

Store at -20C  
#4658

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



**Cell Signaling**  
TECHNOLOGY®

**Orders:** 877-616-CELL (2355)  
orders@cellsignal.com

**Support:** 877-678-TECH (8324)

**Web:** info@cellsignal.com  
cellsignal.com

3 Trask Lane | Danvers | Massachusetts | 01923 | USA

**For Research Use Only. Not for Use in Diagnostic Procedures.**

Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, IP, IF-IC, FC-FP, ChIP, ChIP-seq, C&T	H M R Mk	Endogenous	17	Rabbit IgG	#P68431	8350

### Product Usage Information

For optimal ChIP and ChIP-seq results, use 10 µl of antibody and 10 µg of chromatin (approximately 4 x 10<sup>6</sup> cells) per IP. This antibody has been validated using SimpleChIP® Enzymatic Chromatin IP Kits.

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

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:50
Immunofluorescence (Immunocytochemistry)	1:200 - 1:800
Flow Cytometry (Fixed/Permeabilized)	1:50 - 1:200
Chromatin IP	1:50
Chromatin IP-seq	1:50
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 #80806.

### 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.

### Species predicted to react based on 100% sequence homology:

D. melanogaster, Xenopus, Zebrafish, Bovine, Pig, S. cerevisiae, C. elegans, Rabbit

### 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.

### 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).

**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.

**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 **IP:** Immunoprecipitation **IF-IC:** Immunofluorescence (Immunocytochemistry)  
**FC-FP:** Flow Cytometry (Fixed/Permeabilized) **ChIP:** Chromatin IP **ChIP-seq:** Chromatin IP-seq  
**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

**Trademarks and Patents**

Cell Signaling Technology is a trademark of Cell Signaling Technology, Inc.

XP is a registered trademark of Cell Signaling Technology, Inc.

All other trademarks are the property of their respective owners. Visit [cellsignal.com/trademarks](http://cellsignal.com/trademarks) for more information.

**Limited Uses**

Except as otherwise expressly agreed in a writing signed by a legally authorized representative of CST, the following terms apply to Products provided by CST, its affiliates or its distributors. Any Customer's terms and conditions that are in addition to, or different from, those contained herein, unless separately accepted in writing by a legally authorized representative of CST, are rejected and are of no force or effect.

Products are labeled with For Research Use Only or a similar labeling statement and have not been approved, cleared, or licensed by the FDA or other regulatory foreign or domestic entity, for any purpose. Customer shall not use any Product for any diagnostic or therapeutic purpose, or otherwise in any manner that conflicts with its labeling statement. Products sold or licensed by CST are provided for Customer as the end-user and solely for research and development uses. Any use of Product for diagnostic, prophylactic or therapeutic purposes, or any purchase of Product for resale (alone or as a component) or other commercial purpose, requires a separate license from CST. Customer shall (a) not sell, license, loan, donate or otherwise transfer or make available any Product to any third party, whether alone or in combination with other materials, or use the Products to manufacture any commercial products, (b) not copy, modify, reverse engineer, decompile, disassemble or otherwise attempt to discover the underlying structure or technology of the Products, or use the Products for the purpose of developing any products or services that would compete with CST products or services, (c) not alter or remove from the Products any trademarks, trade names, logos, patent or copyright notices or markings, (d) use the Products solely in accordance with CST Product Terms of Sale and any applicable documentation, and (e) comply with any license, terms of service or similar agreement with respect to any third party products or services used by Customer in connection with the Products.