abcam

Product datasheet

Anti-Histone H3 (di methyl K27) antibody - ChIP Grade ab24684

16 Abreviews 70 References 5 Images

Overview

Product name	Anti-Histone H3 (di methyl K27) antibody - ChIP Grade
Description	Rabbit polyclonal to Histone H3 (di methyl K27) - ChIP Grade
Host species	Rabbit
Specificity	This antibody is specific for Histone H3 di-methylated at residue K27. It does not recognise the mono- or tri-methylated K27 residue or the mono-/di-/tri-methylated H3 K9 residue.
Tested applications	**Suitable for:** WB, IP, IHC-P, ICC/IF, ChIP
Species reactivity	**Reacts with:** Mouse, Cow, Human, Saccharomyces cerevisiae, Caenorhabditis elegans, Drosophila melanogaster, Plants, Cyanidioschyzon merolae
Immunogen	Synthetic peptide corresponding to Histone H3 aa 1-100 (N terminal) (di methyl K27) conjugated to keyhole limpet haemocyanin. (Peptide available as ab1781)
Positive control	WB: Calf thymus histone lysate. IHC-P: Human normal skin tissue.

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

Our Abpromise guarantee covers the use of **ab24684** 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
WB	★★★★★	Use a concentration of 0.5 µg/ml. Detects a band of approximately 18 kDa (predicted molecular weight: 15.2 kDa).Can be blocked with Human Histone H3 (di methyl K27) peptide (ab1781).
IP	★☆☆☆☆	Use at 80 µg/mg of lysate.
IHC-P	★★★★☆	Use a concentration of 1 µg/ml.
ICC/IF	★★★★☆	Use a concentration of 1 µg/ml.
ChIP		Use 2-25 µg for µg of chromatin.

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.

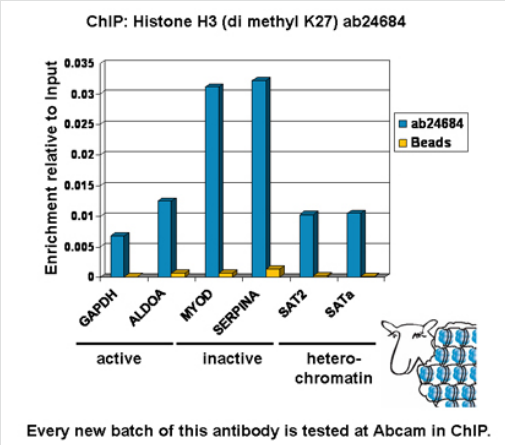
Post-translational modifications

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.

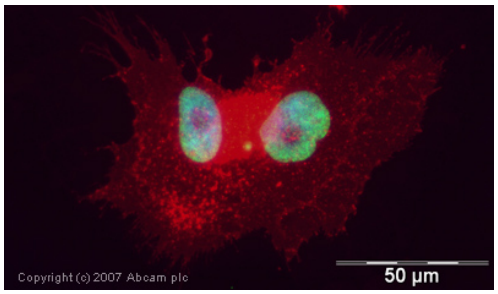
Cellular localization Nucleus. Chromosome.

Images



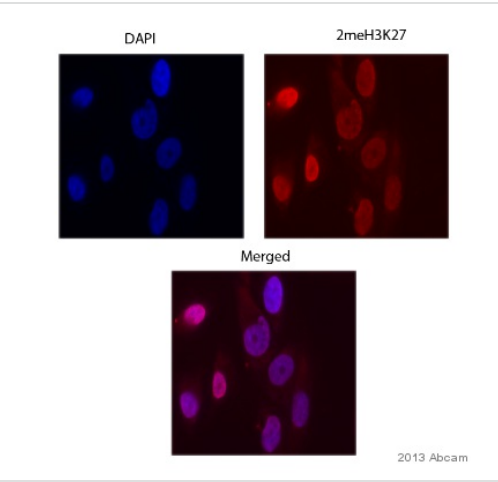
ChIP - Anti-Histone H3 (di methyl K27) antibody - ChIP Grade (ab24684)

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, 2µg of ab24684 (blue), and 20µl 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.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (di methyl K27) antibody - ChIP Grade (ab24684)

