

PRODUCT DATASHEET

MLL2 Complex (Mixed Lineage Leukemia Protein-2 in complex with WDR5, RbBP5, Ash2L, (DPY-30)2)

CATALOG NO.: HMT-15-106

LOT NO .:

DESCRIPTION: Human recombinant MLL2 (residues 5319-5537; Genbank Accession # NM 003482; MW = 27 kDa) in complex with human recombinants WDR5 (22-334; NM 017588; 34 kDa), RbBP5 (1-538; NM 005057; 61 kDa), Ash2L (2-534; NM 001105214; 63 kDa) and DPY-30 (1-99; NM 0325742; 13 kDa; two per complex). All proteins were expressed in E. coli with N-terminal His-tags. Catalyzes the transfer of methyl groups from S-adenosyl-L-methionine (SAM) to the ε-amino function of protein L-lysine residues, specifically lysine-4 of histone H3 (H3K4). The MLL2 subunit of this preparation comprises the catalytic SET domain¹ as well as the WDR5 interaction motif (Win)^{2,3} necessary for assembly of the active complex⁴⁻⁶. Like MLLs 1, 3 and 4, MLL2 complex methylates histone H3 lysine-4 (H3K4), conferring an activating epigenetic mark^{7,8}. MLL2 is required for normal embryonic development, gametogenesis and female fertility^{7,9}. Inactivating MLL2 mutations are prevalent in Kabuki syndrome¹⁰, a developmental disorder, in medulloblastoma¹¹, a childhood cancer, and in the two most common non-Hodgkin lymphomas¹². MLL2 expression is elevated in breast and colon cancer cell lines and in both breast and colon tumor tissue¹³. Possible mechanistic links between MLL2 complex activity and breast carcinogenesis include its association with Pygo2 and consequent activation of Wnt target genes¹⁴ and its association with the JMJD2B H3K9 demethylase in a complex required for estrogen receptor α-activated transcription¹⁵. MLL2 complex may therefore represent a therapeutic target for breast and colon cancer or possibly for solid tumors in general.

PURITY: >90% by SDS-PAGE.

ASSAY CONDITIONS: RBC's MLL2 Complex displays histone methyltransferase activity at MLL2 concentrations of 3 nM-1 µM, 15-90 min. reactions, 30°C, in the HMT HotSpotSM Assay format, with several H3K4-containing substrates (chicken core histones (0.05 mg/mL), calf-thymus histone H3 (5 μM), H3(1-21) peptide (5 μM)) or nucleosomes (0.05 mg/mL as DNA)). Reaction conditions are: 50 mM Tris-HCl, pH 8.5, 50 mM NaCl, 5 mM MgCl₂, 1 mM DTT, 1 mM PMSF, protein or peptide substrate at concentrations indicated above, [3H]-SAM, and a suitable dilution of "RBC MLL Enhancer" (provided). NOTE: Optimal MLL Enhancer dilution depends on substrate and assay system and must be determined by the user. This typically ranges from 1/10 to 1/100 dilutions, i.e. 10% to 1% of the final reaction volume. MLL2 Complex also displays activity in the absence of Enhancer, in a filterbinding assay with nucleosomes or histones as substrate (see Figure below, buffer conditions are the same as for HotSpotSM assays).

SUPPLIED AS: _ μΜ MLL2 Complex, as defined above, (__ μg/μl total protein) in 20 mM Tris-HCl, pH 7.5, 300 mM NaCl, 1 mM TCEP (tris(2carboxyethyl)phosphine HCl), 10% (w/v) glycerol, 1 µM ZnCl₂ as determined by OD₂₈₀.

STORAGE: -70°C. Thaw quickly and store on ice before use. The remaining, unused, undiluted enzyme should be snap frozen, for example in a dry/ice ethanol bath or liquid nitrogen. Minimize freeze/thaws if possible, but very low volume aliquots (<5 µl) or storage of diluted enzyme are not recommended.

REFERENCES: 1) T.A. Milne et al. Mol. Cell 2002 10 1107; 2) A. Patel et al. J. Biol. Chem. 2008 283 32162; 3) J.J. Song & R.E. Kingston J. Biol. Chem. 2008 283 35258; 4) A. Yokoyama et al. Mol. Cell. Biol. 2004 24 5639; 5) Y. Dou et al. Nat. Struct. Mol. Biol. 2006 13 713; 6) A. Patel et al. J. Biol. Chem. 2009 284 24242; 7) S. Glaser et al. Development 2006 133 1423; 8) K.I. Ansari & S.S. Mandal FEBS J. 2010 277 1790; 9) C.V. Andreu-Vieyra et al. PLOS Biol. 2010 8 e1000453; 10) M.C. Hannibal Am. J. Med.Genet. A 2011 155A 1511; 11) D.W. Parsons et al. Science 2011 331 435; 12) R.D. Morin et al. Nature 2011 476 298; 13) T.G. Natarajan et al. Cancer Cell Int. 2010 10 13; 14) J. Chen et al. Mol. Cell. Biol. 2010 30 5621; 15) L. Shi et al. Proc.Natl. Acad. Sci. USA 2011 108 7541



stained SDS-PAGE (4-12% acrylamide) of 4 each of the purified components of the MLL2 complex. MW markers are from top, 120, 90, 70, 60, **50**, 40, 30, 20, 15, 10 kDa. M+W=WDR5/MLL2 and purified as a complex; A=Ash2L; Note that His-DPY-30 (13 kDa) migrates anomalously

blue



Methyltransferase Activity of MLL2 Complex with Nucleosomes or Core Histones. Methylation determined as TCA-precipitable counts in a scintillation/filter plate assay. Reactions were 25 µL, 60 min., 30°C, with 1 µM [3H]-SAM and either HeLa Mono/di-nucleosomes (0.05 mg/mL as DNA; 0.38 µM nucleosome units) or chicken core histones (Millipore; 0.05 mg/mL as protein; ~equal histone H3 molarity to the HeLa nucleosomes).

This product is not intended for therapeutic or diagnostic use in animals or in humans.

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