abcam

Product datasheet

Anti-Histone H3 (tri methyl K4) antibody [mAbcam1012] - ChIP Grade ab1012

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Overview

Product name	Anti-Histone H3 (tri methyl K4) antibody [mAbcam1012] - ChIP Grade
Description	Mouse monoclonal [mAbcam1012] to Histone H3 (tri methyl K4) - ChIP Grade
Host species	Mouse
Specificity	By ELISA the antibody binds to the tri methyl K4 peptide and partially to di and mono methyl K4 peptides. It does not bind to unmodified, mono, di or tri methyl K9 or di or tri methyl K27 peptides. Not suitable for blocking with milk in Western blot (see Application notes).
Tested applications	**Suitable for:** PepArr, ELISA, ChIP, Flow Cyt, ICC/IF, IHC-Fr, WB, ICC
Species reactivity	**Reacts with:** Mouse, Rat, Cow, Human, Zebrafish, Rice **Predicted to work with:** Sheep, Xenopus laevis, Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster, Schizosaccharomyces pombe, Mammals
Immunogen	Synthetic peptide corresponding to Human Histone H3 aa 1-100 (tri methyl K4) conjugated to Keyhole Limpet Haemocyanin (KLH) (Cysteine residue). (Peptide available as ab1342)
General notes	Learn about ChIP assay kits, other ChIP antibodies, protocols and more in the ChIP assay guide.

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 Constituents: PBS, 6.97% L-Arginine
Purity	IgG fraction
Clonality	Monoclonal
Clone number	mAbcam1012
Isotype	IgG2b
Light chain type	kappa

Applications

Our Abpromise guarantee covers the use of **ab1012** 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
PepArr		Use a concentration of 0.5 - 2 µg/ml.
ELISA		Use at an assay dependent concentration.
ChIP	★★★★☆	Use 2-5 µg for 25 µg of chromatin.
Flow Cyt		Use 1µg for 10^6 cells. ab170192 - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★★☆	Use at an assay dependent concentration.
IHC-Fr		Use at an assay dependent concentration. PubMed: 20047469
WB	★★★☆☆	Use a concentration of 1 - 5 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).Can be blocked with Human Histone H3 (tri methyl K4) peptide (ab1342). NOT SUITABLE for blocking with milk. Block in 5% BSA for 1 hour. Our labs have investigated the blocking conditions for this antibody and found that milk significantly decreases the signal and is therefore not a suitable blocking agent for this antibody (see image below).
ICC		Use a concentration of 5 µg/ml.

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.

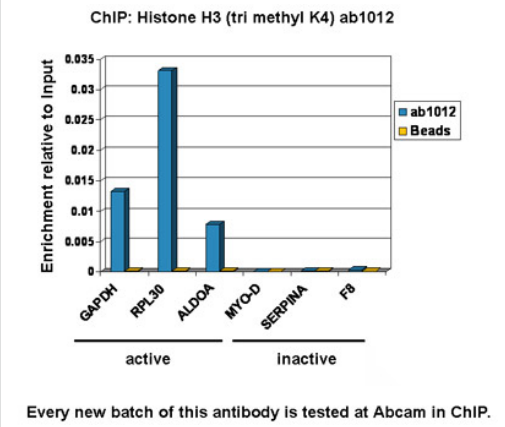
Post-translational modifications

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.

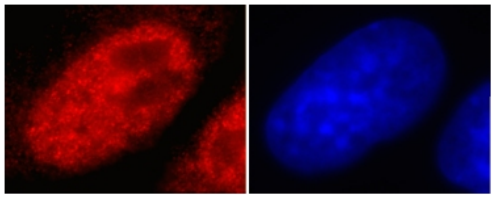
Cellular localization Nucleus. Chromosome.

Images



ChIP - Anti-Histone H3 (tri methyl K4) antibody [mAbcam1012] - ChIP Grade (ab1012)

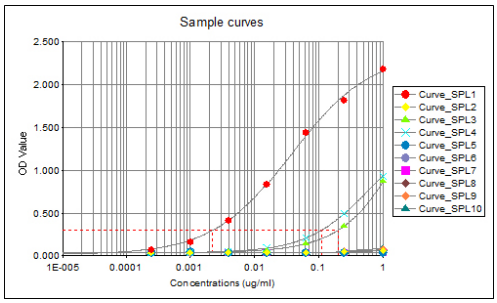
Chromatin was prepared from U2OS cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10min. The ChIP was performed with 25 µg of chromatin, 5 µg of ab1012 (blue), and 20 µl of Protein A/G sepharose beads. No antibody was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Taqman approach). Primers and probes are located in the first kb of the transcribed region.



Immunocytochemistry - Anti-Histone H3 (tri methyl K4) antibody [mAbcam1012] - ChIP Grade (ab1012)

This image is courtesy of Darin McDonald, Cross Cancer Institute

SK-N-SK cells were fixed in 4% paraformaldehdye, permeabilized in 0.5% Triton X-100 and incubated with ab1012 (1/100). The antibody clearly stains the nucleus (red). The cells were counterstained with DAPI (blue). 100x magnification.

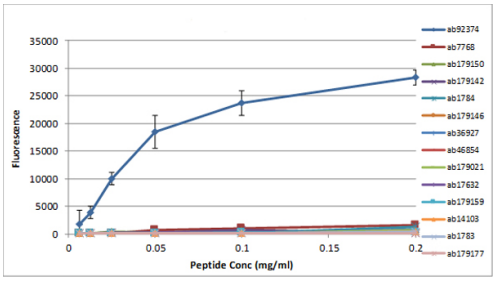


ELISA - Anti-Histone H3 (tri methyl K4) antibody [mAbcam1012] - ChIP Grade (ab1012)

ELISA using ab1012 at varying antibody concentrations.

