Revision 6

Store at -20C

#5737

Tri-Methyl-Histone H4 (Lys20) (D84D2) Rabbit mAb

Cell Signaling TECHNOLOGY®

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, ChIP, ChIP-seq, C&R, C&T	H M R Mk	Endogenous	11	Rabbit IgG	#P62805	8359

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
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.

Specificity/Sensitivity

Tri-Methyl-Histone H4 (Lys20) (D84D2) Rabbit mAb recognizes endogenous levels of histone H4 protein only when tri-methylated at Lys20. This antibody does not cross-react with non-methylated, mono-methylated or di-methylated histone H4 Lys20. This antibody detects a 95 kDa non-specific protein of unkown origin.

Species predicted to react based on 100% sequence homology

Xenopus, Bovine, Pig

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to the amino terminus of histone H4 in which Lys20 is tri-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 **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

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Limited Uses