abcam

Product datasheet

Anti-Histone H2B antibody [mAbcam 64165] - ChIP Grade ab64165

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Overview

Product name	Anti-Histone H2B antibody [mAbcam 64165] - ChIP Grade
Description	Mouse monoclonal [mAbcam 64165] to Histone H2B - ChIP Grade
Host species	Mouse
Tested applications	**Suitable for:** ICC/IF, WB, IP, ChIP, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Human, Recombinant fragment **Predicted to work with:** Mouse, Rat, Chicken, Cow, Xenopus laevis, Caenorhabditis elegans, Drosophila melanogaster, Zebrafish, Orangutan
Immunogen	Synthetic peptide corresponding to Human Histone H2B aa 100 to the C-terminus (C terminal) conjugated to keyhole limpet haemocyanin. (Peptide available as **ab16101**)
Positive control	IP: HeLa whole cell extract. WB: This antibody gave a positive signal on Histone H2B recombinant protein. ChIP: HeLa cells. ICC/IF: HeLa cells. Flow cyt-Intra: HeLa cells.
General notes	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

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

	Constituent: PBS
Purity	Protein G purified
Clonality	Monoclonal
Clone number	mAbcam 64165
Myeloma	Sp2/0
Isotype	IgG2b

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab64165 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
ICC/IF	★★★☆☆ (2)	Use a concentration of 1 µg/ml.
WB	★★★★★ (1)	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 H2B peptide (ab16101)**.
IP		Use a concentration of 5 µg/ml.
ChIP	★★★★★ (1)	Use 5 µg for µg of chromatin.
Flow Cyt (Intra)		Use 1µg for 10^6 cells. **ab170192** - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.

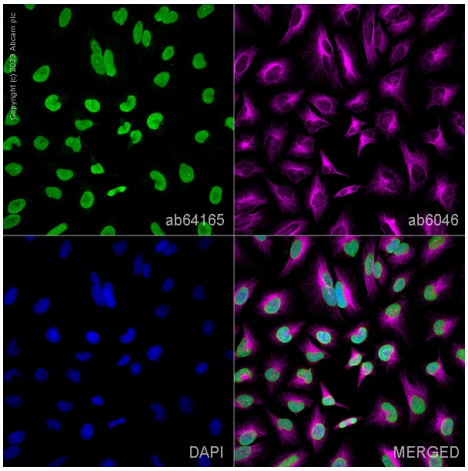
Target

Relevance 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.

Cellular localization Nuclear

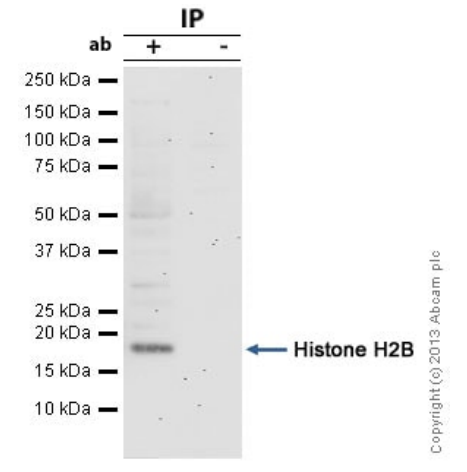
Images



Immunocytochemistry/ Immunofluorescence - Anti-Histone H2B antibody [mAbcam 64165] - ChIP Grade (ab64165)

ab64165 staining Histone H2B in HeLa cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab64165 at 1µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with **ab150117**, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150080**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour magenta). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Immunoprecipitation - Anti-Histone H2B antibody [mAbcam 64165] - ChIP Grade (ab64165)

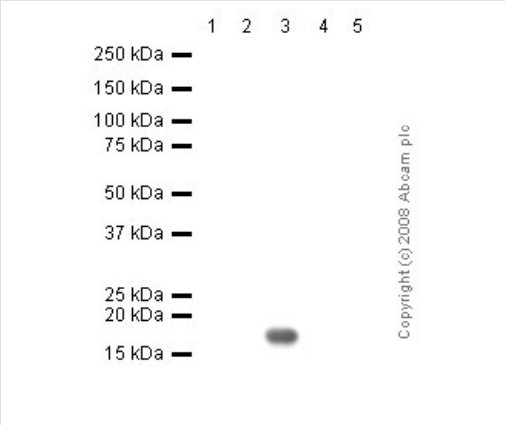
Histone H2B was immunoprecipitated using 0.5mg HeLa whole cell extract, 5µg of Mouse monoclonal to Histone H2B and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

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 ab64165.

Secondary: Goat polyclonal to mouse IgG light chain specific (HRP) at 1/20,000 dilution.

Band: 17kDa; Histone H2B



Western blot - Anti-Histone H2B antibody [mAbcam 64165] - ChIP Grade (ab64165)

All lanes : Anti-Histone H2B antibody [mAbcam 64165] - ChIP Grade (ab64165) at 5 µg/ml

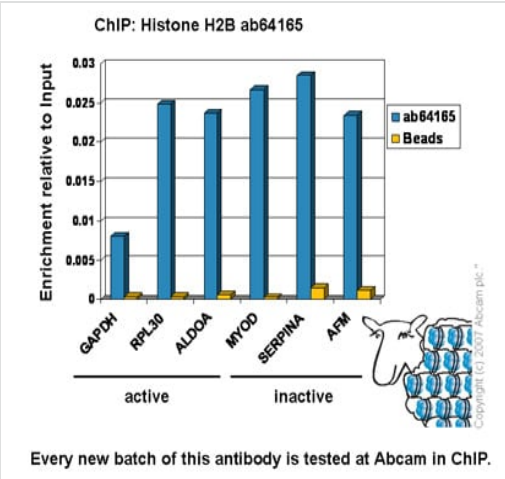
Lane 1 : Histone H1 recombinant protein.
Lane 2 : Histone H2A recombinant protein.
Lane 3 : Histone H2B recombinant protein.
Lane 4 : Histone H3 recombinant protein.
Lane 5 : Histone H4 recombinant protein.

Lysates/proteins at 0.1 µg/ml per lane.

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

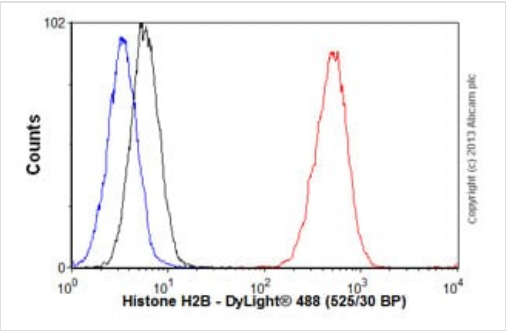
Performed under reducing conditions.

Predicted band size: 15 kDa
Observed band size: 17 kDa



ChIP - Anti-Histone H2B antibody [mAbcam 64165] - ChIP Grade (ab64165)

Chromatin was prepared from HeLa cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10min. The ChIP was performed with 25µg of chromatin, 5µg of ab64165 (blue), and 20µl 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.



Flow Cytometry (Intracellular) - Anti-Histone H2B antibody [mAbcam 64165] - ChIP Grade (ab64165)

Overlay histogram showing HeLa cells stained with ab64165 (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 (ab64165, 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. Unlabelled sample (blue line). Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. 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.

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