Product datasheet

Anti-Histone H3 (di methyl K4, tri methyl K4) antibody [mAbcam 6000] - ChIP Grade ab6000

★★★★★ 7 Abreviews 40 References 画像数 5

製品の概要

製品名	Anti-Histone H3 (di methyl K4, tri methyl K4) antibody [mAbcam 6000] - ChIP Grade
製品の詳細	Mouse monoclonal [mAbcam 6000] to Histone H3 (di methyl K4, tri methyl K4) - ChIP Grade
由来種	Mouse
特異性	This antibody detects a band of the appropriate size when used in Western blotting on a calf thymus histone preparation (see image). It is specifically blocked by peptides corresponding to di methylated K4 of Histone H3 and to tri methylated K4. Slight cross reactivity is observed with mono methyl K4 peptide in ELISA and Western Blot.
アプリケーション	**適用あり:** ICC/IF, WB, ChIP, Flow Cyt (Intra)
種交差性	**交差種:** Cow, Human, Rice **交差が予測される動物種:** Mouse, Rat, Rabbit, Chicken, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster, Schizosaccharomyces pombe, Neurospora crassa, Candida albicans
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab1342**, **ab7768**)
ポジティブ・コントロール	ChIP: Chromatin was prepared from U-2 OS cell. Flow Cyt (Intra): HeLa cells. WB: Calf thymus histone preparation nuclear lysate.
特記事項	This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. 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

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	Protein G purified
ポリ/モノ	モノクローナル
クローン名	mAbcam 6000
ミエローマ	Sp2/0-Ag14
アイソタイプ	IgG2b
軽鎖の種類	kappa

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab6000の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
ICC/IF	★★★★★ (4)	Use a concentration of 1 µg/ml.
WB	★★★★★ (2)	1/500. Predicted molecular weight: 15.2 kDa.Can be blocked with **Human Histone H3 (tri methyl K4) peptide (ab1342)**.
ChIP	★★★★★ (1)	Use at an assay dependent concentration.
Flow Cyt (Intra)		Use 1µg for 10^6 cells. **ab170192** - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.

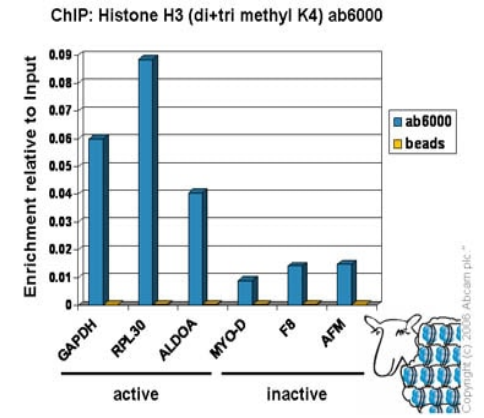
ターゲット情報

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.

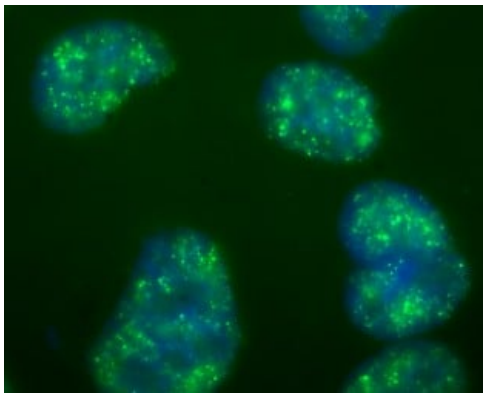
細胞内局在 Nucleus. Chromosome.

画像



ChIP - Anti-Histone H3 (di methyl K4, tri methyl K4) antibody [mAbcam 6000] - ChIP Grade (ab6000)

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 ab6000 (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.



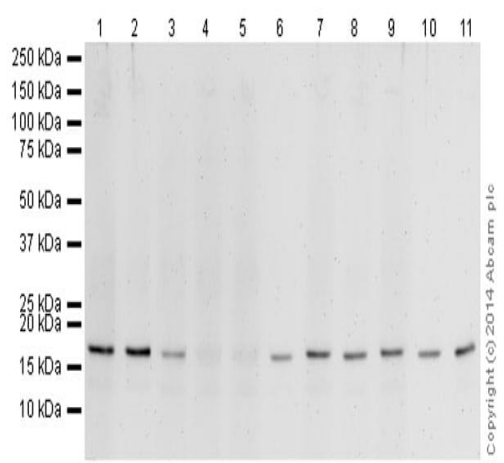
Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (di methyl K4, tri methyl K4) antibody [mAbcam 6000] - ChIP Grade (ab6000)

This image is courtesy of Luke Hughes-Davies and Rhiannon Jade, Gurdon Institute, Cambridge, UK

Immunofluorescent imaging of human cells (U2OS) with ab6000 reveals a diffuse background nuclear staining corresponding to global dimethylation of K4, with multiple foci of brighter staining. The complete lack of nucleolar or cytoplasmic staining background confirms the high specificity of this antibody in this application.

