REACTION BIOLOGY

PRODUCT DATASHEET

MLL3 Complex (Mixed Lineage Leukemia Protein-3 in complex with WDR5, RbBP5, Ash2L, $(DPY-30)_2$)

CATALOG NO.: HMT-15-107 **LOT NO.:**

DESCRIPTION: Human recombinant MLL3 (residues 4689-4911; Genbank Accession # NM_170606; 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 MLL3 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]. Like MLLs 1, 2 and 4, MLL3 complex methylates histone H3 lysine-4 (H3K4), conferring an activating epigenetic mark[7,8]. MLL3 complex (or MLL4 complex) forms a part of the "activating signal cointegrator-2" (ASC-2) Complex or ASCOM[9,10]. ASCOM acts as a coactivator of various nuclear receptors[11-14] and other transcription factors, including the tumor suppressor p53[15]. The chromatin remodeling complex Swi/Snf colocalizes with ASCOM, an interaction mediated by binding of the MLL3 (or MLL4) SET domain to the Swi/Snf subunit INI1[16]. ASCOM can be recruited to the *aP2* promoter by PPARγ and mice homozygous for MLL3 with a partial SET domain deletion have decreased white fat and a partially impaired potential for adipogenesis[13]. Knockdown of MLLs 3 and 4 diminishes estrogen-induced expression of HOXC10, which is overexpressed in breast cancer[17].

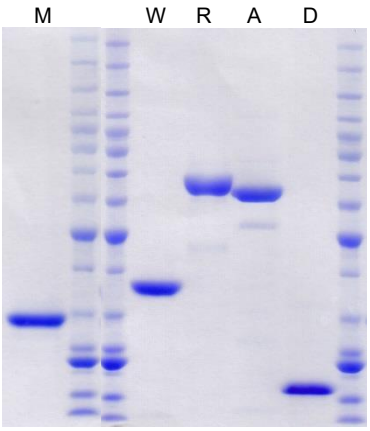
PURITY: >90% by SDS-PAGE.

ASSAY CONDITIONS: RBC's MLL3 Complex displays histone methyltransferase activity at MLL3 concentrations of 10 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)). 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. MLL3 Complex also displays activity in the absence of Enhancer, in a filter-binding assay with recombinant histone H3.3 as substrate (see Figure below; buffer conditions are the same as for HotSpot℠ assays).

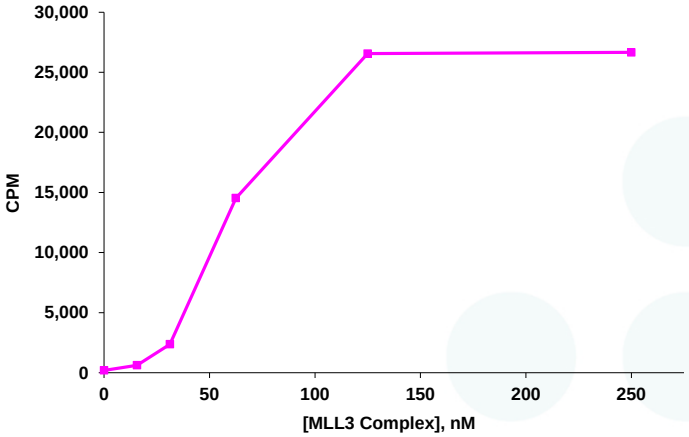
SUPPLIED AS: __ µM MLL3 Complex, as defined above, (__ µg/µl total protein) in 20 mM Tris-HCl, pH 7.5, 300 mM NaCl, 1 mM TCEP (tris(2-carboxyethyl)phosphine HCl), 10% (w/v) glycerol, 1 µM $ZnCl_2$ as determined by OD_{280}.

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 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) Y.H. Goo *et al. Mol. Cell. Biol.* 2003 **23** 140; 10) S. Lee *et al. Proc.Natl. Acad. Sci. USA* 2006 **103** 15392; 11) M.A. Mahajan & H.H. Samuels *Endocr. Rev.* 2005 **26** 583; 12) Q. Li *et al. Mol. Cell. Biol.* 2007 **27** 8073; 13) J. Lee *et al. Proc.Natl. Acad. Sci. USA* 2008 **105** 19229; 14) D. Kim *et al. Mol. Endocrinol.* 2009 **23** 1556; 15) J. Lee *et al. Proc.Natl. Acad. Sci. USA* 2009 **106** 8513; 16) S. Lee *et al. Mol. Endocrinol.* 2009 **23** 610; 17) K.I. Ansari *et al. J. Mol. Endocrinol.* 2011 doi: 10.1530/JME-11-0078



Coomassie blue stained SDS-PAGE (4-12% acrylamide) of 4 µg each of the purified components of the MLL3 complex. MW markers are from top, 120, 90, 70, 60, **50**, 40, 30, **20**, 15, 10 kDa. M=MLL3; R=RbBP5; A=Ash2L; D=DPY-30. Note that His-DPY-30 (13 kDa) migrates anomalously at ~17 kDa.



Methyltransferase Activity of MLL3 Complex. 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 1 µM recombinant histone H3.3 (RBC Cat. # HMT-11-134) as substrates.

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