abcam

Product datasheet

Anti-Histone H2B (mono methyl K5) antibody - ChIP Grade ab12929

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製品の概要

製品名	Anti-Histone H2B (mono methyl K5) antibody - ChIP Grade
製品の詳細	Rabbit polyclonal to Histone H2B (mono methyl K5) - ChIP Grade
由来種	Rabbit
特異性	Mono methylation of Histone H2B K5 is a putative modification site. ab22512 in ELISA specifically recognises mono-methyl K5 histone H2B peptide but not the corresponding unmodified histone H2B peptide.
アプリケーション	**適用あり:** IHC-P, ICC/IF, IP, WB, ChIP
種交差性	**交差種:** Mouse, Rat, Human **交差が予測される動物種:** Chicken, Cow, Xenopus laevis
免疫原	Synthetic peptide within Human Histone H2B aa 1-100 (mono methyl K5) conjugated to Keyhole Limpet Haemocyanin (KLH). The exact sequence is proprietary. (Peptide available as ab13211)
ポジティブ・コントロール	This antibody gave a positive signal in Calf Thymus Histone Preparation Nuclear Lysate. This antibody gave a positive signal in the following Methanol fixed cell lines: HeLa. It also gave a positive signal in FFPE human pancreatic adenocarcinoma tissue sections.

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	Preservative: 0.02% Sodium Azide Constituents: 1% BSA, PBS, pH 7.4
精製度	Immunogen affinity purified
ポリ/モノ	ポリクローナル
アイソタイプ	IgG

アプリケーション

Our Abpromise guarantee covers the use of **ab12929** 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
IHC-P		Use a concentration of 0.1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration. PubMed: 23240083
IP		Use at an assay dependent concentration.
WB	★★★★★	Use a concentration of 1 µg/ml. Can be blocked with Human Histone H2B (mono methyl K5) peptide (ab13211).
ChIP	★★★★★	Use 2-5 µg for 25 µg of chromatin.

ターゲット情報

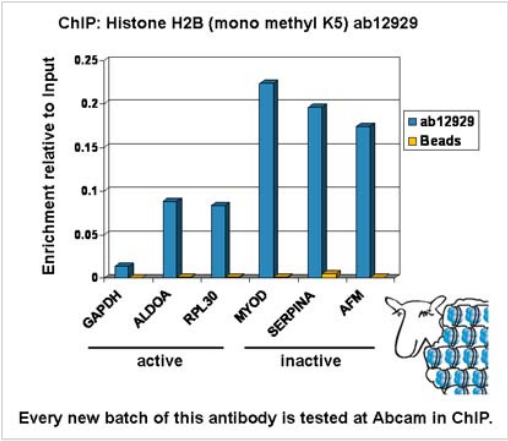
関連性

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. Subunit structure The nucleosome is a histone octamer containing two molecules each of H2A, H2B, H3 and H4 assembled in one H3-H4 heterotetramer and two H2A-H2B heterodimers. The octamer wraps approximately 147 bp of DNA. Post-translational modification Monoubiquitination at Lys-35 (H2BK34Ub) by the MSL1/MSL2 dimer is required for histone H3 'Lys-4' (H3K4me) and 'Lys-79' (H3K79me) methylation and transcription activation at specific gene loci, such as HOXA9 and MEIS1 loci. Similarly, monoubiquitination at Lys-121 (H2BK120Ub) by the RNF20/40 complex gives a specific tag for epigenetic transcriptional activation and is also prerequisite for histone H3 'Lys-4' and 'Lys-79' methylation. It also functions cooperatively with the FACT dimer to stimulate elongation by RNA polymerase II. H2BK120Ub also acts as a regulator of mRNA splicing: deubiquitination by USP49 is required for efficient cotranscriptional splicing of a large set of exons. Phosphorylation at Ser-37 (H2BS36ph) by AMPK in response to stress promotes transcription. Phosphorylated on Ser-15 (H2BS14ph) by STK4/MST1 during apoptosis; which facilitates apoptotic chromatin condensation. Also phosphorylated on Ser-15 in response to DNA double strand breaks (DSBs), and in correlation with somatic hypermutation and immunoglobulin class-switch recombination. GlcNAcylation at Ser-113 promotes monoubiquitination of Lys-121. It fluctuates in response to extracellular glucose, and associates with transcribed genes. Crotonylation (Kcr) is specifically present in male germ cells and marks testis-specific genes in post-meiotic cells, including X-linked genes that escape sex chromosome inactivation in haploid cells. Crotonylation marks active promoters and enhancers and confers resistance to transcriptional repressors. It is also associated with post-meiotically activated genes on autosomes.

細胞内局在

Nuclear

画像



ChIP - Anti-Histone H2B (mono methyl K5) antibody - ChIP Grade (ab12929)

Chromatin was prepared from Hela cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 min. The ChIP was performed with 25 µg of chromatin, 2 µg of ab12929 (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.

