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#14117

Di-Methyl Lysine Motif [dme-K] MultiMab™ Rabbit mAb mix

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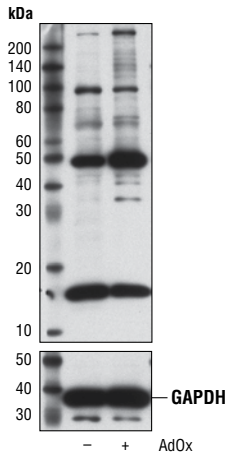
For Research Use Only. Not For Use In Diagnostic Procedures.

Applications	Species Cross-Reactivity*	Isotype
W, IP	All	Rabbit IgG**
Endogenous		

Background: Methylation of lysine residues is a common regulatory posttranslational modification (PTM) that results in the mono-, di-, or tri-methylation of lysine at ε-amine groups by protein lysine methyltransferases (PKMTs). Two PKMT groups are recognized based on structure and catalytic mechanism: class I methyltransferases or seven β strand enzymes, and SET domain-containing class V methyltransferases. Both use the methyl donor S-adenosyl-L-methionine to methylate histone and non-histone proteins. Class I methyltransferases methylate amino acids, DNA, and RNA (1,2). Six methyl-lysine-interacting protein families are distinguished based on binding domains: MBT, PHD finger, Tudor, PWWP, WD40 repeat, and chromodomains. Many of these display differential binding preferences based on lysine methylation state (3). KDM1 subfamily lysine demethylases catalyze demethylation of mono- and di-methyl lysines, while 2-oxoglutarate-dependent JmjC (KDM2-7) subfamily enzymes also modify tri-methyl lysine residues (4).

Most PKMT substrates are histone proteins and transcription factors, emphasizing the importance of lysine methylation in regulating chromatin structure and gene expression. Lys9 of histone H3 is mono- or di-methylated by G9A/GLP and tri-methylated by SETDB1 to activate transcription. JHDM3A-mediated demethylation of the same residue creates mono-methyl Lys9 and inhibits gene transcription (5). Tumor suppressor p53 is regulated by methylation of at least four sites. p53-mediated transcription is repressed following mono-methylation of p53 at Lys370 by SMYD2; di-methylation at the same residue further inhibits p53 by preventing association with 53BP1. Concomitant di-methylation at Lys382 inhibits p53 ubiquitination following DNA damage. Mono-methylation at Lys382 by SET8 suppresses p53 transcriptional activity, while SET7/9 mono-methylation at Lys372 inhibits SMYD2 methylation at Lys370 and stabilizes the p53 protein. Di-methylation at Lys373 by G9A/GLP inhibits p53-mediated apoptosis and correlates with tri-methylation of histone H3 Lys9 at the p21 promoter (1,6). Overexpression of PKMTs is associated with multiple forms of human cancer, which has generated tremendous interest in targeting protein lysine methyltransferases in drug discovery research.

Specificity/Sensitivity: Di-Methyl Lysine Motif [dme-K] MultiMab™ Rabbit mAb mix recognizes endogenous levels of proteins only when di-methylated at a lysine residue. This antibody does not cross-react with endogenous levels of mono- or tri-methyl lysine, or mono- or di-methyl arginine.



Western blot analysis of HeLa cells, untreated (-) or treated with adenosine-2',3'-dialdehyde (AdOx, 100 µM, 24 hr; +), using Di-Methyl Lysine Motif [dme-K] MultiMab™ Rabbit mAb mix (upper) and GAPDH (D16H11) XP® Rabbit mAb #5174 (lower).

Source/Purification: MultiMab™ rabbit monoclonal mix antibodies are prepared by combining individual rabbit monoclonal clones in optimized ratios for the approved applications. Each antibody in the mix is carefully selected based on motif recognition and performance in multiple assays. Each mix is engineered to yield the broadest possible coverage of the modification being studied while ensuring a high degree of specificity for the modification or motif.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol. Store at –20°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	1:100

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

Background References:

(1) Lanouette, S. et al. (2014) *Mol Syst Biol* 10, 724.

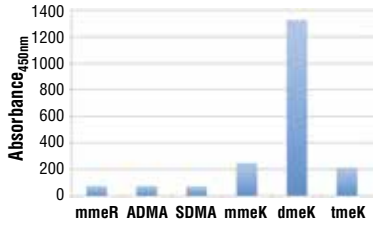
(2) Clarke, S.G. (2013) *Trends Biochem Sci* 38, 243-52.

(3) Herold, J.M. et al. (2011) *Curr Chem Genomics* 5, 51-61.

(4) Thinnes, C.C. et al. (2014) *Biochim Biophys Acta* 1839, 1416-1432.

(5) Klose, R.J. et al. (2006) *Nature* 442, 312-6.

(6) Yost, J.M. et al. (2011) *Curr Chem Genomics* 5, 72-84.



A peptide ELISA was used to determine the specificity of Di-Methyl Lysine Motif [dme-K] MultiMab™ Rabbit mAb mix. The figure demonstrates that the antibody is specific to di-methyl lysine and will not cross-react with mono- or tri-methyl lysine or mono- or di-methyl arginine.

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.

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **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.