REACTION BIOLOGY

PRODUCT DATASHEET

MLL1 Complex (Mixed Lineage Leukemia Protein-1 in complex with WDR5, RbBP5, Ash2L, $(DPY\text{-}30)_2$)

CATALOG NO.: HMT-15-105 **LOT NO.:**

DESCRIPTION: Human recombinant MLL1 (residues 3745-3969; Genbank Accession # NM_005933; MW = 28 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 MLL1 subunit of this preparation comprises the catalytic SET domain[1] as well as the WDR5 interaction motif (*Win*)[2,3] necessary for assembly of the active complex[4-6]. MLL1-containing complexes regulate gene activation, notably including that of the HOX genes, in part by producing methylated H3K4, an activating epigenetic mark (see reviews[7,8]). MLL1 gene rearrangements resulting in chimeric proteins, usually with N-terminal MLL1 sequence, but not the C-terminal, catalytic SET domain, are a feature of aggressive leukemias (see review[9]). However, MLL1 rearrangements that include the SET domain (partial tandem duplications) occur in 5-10% of acute myeloid leukemias (AMLs)[10] and wild-type MLL1 can also be required for leukemic transformation by MLL1 fusion proteins[11,12]. Thus, inhibition of MLL1 methyltransferase activity may represent a strategy for anti-leukemia therapy[11].

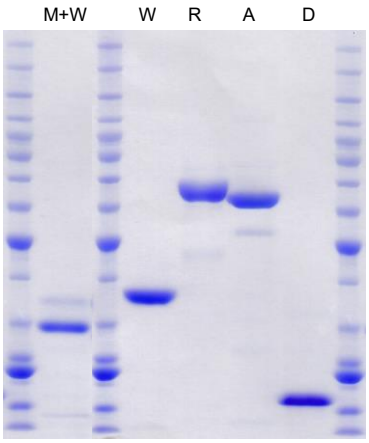
PURITY: >90% by SDS-PAGE.

ASSAY CONDITIONS: RBC's MLL1 Complex displays histone methyltransferase activity at MLL1 concentrations of 150 nM-1 µM, 15-90 min. reactions, 30°C, in the HMT HotSpot℠ 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)) and nucleosomes (0.05 mg/mL). Reaction conditions are: 50 mM Tris-HCl, pH 8.5, 50 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 1 mM PMSF, protein or peptide substrate at concentrations indicated above, [^{3}H]-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. MLL1 Complex also displays activity in the absence of Enhancer, in a filter-binding assay with various types of nucleosomes as substrate (see Figure below; buffer conditions are the same as for HotSpot℠ assays).

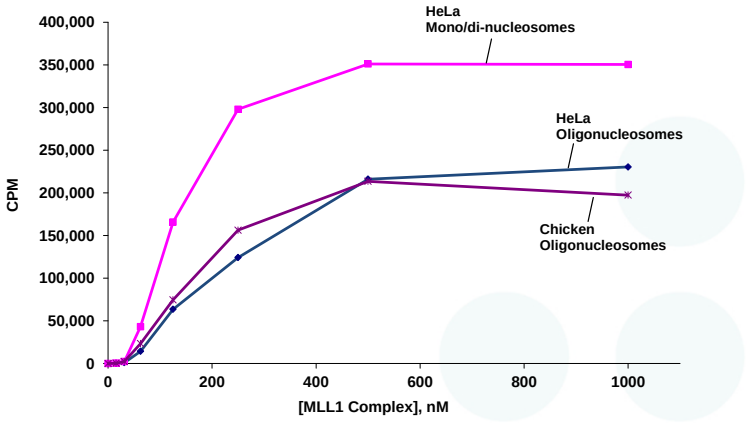
SUPPLIED AS: __ µM MLL1 Complex, as defined above, (__ µg/µl total protein) in 20 mM Tris-HCl, pH 7.5, 300 mM NaCl, 1 mM TCEP (tris(2-carboxyethyl)phosphine HCl), 10% (w/v) glycerol, 1 µM $ZnCl_2$ as determined by OD_{280}.

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 is 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) K.I. Ansari & S.S. Mandal *FEBS J.* 2010 **277** 1790; 8) M.S. Cosgrove & A. Patel *FEBS J.* 2010 **277** 1832; 9) J.L. Hess *Trends Mol. Med.* 2004 10 500; 10) A.M. Dorrance *et al. Blood* 2008 **112** 2508; 11) A.T. Thiel *et al. Cancer Cell* 2010 **17** 148; 12) T.A. Milne *et al. Mol. Cell* 2010 **38** 853



Coomassie blue stained SDS-PAGE (4-12% acrylamide) of 4 µg each of the purified components of the MLL1 complex. MW markers are from top, 220, 120, 90, 70, 60, **50**, 40, 30, **20**, 15, 10 kDa. M+W=WDR5/MLL1 coexpressed and purified as a complex; W=WDR5; R=RbBP5; A=Ash2L; D=DPY-30. Note that His-DPY-30 (13 kDa) migrates anomalously at ~17 kDa.



Methyltransferase Activity of MLL1 Complex with Nucleosomes. Methylation determined as TCA-precipitable counts in a scintillation/filter plate assay. Reactions were 25 µL, 60 min., 30°C, with 1 µM [^{3}H]-SAM and 0.05 mg/mL (as DNA; 0.38 µM) of either HeLa Oligo (RBC Cat. # HMT-35-130), HeLa Mono/di (# HMT-35-123) or Chicken Oligo (# HMT-35-177) as substrate.

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