IF was performed with a standard paraformaldehyde technique (fixed in PBS buffered PFH 4% for 5 minutes, permeabilised with 0.5% triton-PBS for 5 minutes, blocked with 5% milk / 0.2% tween for one hour. Primary antibody used at 1/200 in 5% milk / 0.2% TWEEN for one hour, secondary antibody Alexa 488 for 30 minutes. All blocking and incubation steps carried out at 37 degrees.

Due to the size constraints for images on our website, unfortunately we are unable to show a higher quality image.



Western blot - Anti-Histone H3 (di methyl K4, tri methyl K4) antibody [mAbcam 6000] - ChIP Grade (ab6000)

All lanes : Anti-Histone H3 (di methyl K4, tri methyl K4) antibody [mAbcam 6000] - ChIP Grade (ab6000) at 1 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 0.5 µg
Lane 3 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (mono methyl K4) peptide (**ab1340**) at 0.5 µg
Lane 4 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (di methyl K4) peptide (**ab7768**) at 0.5 µg
Lane 5 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (tri methyl K4) peptide (**ab1342**) at 0.5 µg
Lane 6 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (mono methyl K9) peptide (**ab1771**) at 0.5 µg
Lane 7 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (di methyl K9) peptide (**ab1772**) at 0.5 µg
Lane 8 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (tri methyl K9) peptide (**ab1773**) at 0.5 µg
Lane 9 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (mono methyl K27) peptide (**ab1780**) at 0.5 µg
Lane 10 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (di methyl K27) peptide (**ab1781**) at 0.5 µg
Lane 11 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (tri methyl K27) peptide (**ab1782**) at 0.5 µg

Lysates/proteins at 0.5 µg per lane.

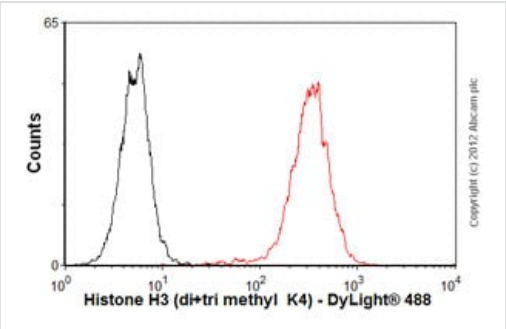
Secondary
All lanes : Goat Anti-Mouse IgG H&L (HRP) preadsorbed (**ab97040**) at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

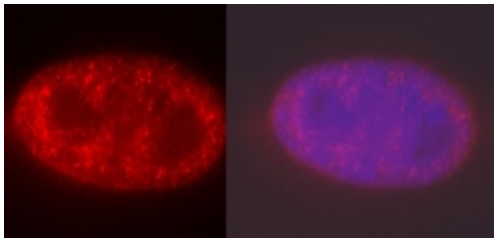
Predicted band size: 15.2 kDa
Observed band size: 17 kDa

Exposure time: 20 minutes



Flow Cytometry (Intracellular) - Anti-Histone H3 (di methyl K4, tri methyl K4) antibody [mAbcam 6000] - ChIP Grade (ab6000)

Overlay histogram showing HeLa cells stained with ab6000 (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 (ab6000, 1µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG2b [PLPV219] (**ab91366**, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (di methyl K4, tri methyl K4) antibody [mAbcam 6000] - ChIP Grade (ab6000)

Left image shows human Hep cell monolayer fixed with 4% formaldehyde, permeabilized using 0.5% Triton X-100, blocked with 5% FCS in PBS (20 min) and incubated with ab6000 for 1 hr (diluted 1/200 in PBS plus 1% FCS). Detection with anti-mouse rhodamin labeled secondary antibody. Unstained areas represent nucleoli and heterochromatin as judged from DAPI overlay of the same cell in the right image.

This image was kindly supplied as part of the review submitted by Christian Schoefer.

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