abcam

Product datasheet

Anti-Histone H3 (tri methyl K4) antibody [mAbcam12209] - ChIP Grade ab12209

7 Abreviews　21 References　画像数 7

製品の概要

製品名	Anti-Histone H3 (tri methyl K4) antibody [mAbcam12209] - ChIP Grade
製品の詳細	Mouse monoclonal [mAbcam12209] to Histone H3 (tri methyl K4) - ChIP Grade
由来種	Mouse
特異性	ab12209 is strongly blocked in Western blotting on histones by tri methyl K4, weakly by di methyl K4 and very weakly by mono methyl K4 peptides. It is not blocked by non-modified peptides. By ELISA the antibody binds to the tri methyl K4 peptide and at high antibody concentrations 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).
アプリケーション	**適用あり:** Flow Cyt, WB, ICC/IF, ChIP, ELISA, IP
種交差性	**交差種:** Mouse, Rat, Human **交差が予測される動物種:** Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster, Schizosaccharomyces pombe, Zebrafish, Mammals, Neurospora crassa
免疫原	Synthetic peptide within Human Histone H3 aa 1-100 (tri methyl K4) conjugated to Keyhole Limpet Haemocyanin (KLH). The exact sequence is proprietary. (Peptide available as ab1342)
ポジティブ・コントロール	This antibody gave a positive signal in methanol fixed/Tween permeabilised HeLa cells within Flow Cytometry.
特記事項	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.

製品の特性

製品の状態	Liquid
保存方法	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.
バッファー	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine

精製度	IgG fraction
ポリ/モノ	モノクローナル
クローン名	mAbcam12209
ミエローマ	Sp2/0-Ag14
アイソタイプ	IgG1
軽鎖の種類	kappa

アプリケーション

Our Abpromise guarantee covers the use of **ab12209** in the following tested applications.

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

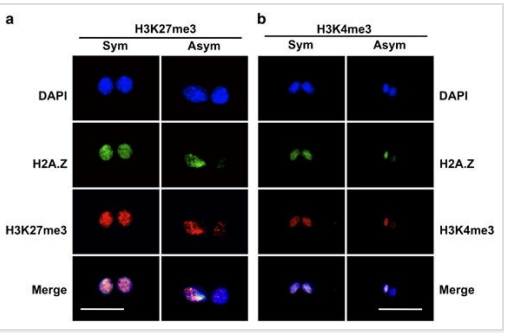
アプリケーション	Abreviews	特記事項
Flow Cyt		Use 1µg for 10^6 cells. ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
WB	★★★★☆	Use a concentration of 1 - 5 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).Can be blocked with Human 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 Western Blot image).
ICC/IF	★★★☆☆	Use a concentration of 5 µg/ml.
ChIP	★★★★☆	Use 2-5 µg for 25 µg of chromatin.
ELISA		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration. PubMed: 22086061

ターゲット情報

機能	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.
配列類似性	Belongs to the histone H3 family.
発生段階	Expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation.
翻訳後修飾	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.
細胞内局在	Nucleus. Chromosome.

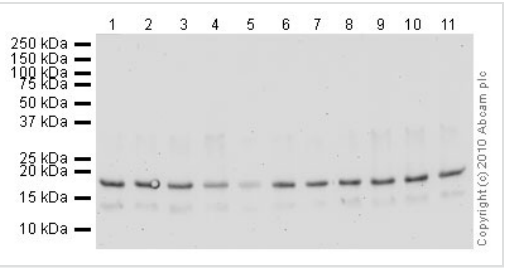
画像



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K4) antibody [mAbcam12209] - ChIP Grade (ab12209)

Image from Huh YH et al., Cell Death Dis 4(5). Doi: 10.1038/cddis.2014.522.

Ab12209 staining Histone H3 (Tri Methyl K4) in Mouse hair follicle DSCs by ICC/IF (Immunocytochemistry/Immunofluorescence). Cells were fixed with 3.7% paraformaldehyde, permeabilised with 0.2% Triton X-100 and blocked with 10% normal goat serum in PBS. Samples were incubated with primary antibody at 1:200 dilution. An Alexa Fluor ® 568 conjugated goat anti-mouse IgG was used as a secondary antibody.



