

Di-Methyl-Histone H3 (Lys9) (D85B4) XP® Rabbit mAb



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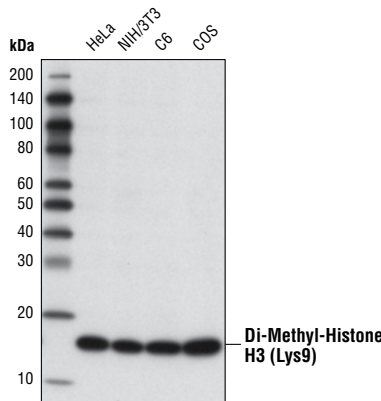
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Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IF-IC, ChIP, F Endogenous	H, M, R, Mk, (Pg, Sc, B, Dm, X, Z, Ce)	17 kDa	Rabbit IgG**

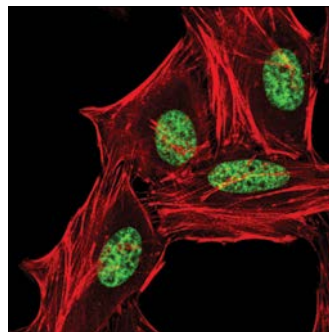
Background: The nucleosome, made up of four core histone proteins (H2A, H2B, H3 and H4), is the primary building block of chromatin. Originally thought to function as a static scaffold for DNA packaging, histones have now been shown to be dynamic proteins, undergoing multiple types of post-translational 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 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 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: Di-Methyl-Histone H3 (Lys9) (D85B4) XP® Rabbit mAb detects endogenous levels of histone H3 only when di-methylated on Lys9. The antibody does not cross-react with non-methylated, mono-methylated or tri-methylated Histone H3 Lys9. In addition, the antibody does not cross-react with histone H3 di-methylated on Lys4, Lys27, or Lys36 and histone H4 di-methylated on Lys20.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to the amino terminus of histone H3 in which Lys9 is di-methylated.



Western blot analysis of extracts from various cell lines using Di-Methyl-Histone H3 (Lys9) (D85B4) XP® Rabbit mAb.



Confocal immunofluorescent analysis of HeLa cells using Di-Methyl-Histone H3 (Lys9) (D85B4) XP® Rabbit mAb (green). Actin filaments have been labeled with DyLight™ 554 Phalloidin #13054 (red).

Entrez-Gene ID #8352
UniProt ID #P68431

Storage: Supplied in 10 mM 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
Immunofluorescence (IF-IC)	1:200
Flow Cytometry	1:100
Chromatin IP	1:50

Optimal ChIP conditions: 10 µl of antibody & 10 µg of chromatin (4 x 10⁶ 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.

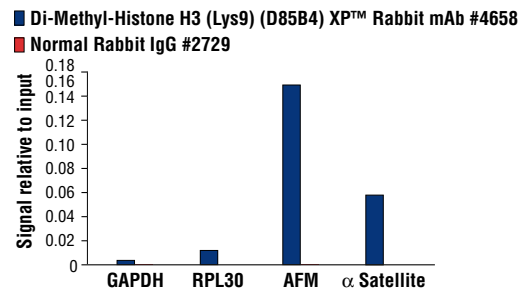
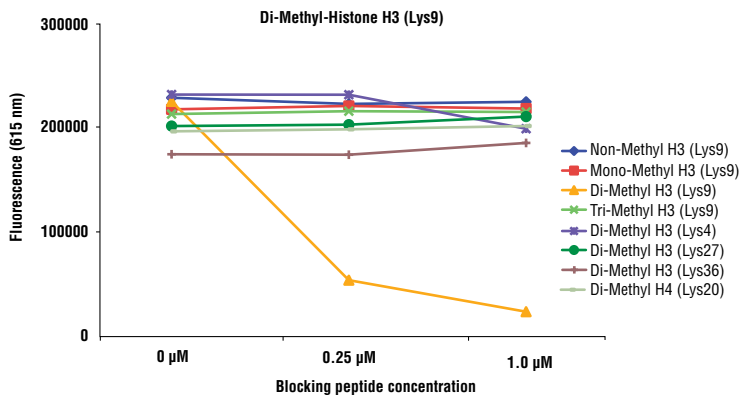
Background References:

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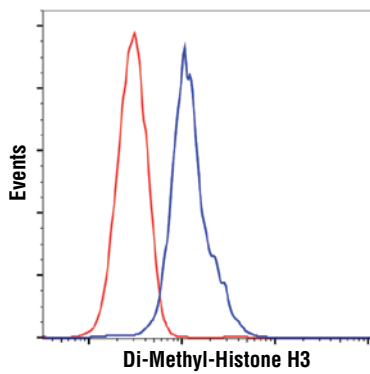
IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

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.



Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells and either Di-Methyl-Histone H3 (Lys9) (D85B4) XP[®] Rabbit mAb or 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 AFM Intron 1 Primers #5098, and SimpleChIP[®] Human α Satellite Repeat Primers #4486. 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.

Di-Methyl Histone H3 (Lys9) (D85B4) XP[®] Rabbit mAb specificity was determined by peptide ELISA. The graph depicts the binding of the antibody to pre-coated di-methyl histone H3 (Lys9) peptide in the presence of increasing concentrations of various competitor peptides. As shown, only the di-methyl histone H3 (Lys9) peptide competed away binding of the antibody.



◀ Flow cytometric analysis of HeLa cells using Di-Methyl-Histone H3 (Lys9) (D85B4) XP[®] Rabbit mAb (blue) compared to concentration-matched Rabbit (DA1E) mAb IgG XP[®] Isotype Control #3900 (red). Anti-rabbit IgG (H+L)₂ Fragment (Alexa Fluor[®] 488 Conjugate) #4412 was used as a secondary antibody.