

**#4069** Store at **-20°C**

# Pan-Methyl Histone H3 (Lys9) Antibody

100 µl  
 (10 western blots)



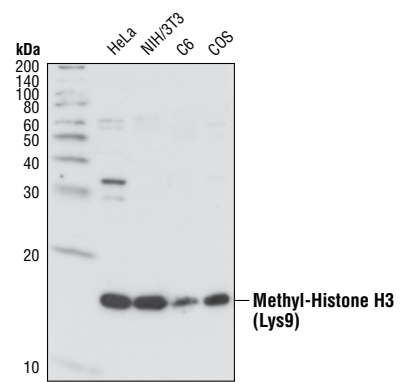
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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP, IF-IC, ChIP Endogenous	H, M, R, Mk, Z	17 kDa	Rabbit**

**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) (5,6,7,8). The recent discovery of histone demethylases such as PADI4, LSD1, JMJD1, JMJD2 and JHDM1 has shown that methylation is a reversible epigenetic mark (9).



Western blot analysis of whole cell lysates from HeLa, NIH/3T3, C6 and COS cells, using Pan-Methyl Histone H3 (Lys9) Antibody.

**Specificity/Sensitivity:** This antibody detects endogenous levels of histone H3 only when mono-, di-, or tri-methylated on Lys9. The antibody does not cross-react with histone H3 mono-methylated, di-methylated or tri-methylated on Lys27.

**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic peptide (KLH-coupled) corresponding to the amino terminus of histone H3 in which Lys9 is di-methylated. Antibodies are purified by protein A and peptide affinity chromatography.

**Entrez-Gene ID** # 8352  
**Swiss-Prot Acc.** # P68431

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at  $-20^{\circ}\text{C}$ . Do not aliquot the antibody.

**\*Species cross-reactivity is determined by western blot.**  
**\*\*Anti-rabbit secondary antibodies must be used to detect this antibody.**

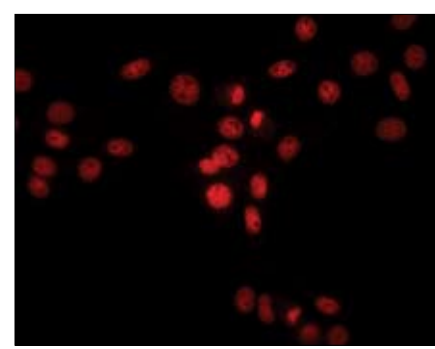
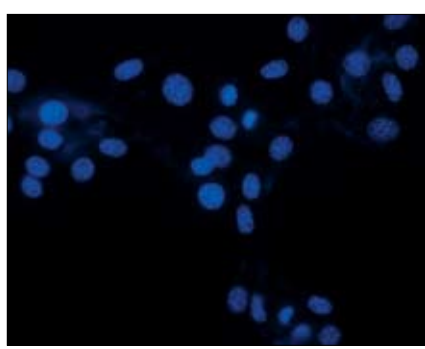
**Recommended Antibody Dilutions:**

Western blotting	1:1000
Immunoprecipitation	1:25
Immunofluorescence (IF-IC)	1:500
Chromatin IP	1:25

For application specific protocols please see the web page for this product at [www.cellsignaling.com](http://www.cellsignaling.com).

Please visit [www.cellsignaling.com](http://www.cellsignaling.com) for a complete listing of recommended companion products.

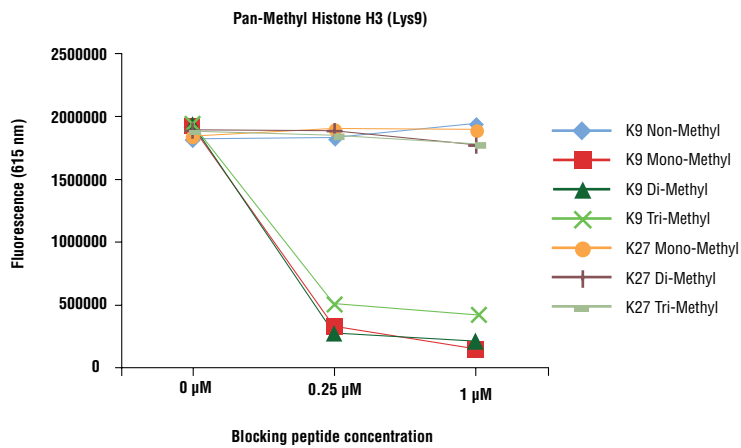
- Background References:**
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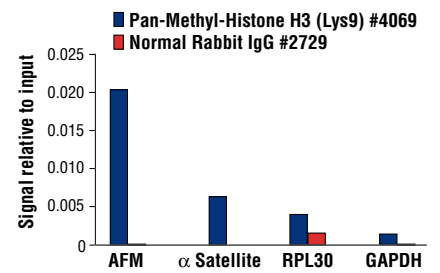
DAPI staining (left) and immunofluorescent analysis (right) of NIH/3T3 cells, using Pan-Methyl Histone H3 (Lys9) Antibody.

**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.



Pan-Methyl Histone H3 (Lys9) Antibody 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 mono-, di- and tri-methyl histone H3 (Lys9) peptides competed away binding of the antibody.



Chromatin immunoprecipitations were performed with cross-linked chromatin from  $2 \times 10^6$  HeLa cells and either 20  $\mu$ l of Pan-Methyl-Histone H3 (Lys9) Antibody #4069 or 1  $\mu$ l of Normal Rabbit IgG #2729, using SimpleChIP™ Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by Real-Time PCR, using primers specific for the transcriptionally inactive AFM gene, the heterochromatic  $\alpha$  Satellite repeat element and the active RPL30 and GAPDH genes. 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.