Curve_SPL1 indicates binding to the tri methyl K4 peptide ab1342. Curve_SPL4 indicates partial binding to the di methyl K4 peptide ab7768. There is very weak cross-reactivity with the mono methyl K4 peptide ab1340 (Curve_SPL3).

Binding to the following peptides was not seen: SPL2 unmodified Histone H3, SPL5 Histone H3 mono methyl K9, SPL6 Histone H3 di methyl K9, SPL7 Histone H3 tri methyl K9, SPL8 Histone H3 mono methyl K27, SPL9 Histone H3 di methyl K27, SPL10 Histone H3 tri methyl K27.



Peptide Array - Anti-Histone H3 (tri methyl K4) antibody [mAbcam1012] - ChIP Grade (ab1012)

All batches of ab1012 are tested in Peptide Array against peptides to different Histone modifications. Six dilutions of each peptide are printed on to the Peptide Array in triplicate and results are averaged before being plotted on to a graph. Results show strong binding to Histone H3 - tri methyl K4 peptide (ab92374), indicating that this antibody specifically recognises the Histone H3 - tri methyl K4 modification.

ab92374 - Histone H3 - tri methyl K4

ab7768 - Histone H3 - di methyl K4

ab179150 - Histone H3 - asymmetric di methyl R2

ab179142 - Histone H3 - mono methyl R2

ab1784 - Histone H3 - di methyl K36

ab179146 - Histone H3 - symmetric di methyl R2

ab36927 - Histone H2A - phospho S122

ab46854 - Histone H3 - tri methyl K18

ab179021 - Histone H2A - symmetric di methyl R29

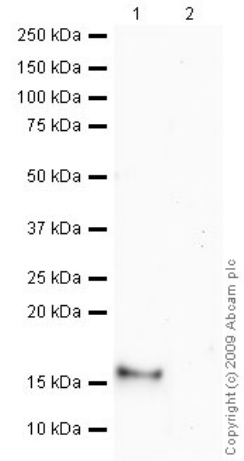
ab17632 - Histone H4 - biotinylated K5

ab179159 - Histone H3 - phospho T3

ab14103 - Histone H3 - phopsho T6

ab1783 - Histone H3 - mono methyl K36

ab179177 - Histone H3 - mono methyl K4 + Histone H3 di methyl K9



Western blot - Anti-Histone H3 (tri methyl K4) antibody [mAbcam1012] - ChIP Grade (ab1012)

Lane 1 : Anti-Histone H3 (tri methyl K4) antibody [mAbcam1012] - ChIP Grade (ab1012) at 1 µg/ml (Blocked in 5% BSA)
Lane 2 : Anti-Histone H3 (tri methyl K4) antibody [mAbcam1012] - ChIP Grade (ab1012) at 1 µg/ml (Blocked in 5% MILK)

All lanes : Calf Thymus Histone Preparation Nuclear Lysate

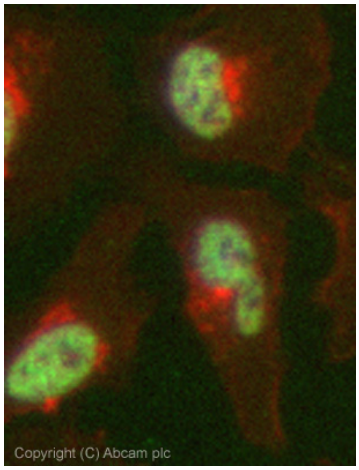
Lysates/proteins at 0.5 µg per lane.

Secondary
All lanes : Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Performed under reducing conditions.

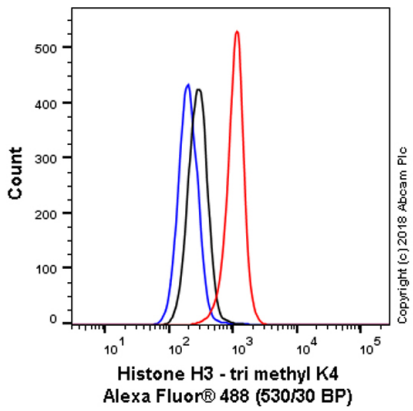
Predicted band size: 15 kDa
Observed band size: 17 kDa
why is the actual band size different from the predicted?

Exposure time: 10 minutes



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K4) antibody [mAbcam1012] - ChIP Grade (ab1012)

ICC/IF image of ab1012 stained HeLa cells. The cells were 4% PFA fixed (10 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 (ab1012, 5µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse IgG (H+L) used at a 1/1000 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 4% PFA fixed (10 min) HepG2, Hek293 and MCF7 cells at 5µg/ml, and in 100% methanol fixed (5 min) HeLa, Hek293, HepG2 and MCF7 cells at 5µg/ml.



Flow Cytometry - Anti-Histone H3 (tri methyl K4) antibody [mAbcam1012] - ChIP Grade (ab1012)

Overlay histogram showing HeLa cells stained with ab1012 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab1012, 1µg/1x10^6) for 30 min at 22°C. The secondary antibody used was Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed (ab150117) at 1/2000 dilution for 30 min at 22°C.

Isotype control antibody (black line) was Mouse IgG2b [7E10G10] isotype control (ab170192) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter.

This antibody gave a positive signal in HeLa cells fixed with 4% formaldehyde (10 min)/permeabilized with 0.1% PBS-Triton X-100 for 15 min used under the same conditions.

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