# abcam

## Product datasheet

## Anti-Histone H2B antibody [IGX4228H] ab213288

Recombinant

1 References 4 Images

Overview

Product name Anti-Histone H2B antibody [IGX4228H]

**Description** Human monoclonal [IGX4228H] to Histone H2B

Host species Human

Tested applications Suitable for: WB, ChIP

Species reactivity Reacts with: Mouse, Cow, Human, Recombinant fragment

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: CTH, HeLa whole cell, HeLa Nuclear, NIH3T3 whole cell, NIH3T3 Nuclear and Histone H2B

Recombinant Protein lysates. ChIP: HeLa and NIH3T3.

**General notes**This product was made using synthetic libraries and phage display technology.

This antibody is a recombinant antibody.

Human monoclonal antibody.

For recommended secondary antibodies -

Rabbit monoclonal Anti-Human lgG1 H&L (Alexa Fluor® 488) - <u>ab200622</u> Rabbit monoclonal Anti-Human lgG1 H&L (Alexa Fluor® 647) - <u>ab200623</u>

Rabbit monoclonal Anti-Human IgG1 H&L (Biotin) - ab201485

Rabbit Anti-Human IgG H&L (HRP) - ab6759

**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 long

term. Avoid freeze / thaw cycle.

**Storage buffer** pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 0.05% BSA, 59% PBS, 40% Glycerol (glycerin, glycerine)

Clonality Monoclonal
Clone number IGX4228H

**Isotype** IgG1

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#### **Applications**

#### The Abpromise guarantee

Our Abpromise guarantee covers the use of ab213288 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
WB		Use a concentration of 1 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 14 kDa).
ChIP		Use 2 µg for 25 µg of chromatin.

#### **Target**

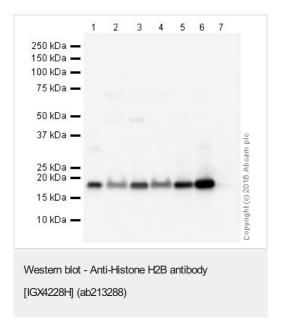
#### Relevance

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**



**All lanes :** Anti-Histone H2B antibody [IGX4228H] (ab213288) at 1  $\mu$ g/ml

Lane 1: CTH (Calf Thymus Histone) at 0.5 µg

Lane 2 : HeLa (Human epithelial carcinoma cell line) Whole Cell

Lysate at 20 µg

Lane 3: HeLa (Human epithelial carcinoma cell line) Nuclear

Lysate at 10 µg

Lane 4: NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell

Lysate at 20 µg

Lane 5: NIH 3T3 (Mouse embryonic fibroblast cell line) Nuclear

Lysate at 10 µg

Lane 6: Histone H2B Recombinant Protein at 0.1 µg

Lane 7: Histone H3 Recombinant Protein at 0.1 µg

### Secondary

**All lanes :** HRP conjugated Goat Anti-Human IgG (H+L) at 1/10000 dilution

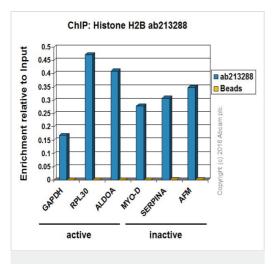
Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size:** 14 kDa **Observed band size:** 17 kDa

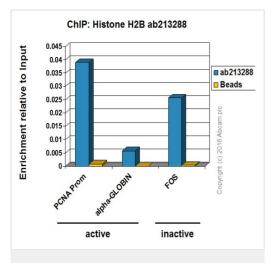
Exposure time: 30 seconds

This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% milk before being incubated with ab213288 overnight at 4°C. Antibody binding was detected using an anti-human antibody conjugated to HRP, and visualised using ECL development solution **ab133406**.



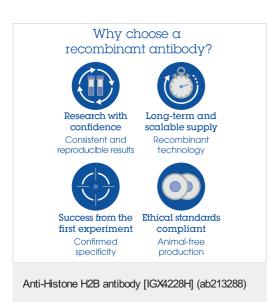
ChIP - Anti-Histone H2B antibody [IGX4228H] (ab213288)

Chromatin was prepared from HeLa cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 2µg of ab213288 (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.



ChIP - Anti-Histone H2B antibody [IGX4228H] (ab213288)

Chromatin was prepared from NIH3T3 cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 2µg of ab213288 (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 (Sybr green approach). Primers and probes are located in the first kb of the transcribed region.



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