Western blot - Anti-Histone H3 (tri methyl K4) antibody [mAbcam12209] - ChIP Grade (ab12209)

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 Human Histone H3 (unmodified) peptide (ab7228) at 0.25 µg/ml
Lane 3 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (mono methyl K4) peptide (ab1340) at 0.25 µg/ml
Lane 4 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (di methyl K4) peptide (ab7768) at 0.25 µg/ml
Lane 5 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (tri methyl K4) peptide (ab1342) at 0.25 µg/ml
Lane 6 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (mono methyl K9) peptide (ab1771) at 0.25 µg/ml
Lane 7 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (di methyl K9) peptide (ab1772) at 0.25 µg/ml
Lane 8 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (tri methyl K9) peptide (ab1773) at 0.25 µg/ml
Lane 9 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (mono methyl K27) peptide (ab1780) at 0.25 µg/ml
Lane 10 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (di methyl K27) peptide (ab1781) at 0.25 µg/ml

Lane 11 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (tri methyl K27) peptide (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

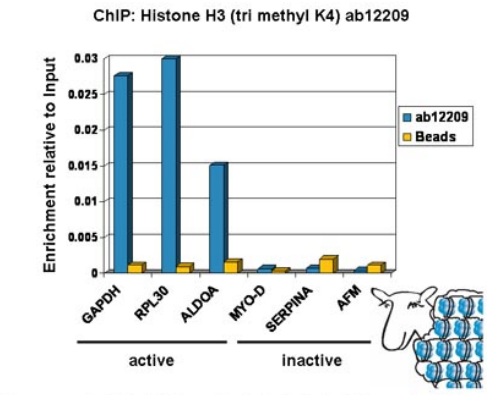
Performed under reducing conditions.

Predicted band size: 15 kDa

Observed band size: 17 kDa

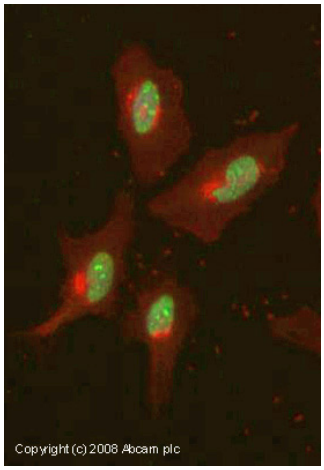
why is the actual band size different from the predicted?

Exposure time: 16 minutes



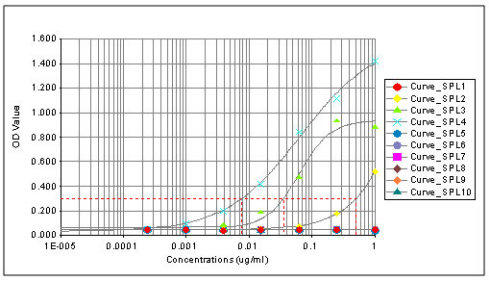
ChIP - Anti-Histone H3 (tri methyl K4) antibody [mAbcam12209] - ChIP Grade (ab12209)

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.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K4) antibody [mAbcam12209] - ChIP Grade (ab12209)

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).

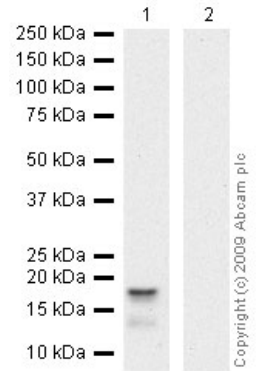


ELISA - Anti-Histone H3 (tri methyl K4) antibody [mAbcam12209] - ChIP Grade (ab12209)

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.



Western blot - Anti-Histone H3 (tri methyl K4) antibody [mAbcam12209] - ChIP Grade (ab12209)

Lane 1 : Anti-Histone H3 (tri methyl K4) antibody [mAbcam12209] - ChIP Grade (ab12209) at 1 µg/ml (BLOCKED WITH 5% BSA)
Lane 2 : Anti-Histone H3 (tri methyl K4) antibody [mAbcam12209] - ChIP Grade (ab12209) at 1 µg/ml (BLOCKED WITH 5% MILK)

All lanes : Calf Thymus Histone Preparation Nuclear Lysate

Lysates/proteins at 0.5 µg per lane.

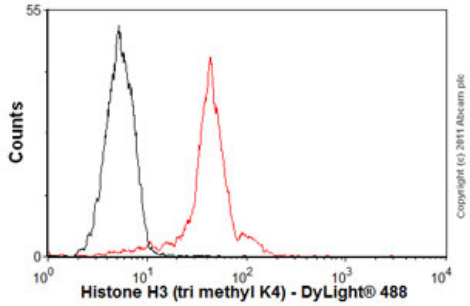
Secondary
All lanes : Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

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: 12 minutes



Flow Cytometry - Anti-Histone H3 (tri methyl K4) antibody [mAbcam12209] - ChIP Grade (ab12209)

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.

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