

Histone H2B (D2H6) Rabbit mAb



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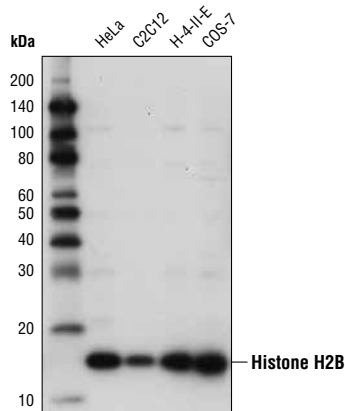
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rev. 03/21/18

For Research Use Only. Not For Use In Diagnostic Procedures.

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IHC-P, ChIP Endogenous	H, M, R, Mk, (C, Z, B)	14 kDa	Rabbit IgG**

Background: The nucleosome, made up of four core histone proteins (H2A, H2B, H3, and H4), is the primary building block of chromatin. Originally thought to function as a static scaffold for DNA packaging, histones have now been shown to be dynamic proteins, undergoing multiple types of post-translational modifications, including acetylation, phosphorylation, methylation, and ubiquitination (1,2). The p300/CBP histone acetyltransferases acetylate multiple lysine residues in the amino terminal tail of histone H2B (Lys5, 12, 15, and 20) at gene promoters during transcriptional activation (1-3). Hyper-acetylation of the histone tails neutralizes the positive charge of these domains and is believed to weaken histone-DNA and nucleosome-nucleosome interactions, thereby destabilizing chromatin structure and increasing the access of DNA to various DNA-binding proteins (4,5). In addition, acetylation of specific lysine residues creates docking sites that facilitate recruitment of many transcription and chromatin regulatory proteins that contain a bromodomain, which binds to acetylated lysine residues (6). Histone H2B is mono-ubiquitinated at Lys120 during transcriptional activation by the RAD6 E2 protein in conjunction with the BRE1A/BRE1B E3 ligase (also known as RNF20/RNF40) (7). Mono-ubiquitinated histone H2B Lys120 is associated with the transcribed region of active genes and stimulates transcriptional elongation by facilitating FACT-dependent chromatin remodeling (7-9). In addition, it is essential for subsequent methylation of histone H3 Lys4 and Lys79, two additional histone modifications that regulate transcriptional initiation and elongation (10). In response to metabolic stress, AMPK is recruited to responsive genes and phosphorylates histone H2B at Lys36, both at promoters and in transcribed regions of genes, and may regulate transcriptional elongation (11). In response to multiple apoptotic stimuli, histone H2B is phosphorylated at Ser14 by the Mst1 kinase (12). Upon induction of apoptosis, Mst1 is cleaved and activated by caspase-3, leading to global phosphorylation of histone H2B during chromatin condensation. Interestingly, histone H2B is rapidly phosphorylated at irradiation-induced DNA damage foci in mouse embryonic fibroblasts (13). In this case, phosphorylation at Ser14 is rapid, depends on prior phosphorylation of H2AX Ser139, and occurs in the absence of apoptosis, suggesting that Ser14 phosphorylation may have distinct roles in DNA-damage repair and apoptosis.



Western blot analysis of extracts from various cell lines using Histone H2B (D2H6) Rabbit mAb.

Specificity/Sensitivity: Histone H2B (D2H6) Rabbit mAb recognizes endogenous levels of total H2B protein. This antibody does not cross-react with other histone proteins.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Val118 of human histone H2B protein.

Entrez-Gene ID #3018
Swiss-Prot Acc. #P33778

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

*Species cross-reactivity is determined by western blot.

**Anti-rabbit secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

Western blotting 1:1000
Immunohistochemistry (Paraffin) 1:1600†

Unmasking buffer: Citrate
Antibody diluent: SignalStain® Antibody Diluent #8112
Detection reagent: SignalStain® Boost (HRP, Rabbit) #8114

†Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.

Chromatin IP 1:50
Optimal ChIP conditions: 10 µl of antibody & 10 µg of chromatin (4 x 10⁶ cells) per IP. Antibody validated using SimpleChIP® Enzymatic ChIP Kits

For product specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended complementary products.

Background References:

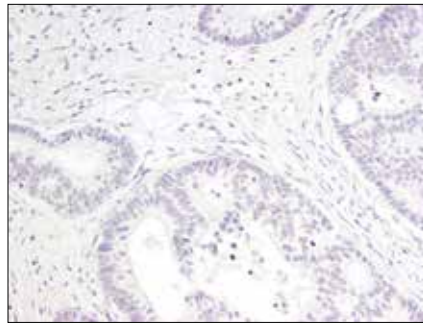
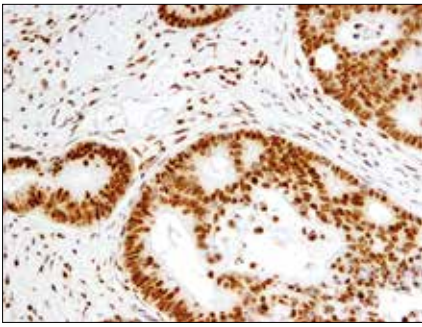
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IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

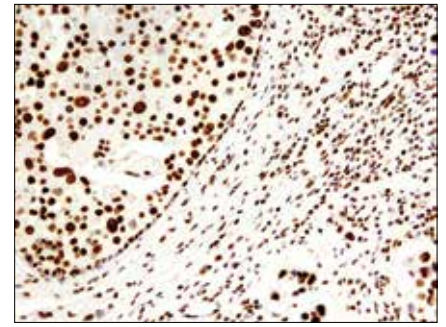
Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA—Peptide

Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine

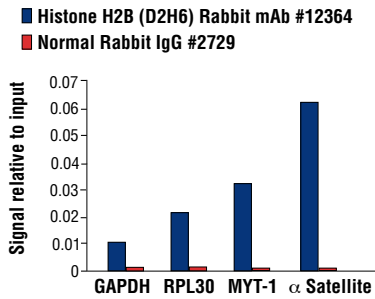
Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



Immunohistochemical analysis of paraffin-embedded human colon carcinoma using Histone H2B (D2H6) Rabbit mAb in the presence of control peptide (left) or antigen-specific peptide (right).



Immunohistochemical analysis of paraffin-embedded human breast carcinoma using Histone H2B (D2H6) Rabbit mAb.



Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells and either Histone H2B (D2H6) Rabbit mAb or Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human GAPDH Exon 1 Primers #5516, SimpleChIP® Human RPL30 Exon 3 Primers #7014, SimpleChIP® Human MYT-1 Exon 1 Primers #4493, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.