abcam

Product datasheet

Anti-Histone H3 (tri methyl K4) antibody [mAbcam12209] - ChIP Grade – BSA and Azide free ab237971

5 Images

Overview

Product name	Anti-Histone H3 (tri methyl K4) antibody [mAbcam12209] - ChIP Grade – BSA and Azide free
Description	Mouse monoclonal [mAbcam12209] to Histone H3 (tri methyl K4) - ChIP Grade – BSA and Azide free
Host species	Mouse
Tested applications	**Suitable for:** ChIP, WB, Flow Cyt, ICC/IF, ELISA
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Histone H3 aa 1-100 (tri methyl K4) conjugated to keyhole limpet haemocyanin. The exact sequence is proprietary.
Positive control	Flow Cyt: HeLa cells; ICC/IF: HeLa cells; WB: Calf Thymus Histone Preparation Nuclear lysate;ChIP: U2OS cells.
General notes	Ab237971 is a PBS only version of ab12209. This antibody clone is manufactured by Abcam. If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact orders@abcam.com or you can find further information here. Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough. Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility. We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee. In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for. We are also updating the applications & species that this product has been "predicted to work with," however this information is not covered by our Abpromise guarantee. Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

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, as well as customer reviews and Q&As.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Store at +4°C. Do Not Freeze.
Storage buffer	Constituent: PBS
Carrier free	Yes
Purity	IgG fraction
Clonality	Monoclonal
Clone number	mAbcam12209
Myeloma	Sp2/0-Ag14
Isotype	IgG1
Light chain type	kappa

Applications

Our Abpromise guarantee covers the use of **ab237971** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

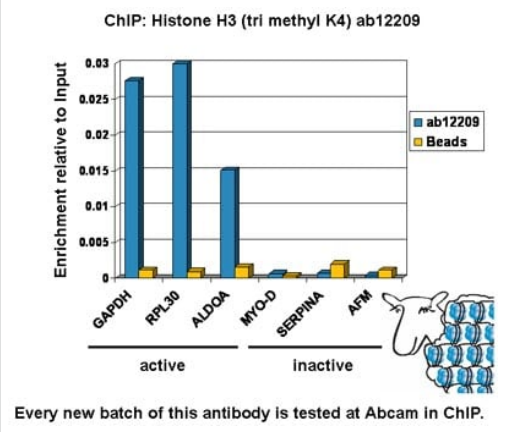
Application	Abreviews	Notes
ChIP		Use 2-5 µg for 25 µg of chromatin.
WB		Use a concentration of 1 - 5 µg/ml. Predicted molecular weight: 15 kDa. NOT SUITABLE for blocking with milk. Block in 5% BSA for 1 hour. Our labs have investigated the blocking conditions for this antibody and found that milk significantly decreases the signal and is therefore not a suitable blocking agent for this antibody (see Western Blot image).
Flow Cyt		Use 1µg for 10^6 cells.
ICC/IF		Use a concentration of 5 µg/ml.
ELISA		Use at an assay dependent concentration.

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.

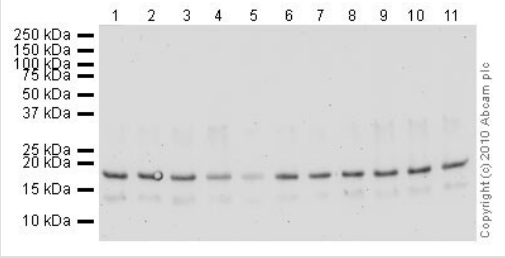
Images



ChIP - Anti-Histone H3 (tri methyl K4) antibody [mAbcam12209] - ChIP Grade – BSA and Azide free (ab237971)

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, 2 µg of ab12209 (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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, L-arginine, and sodium azide (ab12209).



