Di-Methyl-Histone H3 (Lys36) (C75H12) Rabbit mAb

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 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 JHD1 has shown that methylation is a reversible epigenetic marker (9).

Specificity/Sensitivity: Di-Methyl-Histone H3 (Lys36) (C75H12) Rabbit mAb detects endogenous levels of histone H3.1, histone H3.2, and histone H3.3, only when di-methylated on Lys36. The antibody does not cross-react with non-methylated, mono-methylated, or tri-methylated Lys36. In addition, the antibody does not cross-react with di-methylated histone H3 (Lys4, Lys9, Lys27, Lys79) or di-methylated histone H4 Lys20.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to the amino terminus of histone H3 in which Lys36 is di-methylated.
Immunohistochemical analysis of paraffin-embedded human gastric carcinoma using Di-Methyl-Histone H3 (Lys36) (C75H12) Rabbit mAb in the presence of non-methyl peptide (left) or K36 di-methyl peptide (right).

Blocking Peptides

- A None
- B Non-Methyl H3 (Lys36)
- C Mono-Methyl H3 (Lys36)
- D Di-Methyl H3 (Lys36)
- E Tri-Methyl H3 (Lys36)
- F Di-Methyl H3 (Lys4)
- G Di-Methyl H3 (Lys9)
- H Di-Methyl H3 (Lys27)
- I Di-Methyl H4 (Lys20)

Flow cytometric analysis of HeLa cells using Di-Methyl-Histone H3 (Lys36) (C75H12) XP® Rabbit mAb (blue) compared to concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (red). Anti-rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.

Antibody specificity was determined by Western blotting. HeLa and NIH/3T3 cell extracts were probed with Di-Methyl Histone H3 (Lys36) (C75H12) Rabbit mAb alone (Panel A) or Di-Methyl-Histone H3 (Lys36) (C75H12) Rabbit mAb pre-adsorbed with 1.5 μM of various competitor peptides (Panels B-I). As shown, only the di-methyl histone H3 (Lys36) peptide competed away binding of the antibody.