#8173 Store at -20C

Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb

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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, C&R, C&T	H M R Mk	Endogenous	17	Rabbit IgG	#P68431	8350

Product Usage Information

For optimal ChIP and ChIP-seq results, use 5 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.

The CUT&RUN dilution was determined using CUT&RUN Assay Kit #86652.

The CUT&Tag dilution was determined using CUT&Tag Assay Kit #77552.

Application	Dilution
Western Blotting	1:1000
Immunofluorescence (Immunocytochemistry)	1:50 - 1:200
Flow Cytometry (Fixed/Permeabilized)	1:50 - 1:200
Chromatin IP	1:100
Chromatin IP-seq	1:100
CUT&RUN	1:100
CUT&Tag	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 #87261.

Specificity / Sensitivity

Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb recognizes endogenous levels of histone H3 protein only when acetylated at Lys27. This antibody does not cross react with histone H3 acetylated at Lys9, 14, 18, 23, or 56. This antibody shows some cross-reactivity with acetyl-histone H2B lysine 5.

Species predicted to react based on 100% sequence homology:

Hamster, Xenopus, Zebrafish, Horse, Guinea Pig, Rabbit

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding acetylated Lys27 of human histone H3 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). Histone acetylation occurs mainly on the amino-terminal tail domains of histones H2A (Lys5), H2B (Lys5, 12, 15, and 20), H3 (Lys9, 14, 18, 23, 27, 36, and 56), and H4 (Lys5, 8, 12, and 16) and is important for the regulation of histone deposition, transcriptional activation, DNA replication, recombination, and DNA repair (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 accessibility of DNA to various DNA-binding proteins (4,5). In addition, acetylation of specific lysine residues creates docking sites for a protein module called the bromodomain, which binds to acetylated lysine residues (6). Many transcription and chromatin regulatory proteins contain bromodomains and may be recruited to gene promoters, in part, through binding of acetylated histone tails. Histone acetylation is mediated by histone acetyltransferases (HATs), such as CBP/p300, GCN5L2, PCAF, and Tip60, which are

recruited to genes by DNA-bound protein factors to facilitate transcriptional activation (3). Deacetylation, which is mediated by histone deacetylases (HDAC and sirtuin proteins), reverses the effects of acetylation and generally facilitates transcriptional repression (7,8).

Background References

1. Peterson, C.L. and Laniel, M.A. (2004) *Curr Biol* 14, R546-51.
2. Jaskelioff, M. and Peterson, C.L. (2003) *Nat Cell Biol* 5, 395-9.
3. Roth, S.Y. et al. (2001) *Annu Rev Biochem* 70, 81-120.
4. Workman, J.L. and Kingston, R.E. (1998) *Annu Rev Biochem* 67, 545-79.
5. Hansen, J.C. et al. (1998) *Biochemistry* 37, 17637-41.
6. Yang, X.J. (2004) *Bioessays* 26, 1076-87.
7. Haberland, M. et al. (2009) *Nat Rev Genet* 10, 32-42.
8. Haigis, M.C. and Sinclair, D.A. (2010) *Annu Rev Pathol* 5, 253-95.

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
C&R: CUT&RUN **C&T:** CUT&Tag

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