Western blot - Anti-Histone H3 (tri methyl K4) antibody [mAbcam12209] - ChIP Grade – BSA and Azide free (ab237971)

All lanes : Anti-Histone H3 (tri methyl K4) antibody [mAbcam12209] - ChIP Grade (ab12209) at 2 µg/ml

Lane 1 : Calf Thymus Histone Preparation Nuclear Lysate
Lane 2 : Calf Thymus Histone Preparation Nuclear Lysate with ab7228 at 0.25 µg/ml
Lane 3 : Calf Thymus Histone Preparation Nuclear Lysate with ab1340 at 0.25 µg/ml
Lane 4 : Calf Thymus Histone Preparation Nuclear Lysate with ab7768 at 0.25 µg/ml
Lane 5 : Calf Thymus Histone Preparation Nuclear Lysate with ab1342 at 0.25 µg/ml
Lane 6 : Calf Thymus Histone Preparation Nuclear Lysate with ab1771 at 0.25 µg/ml
Lane 7 : Calf Thymus Histone Preparation Nuclear Lysate with ab1772 at 0.25 µg/ml
Lane 8 : Calf Thymus Histone Preparation Nuclear Lysate with ab1773 at 0.25 µg/ml
Lane 9 : Calf Thymus Histone Preparation Nuclear Lysate with ab1780 at 0.25 µg/ml
Lane 10 : Calf Thymus Histone Preparation Nuclear Lysate with ab1781 at 0.25 µg/ml
Lane 11 : Calf Thymus Histone Preparation Nuclear Lysate with ab1782 at 0.25 µg/ml

Lysates/proteins at 0.5 µg per lane.

Secondary
All lanes : Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed

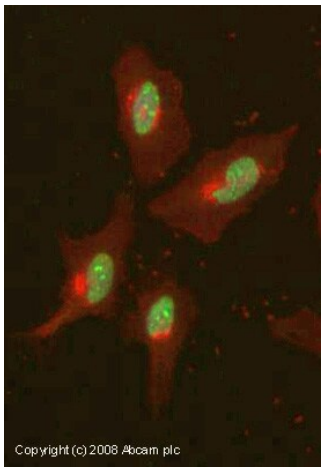
(HRP) at 1/3000 dilution

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: 16 minutes

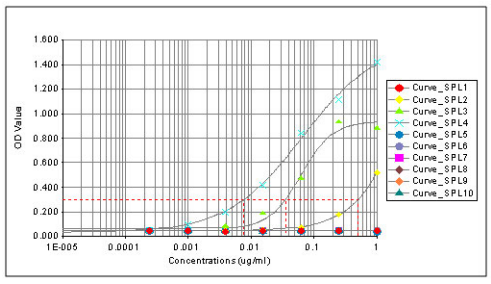
This data was developed using the same antibody clone in a different buffer formulation containing PBS, L-arginine, and sodium azide (ab12209).



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K4) antibody [mAbcam12209] - ChIP Grade – BSA and Azide free (ab237971)

ICC/IF image of ab12209 stained human HeLa cells. The cells were 4% PFA fixed (10 min), permabilised in 0.1% PBS-Tween (20 min) and incubated with the antibody (ab12209, 5µg/ml) for 1h at room temperature. 1% BSA / 10% normal goat serum / 0.3M glycine was used to block non-specific protein-protein interactions. 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). DAPI was used to stain the cell nuclei (blue).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, L-arginine, and sodium azide (ab12209).



ELISA - Anti-Histone H3 (tri methyl K4) antibody [mAbcam12209] - ChIP Grade – BSA and Azide free (ab237971)

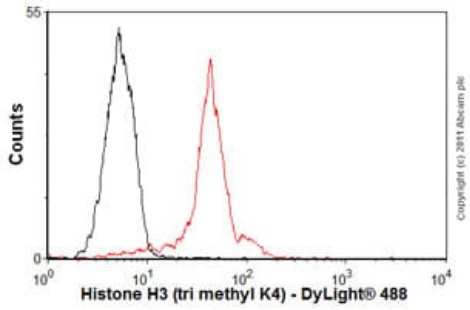
ELISA using ab12209 at varying antibody concentrations.

Curve_SPL4 indicates binding to the tri methyl K4 peptide ab1342. In addition, SPL3 indicates partial binding to the di methyl K4 peptide ab7768. There is very weak cross-reactivity with the mono methyl K4 peptide ab1340 (Curve_SPL2).

Binding to the following peptides was not seen:
SPL1 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, L-arginine, and sodium

azide (ab12209).



Flow Cytometry - Anti-Histone H3 (tri methyl K4) antibody [mAbcam12209] - ChIP Grade – BSA and Azide free (ab237971)

Overlay histogram showing HeLa cells stained with ab12209 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab12209, 1µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was a goat anti-mouse DyLight® 488 (IgG; H+L) (ab96879) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, L-arginine, and sodium azide (ab12209).

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