

#5546 Store at -20C**Ubiquityl-Histone H2B (Lys120)
(D11) XP® Rabbit mAb****Cell Signaling**
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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, IF-IC, FC-FP, ChIP, ChIP-seq	H M R Mk	Endogenous	23	Rabbit IgG	#P33778	3018

Product Usage Information

For optimal ChIP and ChIP-seq results, use 2.5 µl of antibody and 10 µg of chromatin (approximately 4 x 10⁶ cells) per IP. This antibody has been validated using SimpleChIP® Enzymatic Chromatin IP Kits.

Application	Dilution
Western Blotting	1:1000
Immunofluorescence (Immunocytochemistry)	1:800 - 1:3200
Flow Cytometry (Fixed/Permeabilized)	1:400 - 1:1600
Chromatin IP	1:200
Chromatin IP-seq	1:200

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.

Specificity / Sensitivity

Ubiquityl-Histone H2B (Lys120) (D11) XP® Rabbit mAb detects endogenous levels of histone H2B protein only when ubiquitylated on Lys120. The antibody does not cross-react with other ubiquitylated proteins or free ubiquitin.

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to the carboxy terminus of the human histone H2B protein in which Lys120 is mono-ubiquitylated.

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 ubiquitylation (1). Ubiquitin is a conserved 76 amino acid peptide unit that can be covalently linked to many cellular proteins by the ubiquitylation process. Three components are involved in this protein-ubiquitin conjugation process. Ubiquitin is first activated by forming a thiolester complex with the activation component E1; the activated ubiquitin is subsequently transferred to the ubiquitin-carrier protein E2, then from E2 to ubiquitin ligase E3 for final delivery to the epsilon-NH₂ of the target protein lysine residue (2). Histone H2B is mono-ubiquitylated on lysine 120 during transcriptional activation by the RAD6 E2 protein in conjunction with the BRE1A/BRE1B E3 ligase (also known as RNF20/RNF40) (3). The RAD6/BRE1 complex is recruited to gene promoters during activation by the PAF complex, an RNA polymerase II-associated protein complex that regulates transcriptional elongation (3-5). Mono-ubiquitylated histone H2B lysine 120 is associated with the transcribed region of active genes (3,6). Mono-ubiquitylation of histone H2B stimulates transcriptional elongation by facilitating FACT-dependent chromatin remodeling (7,8). In addition, it is essential for subsequent methylation of histone H3 lysines 4 and 79, two additional histone modifications that regulate transcriptional initiation and elongation (9). Interestingly, de-ubiquitylation of histone H2B lysine 120 by USP22, a subunit of the human SAGA histone acetyltransferase complex, is a required step in transcriptional activation (10). Thus, it appears that the ubiquitylation state of histone H2B is dynamic during transcription and may serve as an intermediate step in transcriptional activation.

Background References

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2. Liu, F. and Walters, K.J. (2010) *Trends Biochem Sci* 35, 352-60.
3. Kim, J. et al. (2009) *Cell* 137, 459-71.
4. Wood, A. et al. (2003) *J Biol Chem* 278, 34739-42.
5. Xiao, T. et al. (2005) *Mol Cell Biol* 25, 637-51.
6. Minsky, N. et al. (2008) *Nat Cell Biol* 10, 483-8.
7. Pavri, R. et al. (2006) *Cell* 125, 703-17.
8. Fleming, A.B. et al. (2008) *Mol Cell* 31, 57-66.
9. Shilatifard, A. (2006) *Annu Rev Biochem* 75, 243-69.
10. Wyce, A. et al. (2004) *Novartis Found Symp* 259, 63-73; discussion 73-7, 163-9.

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 **IF-IC:** Immunofluorescence (Immunocytochemistry)
FC-FP: Flow Cytometry (Fixed/Permeabilized) **ChIP:** Chromatin IP **ChIP-seq:** Chromatin IP-seq

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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