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#74073

Tri-Methyl-Histone H3 (Lys79) (E8B3M) Rabbit mAb

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Entrez-Gene ID #8350
UniProt ID #P68431

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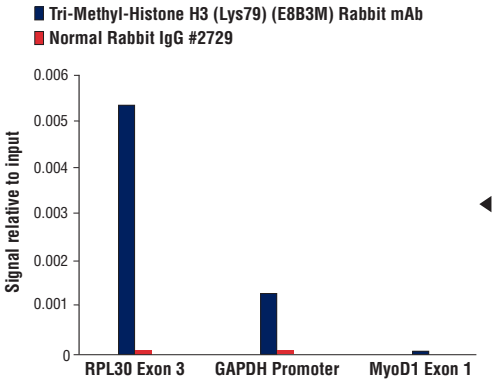
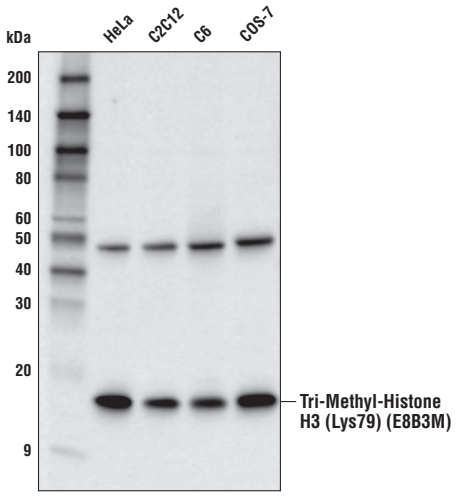
Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, ChIP, ChIP-seq	H, M, R, Mk	17 kDa	Rabbit IgG**
Endogenous			

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). Histone methylation is a major determinant for the formation of active and inactive regions of the genome and is crucial for the proper programming of the genome during development (2,3). Arginine methylation of histones H3 (Arg2, 17, 26) and H4 (Arg3) promotes transcriptional activation and is mediated by a family of protein arginine methyltransferases (PRMTs), including the co-activators PRMT1 and CARM1 (PRMT4) (4). In contrast, a more diverse set of histone lysine methyltransferases has been identified, all but one of which contain a conserved catalytic SET domain originally identified in the *Drosophila* Su(var)3-9, Enhancer of zeste, and Trithorax proteins. Lysine methylation occurs primarily on histones H3 (Lys4, 9, 27, 36, 79) and H4 (Lys20) and has been implicated in both transcriptional activation and silencing (4). Methylation of these lysine residues coordinates the recruitment of chromatin modifying enzymes containing methyl-lysine binding modules such as chromodomains (HP1, PRC1), PHD fingers (BPTF, ING2), tudor domains (53BP1), and WD-40 domains (WDR5) (5-8). The discovery of histone demethylases such as PADI4, LSD1, JMJD1, JMJD2, and JHDM1 has shown that methylation is a reversible epigenetic marker (9).



Specificity/Sensitivity: Tri-Methyl-Histone H3 (Lys79) (E8B3M) Rabbit mAb recognizes endogenous levels of histone H3 protein when tri-methylated at Lys79. This antibody shows some cross-reactivity to histone H3 that is di-methylated on Lys79, but does not cross-react with non-methylated or mono-methylated histone H3 Lys79. The antibody does not cross-react with histone H3 methylated at Lys4, Lys9, Lys27, or Lys36, and does not cross-react with any other methylated histone proteins.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide containing histone H3 tri-methyl lysine 79.



Western blot analysis of extracts from various cell lines using Tri-Methyl-Histone H3 (Lys79) (E8B3M) Rabbit mAb.

◀ *Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells and either Tri-Methyl-Histone H3 (Lys79) (E8B3M) Rabbit mAb or Normal Rabbit IgG #2729 using SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human RPL30 Exon 3 Primers #7014, SimpleChIP® Human GAPDH Promoter Primers #4471, and SimpleChIP® Human MyoD1 Exon 1 Primers #4490. 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.*

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
Chromatin IP / Chromatin IP-seq	1:50

Optimal ChIP / ChIP-seq conditions: 10 µl of antibody & 10 µg of chromatin (4 x 10^6 cells) per IP. Antibody validated using SimpleChIP® Enzymatic ChIP Kits.

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com.

Background References:

(1) Peterson, C.L. and Laniel, M.A. (2004) *Curr Biol* 14, R546-51.

(2) Kubicek, S. et al. (2006) *Ernst Schering Res Found Workshop*, 1-27.

(3) Lin, W. and Dent, S.Y. (2006) *Curr Opin Genet Dev* 16, 137-42.

(4) Lee, D.Y. et al. (2005) *Endocr Rev* 26, 147-70.

(5) Daniel, J.A. et al. (2005) *Cell Cycle* 4, 919-26.

(6) Shi, X. et al. (2006) *Nature* 442, 96-9.

(7) Wysocka, J. et al. (2006) *Nature* 442, 86-90.

(8) Wysocka, J. et al. (2005) *Cell* 121, 859-72.

(9) Trojer, P. and Reinberg, D. (2006) *Cell* 125, 213-7.

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.

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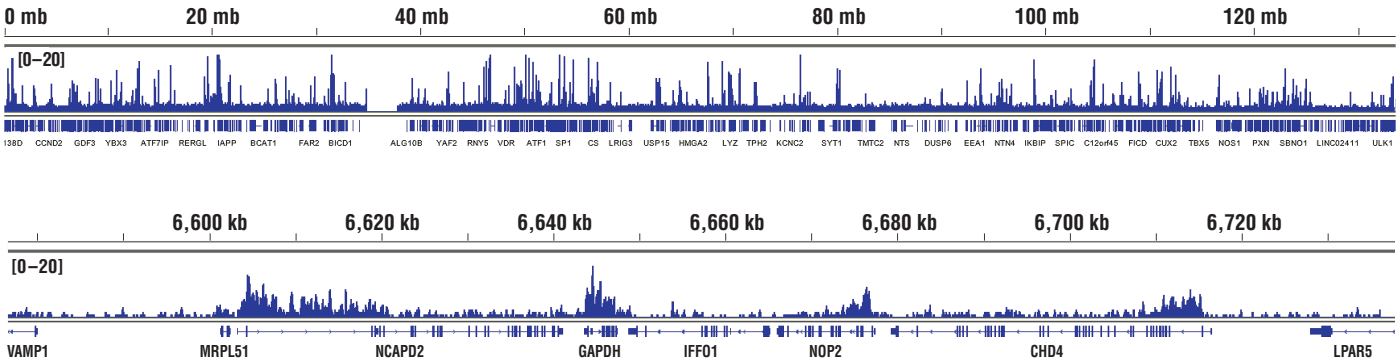
Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **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.



Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells and Tri-Methyl-Histone H3 (Lys79) (E8B3M) Rabbit mAb, using SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. DNA Libraries were prepared using SimpleChIP® ChIP-seq DNA Library Prep Kit for Illumina® #56795. The figure shows binding across chromosome 12 (upper), including GAPDH (lower), a known target gene of H3K79me3 (see additional figures containing ChIP-qPCR data).