-Histone H3 (mono methyl K56) antibody
Description	Rabbit polyclonal to Histone H3 (mono methyl K56)
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, WB
Species reactivity	**Reacts with:** Cow, Human **Predicted to work with:** Mouse, Rat, Rabbit, Chicken, Pig, Drosophila melanogaster, Zebrafish, Chinese hamster
Immunogen	Synthetic peptide corresponding to Human Histone H3 (methylated) aa 50 to the C-terminus conjugated to Keyhole Limpet Haemocyanin (KLH). Database link: **P68431** (Peptide available as **ab150460**)
Positive control	This antibody gave a positive signal in Calf Thymus Histone lysate as well as the following whole cell lysates: HeLa; HeLa Nuclear; HeLa Histone Nuclear. This antibody gave a positive result when used in the following methanol fixed cell lines: MCF-7 and HepG2.
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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising

agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

Purity Immunogen affinity purified

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab66857 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
ICC/IF	★★★☆☆ (1)	Use a concentration of 1 µg/ml.
WB		Use a concentration of 1 µg/ml. Detects a band of approximately 18 kDa (predicted molecular weight: 15 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 Belongs to the histone H3 family.

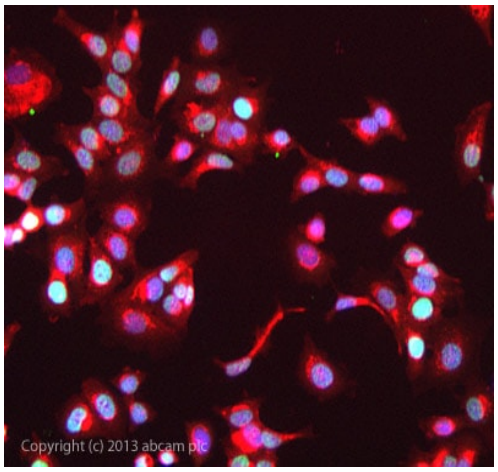
Developmental stage Expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation.

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.

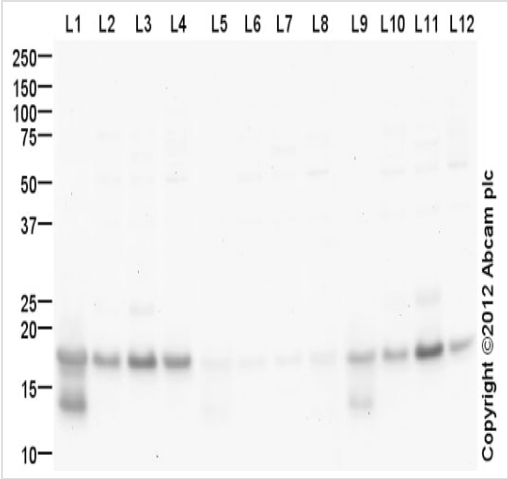
Cellular localization Nucleus. Chromosome.

Images



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (mono methyl K56) antibody (ab66857)

ab66857 stained MCF-7 cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab66857 at 1µg/ml overnight at +4°C. The secondary antibody (green) was a goat **anti-rabbit DyLight® 488** (**ab96899**) IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM. This antibody also gave a positive result in Methanol fixed (100%) HepG2 cells.



Western blot - Anti-Histone H3 (mono methyl K56) antibody (ab66857)

All lanes : Anti-Histone H3 (mono methyl K56) antibody (ab66857) at 1 µg/ml

Lane 1 : Calf Thymus Histone Preparation Nuclear Lysate
Lane 2 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 3 : HeLa (Human epithelial carcinoma cell line) Nuclear Lysate
Lane 4 : HeLa Histone Preparation Nuclear Lysate
Lane 5 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (mono methyl K56) peptide (**ab150460**) at 1 µg/ml
Lane 6 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate with Human Histone H3 (mono methyl K56) peptide (**ab150460**) at 1 µg/ml
Lane 7 : HeLa (Human epithelial carcinoma cell line) Nuclear Lysate with Human Histone H3 (mono methyl K56) peptide (**ab150460**) at 1 µg/ml
Lane 8 : HeLa Histone Preparation Nuclear Lysate with Human Histone H3 (mono methyl K56) peptide (**ab150460**) at 1 µg/ml
Lane 9 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 peptide (**ab172484**) at 1 µg/ml
Lane 10 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate with Human Histone H3 peptide (**ab172484**) at 1 µg/ml
Lane 11 : HeLa (Human epithelial carcinoma cell line) Nuclear Lysate with Human Histone H3 peptide (**ab172484**) at 1 µg/ml
Lane 12 : HeLa Histone Preparation Nuclear Lysate with Human Histone H3 peptide (**ab172484**) at 1 µg/ml

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (**ab97080**) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 15 kDa

Observed band size: 18 kDa
Additional bands at: 14 kDa, 23 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 2 minutes

This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 5% Bovine Serum Albumin before being incubated with ab66857 overnight at 4°C. Antibody binding was detected using an **anti-rabbit HRP** secondary antibody, and visualised using ECL development solution.

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