REACTION BIOLOGY

PRODUCT DATASHEET

SET8 (PR-Set7, SETD8, KMT5A)

CATALOG NO.: HMT-11-118 **LOT NO.:**

DESCRIPTION: Human recombinant SET8 (residues 190-352; Genbank Accession # NM_020382; MW = 21.8 kDa) expressed in *E. coli* with an N-terminal His-tag. Catalyzes the transfer of methyl groups from S-adenosyl-L-methionine (SAM) to the ε-amino function of protein L-lysine residues, specifically the monomethylation of lysine-20 of histone H4 (H4K20me1)[1-3] and of lysine-382 of p53 (p53K382me1)[4]. SET8 and H4K20me1 are essential to chromosome condensation, entry into mitosis and maintenance of genomic stability[5] and are implicated in the licensing of replication origins[6]. Monomethylation of H4K20 and cellular SET8 levels oscillate with the cell cycle, peaking at the G2/M transition, declining late in mitosis, with SET8 protein becoming nearly undetectable in S phase (see reviews[7-9]). Apart from its role in cell cycle progression, there is evidence SET8 can regulate transcription of specific genes by H4K20 monomethylation in promoter elements[10] or gene bodies[11] and by methylation of a transcription factor, p53[4,12]. Multiple lines of evidence suggest that SET8 may be a promising target for anti-cancer therapy. These include the requirement for SET8 in cell cycle progression[5,6], SET8's activation of Wnt target genes[10], its negative regulation of the p53 tumor suppressor[4,12], the association of increased SET8 levels with breast cancer metastasis[13] and the association of decreased levels with longer survival in hepatocellular carcinoma patients[14].

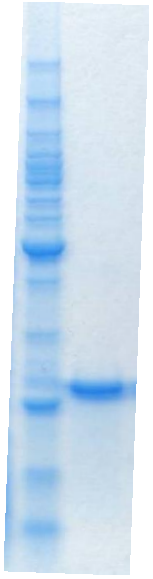
PURITY: >90% by SDS-PAGE.

ASSAY CONDITIONS: RBC's SET8 displays histone methyltransferase activity at enzyme concentrations of 15.6 nM and above, with HeLa Nucleosomes (Cat. #HMT-35-123) and [^{3}H]-SAM as substrates. Activity was determined as TCA-precipitated counts in a scintillation/filter plate assay (Multiscreen FB, Topcount). Reaction conditions: 50 mM Tris-HCl, pH 8.5, 50 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 1 mM PMSF, 30°C, 60 min. with substrates as indicated above.

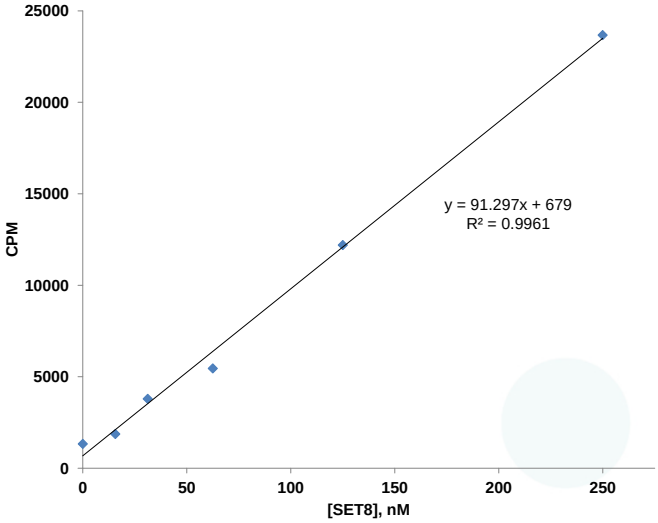
SUPPLIED AS: ___ µg/µl total protein in 20 mM Tris-HCl, pH 7.5, 300 mM NaCl, 10% glycerol (v/v), 1 mM TCEP as determined by OD_{280}

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) K. Nishioka *et al. Mol. Cell* 2002 **9** 1201; 2) J. Fang *et al. Curr. Biol.* 2002 **12** 1086; 3) B. Xiao *et al. Genes Dev.* 2005 **19** 1444; 4) X. Shi *et al. Mol. Cell* 2007 **27** 636; 5) S. Houston *et al. J. Biol. Chem.* 2008 **283** 19478; 6) M. Tardat *et al. Nat. Cell Biol.* 2010 **12** 1086; 7) S. Wu & J.C. Rice *Cell Cycle* 2011 **10** 68; 8) J. Brustel *et al. Trends Cell Biol.* 2011 **21** 452; 9) D.B. Beck *et al. Genes Dev.* 2012 **26** 325; 10) Z. Li *et al. Proc. Natl. Acad. Sci. USA* 2011 **108** 3116; 11) L.M. Congdon *et al. J. Cell. Biochem.* 2010 **110** 609; 12) L.E. West *et al. J. Biol. Chem.* 2010 **285** 37725; 13) F. Yang *et al. EMBO J.* 2011 **31** 110; 14) Z. Guo *et al. Int. J. Cancer* 2012 doi: 10.1002/ijc.27352



Coomassie blue stained SDS-PAGE (4-20% acrylamide) of 4 µg of purified SET8. MW markers at left, from top: 220, 160, 120, 100, 90, 80, 70, 60, **50**, 40, 30, 25, **20**, 15, 10 kDa.



Methyltransferase Activity of SET8. Methylation determined as TCA-precipitable counts in a scintillation/filter plate assay. Reactions were 60 min., 30°C, with 1 µM [^{3}H]-SAM and HeLa Nucleosomes (Cat. #HMT-35-123; 0.05 mg/mL as DNA) as substrates.

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