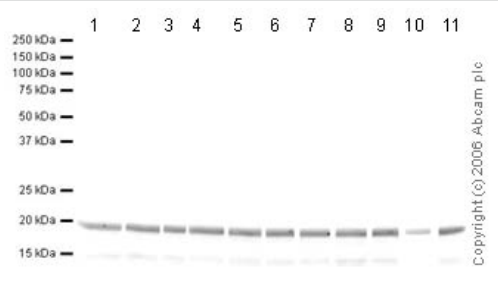
ICC/IF image of ab24684 stained human HeLa cells. The cells were PFA fixed (10 min), permabilised in TBS-T (20 min) and incubated with the antibody (ab24684, 1µg/ml) for 1h at room temperature. 1%BSA / 10% normal goat serum / 0.3M glycine was used to quench autofluorescence and block non-specific protein-protein interactions. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red). DAPI was used to stain the cell nuclei (blue).



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (di methyl K27) antibody - ChIP Grade (ab24684)

This image is courtesy of an anonymous abreview.

Immunocytochemistry/ Immunofluorescence analysis of IMR32, Human Neuroblatoma cells labeling Histone H3 (di methyl K27) with ab24684 at 1/100 dilution. The cells were fixed with paraformaldehyde and permeabilized with 0.3% triton X 100. The cells were blocked with 1% BSA for 1 hour at 25°C, followed by incubation with primary antibody in 1% BSA containing TBST for 16 hours at 4°C. A Goat Anti Rabbit-IgG Cy3® was used as the secondary antibody at 1/1000 dilution.



Western blot - Anti-Histone H3 (di methyl K27) antibody - ChIP Grade (ab24684)

All lanes : Anti-Histone H3 (di methyl K27) antibody - ChIP Grade (ab24684) at 1 µg/ml

Lane 1 : Calf thymus histone lysate
Lane 2 : Calf thymus histone lysate with Human Histone H3 peptide (ab17163) at 0.5 µg/ml
Lane 3 : Calf thymus histone lysate with Human Histone H3 (mono methyl K4) peptide (ab1340) at 0.5 µg/ml
Lane 4 : Calf thymus histone lysate with Human Histone H3 (di methyl K4) peptide (ab7768) at 0.5 µg/ml
Lane 5 : Calf thymus histone lysate with Human Histone H3 (tri methyl K4) peptide (ab1342) at 0.5 µg/ml
Lane 6 : Calf thymus histone lysate with Human Histone H3 (mono methyl K9) peptide (ab1771) at 0.5 µg/ml
Lane 7 : Calf thymus histone lysate with Human Histone H3 (di methyl K9) peptide (ab1772) at 0.5 µg/ml

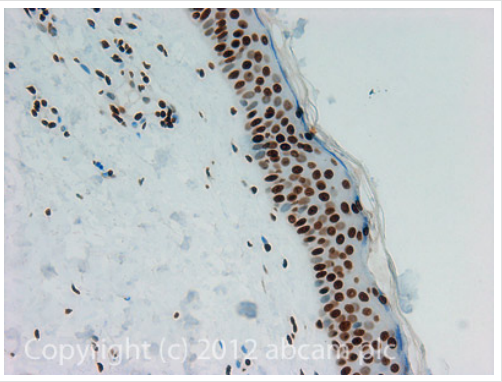
Lane 8 : Calf thymus histone lysate with Human Histone H3 (tri methyl K9) peptide (ab1773) at 0.5 µg/ml
Lane 9 : Calf thymus histone lysate with Human Histone H3 (mono methyl K27) peptide (ab1780) at 0.5 µg/ml
Lane 10 : Calf thymus histone lysate with Human Histone H3 (di methyl K27) peptide (ab1781) at 0.5 µg/ml
Lane 11 : Calf thymus histone lysate with Human Histone H3 (tri methyl K27) peptide (ab1782) at 0.5 µg/ml

Secondary
All lanes : Goat polyclonal to Rabbit IgG H&L (HRP) at 1/5000 dilution

Predicted band size: 15.2 kDa
Observed band size: 17 kDa
why is the actual band size different from the predicted?

Exposure time: 5 seconds

The peptide competition assay shows that this antibody is very specific for Histone H3 di-methylated at K27. It does not recognise Histone H3 mono, di, or tri methylated at K4, Histone H3 mono, di, or tri methylated at K9, or Histone H3 mono, or tri methylated at K27.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (di methyl K27) antibody - ChIP Grade (ab24684)

IHC image of Histone H3 (di methyl K27) staining in Human normal skin formalin fixed paraffin embedded tissue section, performed on a Leica BondTM system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab24684, 1µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

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