Revision 11

Store at -20C

#5326

Mono-Methyl-Histone H3 (Lys4) (D1A9) 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:
W, IF-IC, FC-FP, ChIP, ChIP-seq, C&R, 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 10^6 cells) per IP. This antibody has been validated using SimpleChIP® Enzymatic Chromatin IP Kits.

The CUT&RUN dilution was determined using CUT&RUN Assay Kit #86652.

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

Application	Dilution
Western Blotting	1:1000
Immunofluorescence (Immunocytochemistry)	1:400 - 1:1600
Flow Cytometry (Fixed/Permeabilized)	1:200 - 1:800
Chromatin IP	1:50
Chromatin IP-seq	1:50
CUT&RUN	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 #53138.

Specificity/Sensitivity

Mono-Methyl-Histone H3 (Lys4) (D1A9) XP® Rabbit mAb detects endogenous levels of histone H3 only when mono-methylated on Lys4. The antibody does not cross-react with non-methylated, di-methylated or tri-methylated histone H3 Lys4. In addition, the antibody does not cross-react with methylated histone H3 Lys9, Lys27, Lys36 or methylated histone H4 Lys20.

Species predicted to react based on 100% sequence homology

D. melanogaster

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to the amino terminus of histone H3 in which lysine 4 is mono-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

W: Western Blotting **IF-IC:** Immunofluorescence (Immunocytochemistry) **FC-FP:** Flow Cytometry (Fixed/Permeabilized) **ChIP:** Chromatin IP **ChIP-seq:** Chromatin IP-seq **C&R:** CUT&RUN **C&T:** CUT&Tag

Cross-Reactivity Key

H: Human **M:** Mouse **R:** Rat **Mk:** Monkey

Trademarks and Patents

Limited Uses