#9733 Store at -20°C

Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb

☑ 100 µl
(10 western blots)

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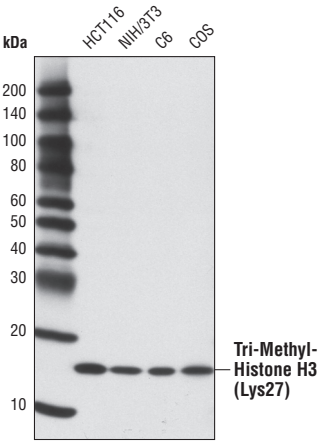
Entrez-Gene ID #8352
UniProt ID #P68431

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P, IF-IC, ChIP, F Endogenous	H, M, R, Mk, (X, Z)	17 kDa	Rabbit IgG**

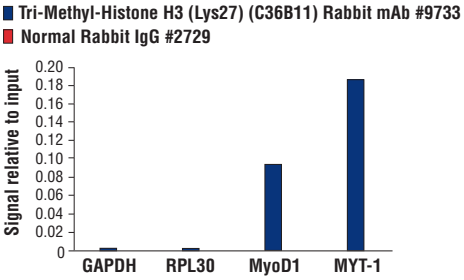
Background: The nucleosome, made up of four core histone proteins (H2A, H2B, H3 and H4), is the primary building block of chromatin. Originally thought to function as a static scaffold for DNA packaging, histones have now been shown to be dynamic proteins, undergoing multiple types of post-translational modifications, including acetylation, phosphorylation, methylation and ubiquitination (1). 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 have 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 recent discovery of histone demethylases such as PADI4, LSD1, JMJD1, JMJD2 and JHDM1 has shown that methylation is a reversible epigenetic mark (9).

Specificity/Sensitivity: Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb detects endogenous levels of histone H3 only when tri-methylated on Lys27. The antibody does not cross-react with non-methylated, mono-methylated or di-methylated Lys27. In addition, the antibody does not cross-react with mono-methylated, di-methylated or tri-methylated histone H3 at Lys4, Lys9, Lys36 or Histone H4 at Lys20.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to the amino terminus of histone H3 in which Lys27 is tri-methylated.



Western blot analysis of various cell lines using Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb.



Chromatin immunoprecipitations were performed with cross-linked chromatin from 4 x 10^6 HeLa cells and either 10 µl of Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb, or 2 µl of 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 MyoD1 Exon 1 Primers #4490, and SimpleChIP® Human MYT-1 Exon 1 Primers #4493. 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
Immunoprecipitation	1:50
Immunohistochemistry (Paraffin)	1:200†
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.*

Immunofluorescence (IF-IC)	1:1600
Chromatin IP	1:50
Flow Cytometry	1:200

For application 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 companion products.

Background References:

(1) Peterson, C.L. and Laniel, M.A. (2004) Curr. Biol. 14, R546–R551.

(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, 137–142.

(4) Lee, D.Y. et al. (2005) Endocr. Rev. 26, 147–170.

(5) Daniel, J.A. et al. (2005) Cell Cycle 4, 919–926.

(6) Shi, X. et al. (2006) Nature 442, 96–99.

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

(8) Wysocka, J. et al. (2005) Cell 121, 859–872.

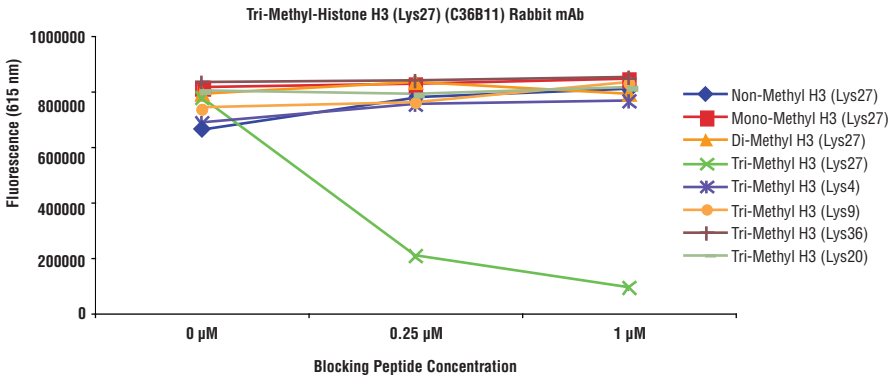
(9) Trojer, P. and Reinberg, D. (2006) Cell 125, 213–217.

U.S. Patent No. 5,675,063

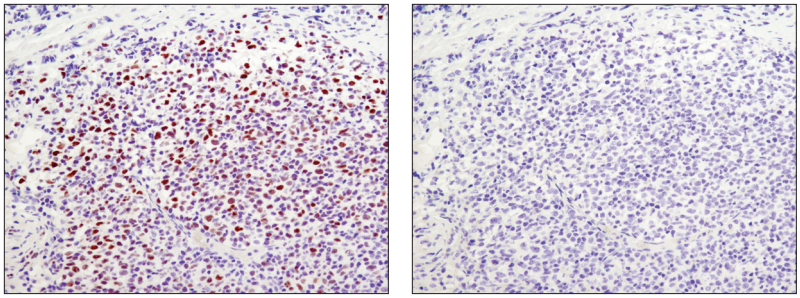
IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

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

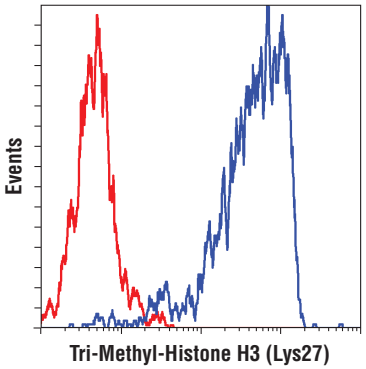
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



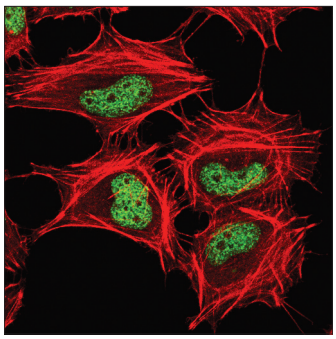
Tri-Methyl Histone H3 (Lys27) (C36B11) Rabbit mAb specificity was determined by peptide ELISA. The graph depicts the binding of the antibody to pre-coated tri-methyl histone H3 (Lys27) peptide in the presence of increasing concentrations of various competitor peptides. As shown, only the tri-methyl histone H3 (Lys27) peptide competed away binding of the antibody.



Immunohistochemical analysis of paraffin-embedded human lymphoma using Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb in the presence of non-methyl peptide (left) or K27 tri-methyl peptide (right).



Flow cytometric analysis of human whole blood cells using Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb (blue) and Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (red). Anti-rabbit IgG (H+L), F(ab')$_2$ Fragment (Alexa Fluor® 647 Conjugate) #4414 was used as a secondary antibody. Analysis was performed on cells in the lymphocyte gate.



Confocal immunofluorescent analysis of HeLa cells using Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb (green). Actin filaments have been labeled with DY-554 phalloidin (red).