Revision 3

#12799 Store at -20C

Acetyl-Histone H2B (Lys5) (D5H1S) XP® Rabbit mAb

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, IP, IHC-P, IF-IC, ChIP	H M R Mk	Endogenous	14	Rabbit IgG	#P33778	3018

Product Usage Information

For optimal ChIP results, use 10 µl of antibody and 10 µg of chromatin (approximately 4 x 10^6 cells) per IP. This antibody has been validated using SimpleChIP® Enzymatic Chromatin IP Kits.

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:100
Immunohistochemistry (Paraffin)	1:1000
Immunofluorescence (Immunocytochemistry)	1:400
Chromatin IP	1:50

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.

For a carrier free (BSA and azide free) version of this product see product #99333.

Specificity / Sensitivity

Acetyl-Histone H2B (Lys5) (D5H1S) XP® Rabbit mAb recognizes endogenous levels of histone H2B only when acetylated at Lys5. This antibody does not cross-react with other acetylated histones.

Species predicted to react based on 100% sequence homology:

Hamster, Chicken, Zebrafish, Bovine, Horse

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding acetylated Lys5 of human histone H2B protein.

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.

Background References

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5. Hansen, J.C. et al. (1998) *Biochemistry* 37, 17637-41.
6. Yang, X.J. (2004) *Bioessays* 26, 1076-87.
7. Kim, J. et al. (2009) *Cell* 137, 459-71.
8. Minsky, N. et al. (2008) *Nat Cell Biol* 10, 483-8.
9. Pavri, R. et al. (2006) *Cell* 125, 703-17.
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11. Bungard, D. et al. (2010) *Science* 329, 1201-5.
12. Cheung, W.L. et al. (2003) *Cell* 113, 507-17.
13. Fernandez-Capetillo, O. et al. (2004) *J Exp Med* 199, 1671-7.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

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

Applications Key

WB: Western Blotting **IP:** Immunoprecipitation **IHC-P:** Immunohistochemistry (Paraffin)
IF-IC: Immunofluorescence (Immunocytochemistry) **ChIP:** Chromatin IP

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **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
GP: Guinea Pig **Rab:** rabbit **All:** all species expected

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Limited Uses