

## SET1B Complex (SET1B (SETD1B) in complex with WDR5, RbBP5, Ash2L, (DPY-30)<sub>2</sub>)

**CATALOG NO.:** HMT-15-117

**LOT NO.:**

**DESCRIPTION:** Human recombinant SET1B (residues 1629-1923 (C-term.); Genbank Accession # NM\_015048; MW = 66 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*, SET1B with an N-terminal GST-tag and all others with N-terminal His-tags. This recombinant SET1B Complex 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), and contains, in addition to SET1B, the four additional proteins (WDR5, RbBP5, Ash2L, DPY-30) common to complexes of all six members of the human MLL/SET1 family (MLLs 1-4, SET1A, SET1B)<sup>1-6</sup>. Human SET1-containing complexes (SET1A and SET1B) appear to be responsible for widespread H3K4 trimethylation<sup>4,7</sup>, a mark associated with active gene transcription. In contrast, the H3K4 methylating activities of other MLL/SET1 family members are more narrowly focused, as for example in the case of MLL1 and MLL2 and the HOX genes<sup>8</sup>.

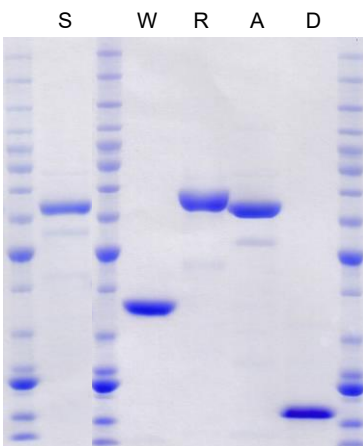
**PURITY:** >90% by SDS-PAGE.

**ASSAY CONDITIONS:** RBC's SET1B Complex displays histone methyltransferase activity at SET1B concentrations of 15.6 nM-1 μM, 60 min. reactions, 30°C, as TCA-precipitated counts in a scintillation/filter plate assay (Multiscreen FB, Topcount), with several H3K4-containing substrates (chicken core histones (0.05 mg/mL), HeLa oligo or mono/di-nucleosomes (0.05 mg/mL as [DNA]), recombinant histone H3.3 (0.77 μM)). Reaction conditions are: 50 mM Tris-HCl, pH 8.5, 50 mM NaCl, 5 mM MgCl<sub>2</sub>, 1 mM DTT, 1 mM PMSF, protein substrate at concentrations indicated above. NOTE: Supplied with "RBC MLL Enhancer", addition of which may increase activity, especially at low concentrations of SET1B Complex. Optimal 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. SET1B Complex also displays activity in the absence of Enhancer, in a filter-binding assay with nucleosomes, core histones or histone H3 as substrate (see Figure below).

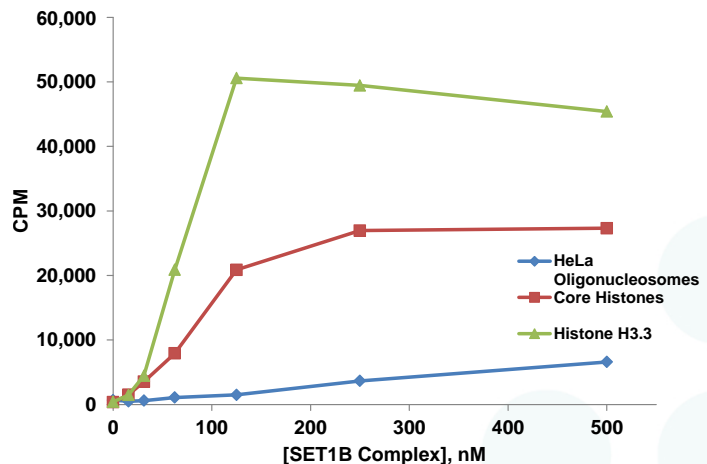
**SUPPLIED AS:** μM SET1B Complex, as defined above, ( μg/μl total protein) in 20 mM Tris-HCl, pH 7.5, 300 mM NaCl, 1 mM TCEP (tris(2-carboxyethyl)phosphine HCl), 10% (w/v) glycerol, 1 μM ZnCl<sub>2</sub> as determined by OD<sub>280</sub>.

**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) J. Wysocka *et al. Genes Dev.* 2003 **17** 896; 2) J. Lee & D.G. Skalnik *J. Biol. Chem.* 2005 **280** 41725; 3) J. Lee & D.G. Skalnik *Mol. Cell. Biol.* 2008 **28** 609; 4) M. Wu *et al. Mol. Cell. Biol.* 2008 **28** 7337; 5) Y. Cho *et al. J. Biol. Chem.* 2007 **282** 20395; 6) Y. Takahashi *et al. Proc. Natl. Acad. Sci. USA* 2011 **108** 20526; 7) J. Lee *et al. J. Biol. Chem.* 2007 **282** 13419; 8) P. Wang *et al. Mol. Cell. Biol.* 2009 **29** 6074; 9) F.E. El Mansouri *et al. Arthritis & Rheumatism* 2011 **63** 168; 10) S. Yadav *et al. Biochem. Pharmacol.* 2009 **77** 1635



**Coomassie blue stained SDS-PAGE (4-12% acrylamide) of 4 μg each of the purified components of the SET1B complex.** MW markers are from top, 220, 120, 90, 70, 60, 50, 40, 30, 20, 15, 10 kDa. S=SET1B; W=WDR5; R=RbBP5; A=Ash2L; D=DPY-30. Note that His-DPY-30 (13 kDa) migrates anomalously at ~17 kDa.



**Methylation Activity of SET1B Complex with Core Histones, Oligonucleosomes and Histone H3.3.** Assays were performed with a scintillation/filter plate assay. Incubations were 60 min., 30°C with chicken core histones (0.05 mg/mL) or HeLa Oligonucleosomes (0.05 mg/mL as [DNA]) or recombinant histone H3.3 (0.77 μM; H3 concentration equal to those in the core histones or oligonucleosomes) and 1 μM [<sup>3</sup>H]-SAM.

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

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