Western blot - Anti-Histone H2B (mono methyl K5) antibody - ChIP Grade (ab12929)

All lanes : Anti-Histone H2B (mono methyl K5) antibody - ChIP Grade (ab12929) at 1 µg/ml

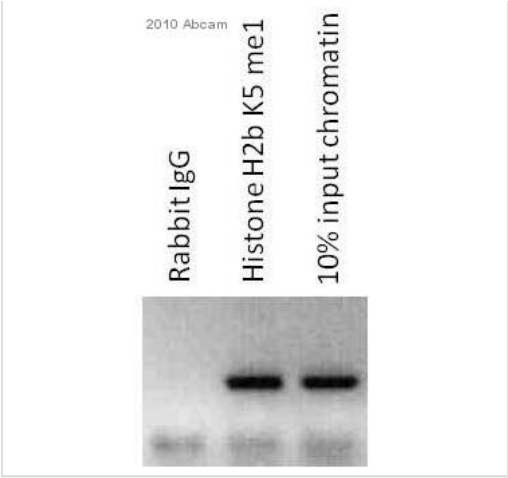
Lane 1 : Calf Thymus Histone Preparation Nuclear Lysate (ab121)
Lane 2 : Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone H2B (mono methyl K5) peptide (ab13211)
Lane 3 : Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone H2B peptide (ab13212)

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : IRDye 680 Conjugated Goat Anti-Rabbit IgG (H+L) at 1/10000 dilution

Performed under reducing conditions.

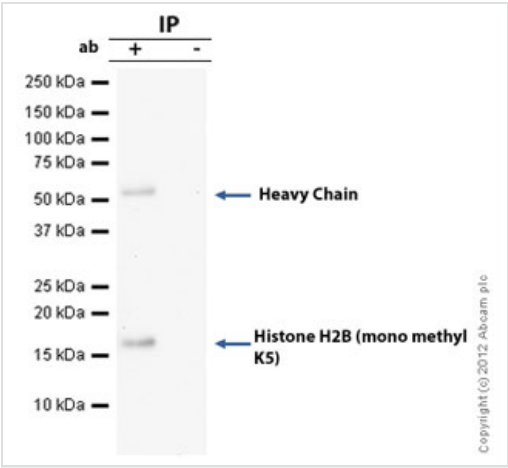
Predicted band size: 14 kDa



ChIP - Anti-Histone H2B (mono methyl K5) antibody - ChIP Grade (ab12929)

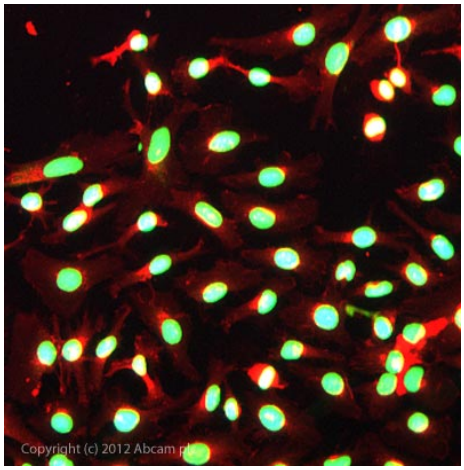
This image is courtesy of an anonymous abreview.

ab12929 at a 1/600 dilution for ChIP analysis of mouse dorsal skin epidermis whole tissue lysate, incubated for 15 hours at 4°C with ChIP dilution buffer. Cross-linking (X-ChIP) using 1% formaldehyde for 10 minutes.
Detection step: Semiquantitative PCR.
Negative control: Rabbit IgG.
Cells untreated.



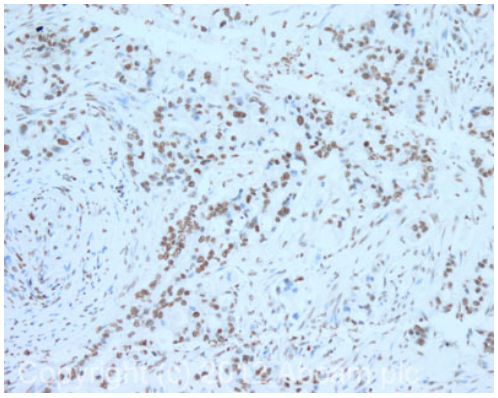
Immunoprecipitation - Anti-Histone H2B (mono methyl K5) antibody - ChIP Grade (ab12929)

Histone H2B (mono methyl K5) was immunoprecipitated using 0.5mg Hela whole cell extract, 5µg of Rabbit polyclonal to Histone H2B (mono methyl K5) - ChIP Grade and 50µl of protein G magnetic beads (+). No antibody was added to the control (-). The antibody was incubated under agitation with Protein G beads for 10min, Hela whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab12929.
Secondary: Clean blot (HRP conjugate) at 1/1000 dilution.
Band: 17kDa: Histone H2B (mono methyl K5).



Immunocytochemistry/ Immunofluorescence - Anti-Histone H2B (mono methyl K5) antibody - ChIP Grade (ab12929)

ICC/IF image of ab12929 stained HeLa cells. The cells were 100% methanol fixed (5 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 ab12929 at 1µg/ml overnight at +4°C. The secondary antibody (green) was a goat anti-rabbit DyLight® 488 (ab96899) 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2B (mono methyl K5) antibody - ChIP Grade (ab12929)

IHC image of Histone H2B (mono methyl K5) staining in human pancreatic adenocarcinoma formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ 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 ab12929, 0.1µg/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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