

NSD2-E1099K-SET (His)

CATALOG NO.: HMT-11-378

LOT NO.:

DESCRIPTION: Mutant human recombinant NSD2 (residues 934-1241) with lysine (K) substituted for glutamate-1099 (E1099) and expressed in *E. coli* cells with an N-terminal His-tag. (Otherwise contains wild-type residues 934-1098, 1100-1241 as at Genbank Accession # NM_001042424; MW = 37.4 kDa). Catalyzes the transfer of methyl groups from S-adenosyl-L-methionine (SAM) to the ϵ -amino function of protein L-lysine residues, specifically lysine-36 of histone H3 (H3K36)¹ (see also review²). H4K20 methylation by NSD2 may be linked to double strand breaks and the DNA damage response³, whereas its principal regulatory functions appear to occur via methylation of H3K36^{4,5}, a mark associated with active transcription. NSD2 is overexpressed in multiple myelomas with the t(4;14) translocation⁶. NSD2 knockdown in such cells induces apoptosis⁴, while overexpression of catalytically active NSD2 promotes oncogenic transformation and tumor formation even in the absence of the translocation⁵. In addition to t(4;14)+ multiple myelomas, NSD2 expression is elevated in a variety of cancers (bladder^{7,8}, breast⁷, prostate⁷, kidney⁷, lung^{7,8}, pancreas⁷, colon⁸, stomach⁸, anal canal⁸, female genitals⁸, skin⁸, neuroblastoma⁹) and its carcinogenic effects may be mediated by interaction with β -catenin and effects on the WNT pathway⁷. Like cells in which translocations drive NSD2 overexpression, tumor lines bearing an NSD2-E1099K mutant allele display elevated levels of H3K36 dimethylation.^{10,11} A truncated, SET-domain E1099K construct (residues 955-1365) has been reported to exhibit higher methylation activity toward nucleosomes *in vitro*.¹¹ Although observed in other cancers, the E1099K mutation appears to be most prevalent in pediatric acute lymphoblastic leukemia (ALL), the most common childhood cancer.^{10,11} Knockdown of NSD2-E1099K in ALL lines inhibits proliferation¹¹, implicating, as in the other cancers mentioned above, NSD2 as a promising therapeutic target in ALL.

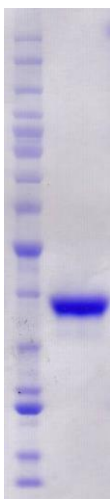
PURITY: >90% by SDS-PAGE

ASSAY CONDITIONS: RBC's NSD2-E1099K-SET displays histone methyltransferase activity at concentrations of ≥ 15.6 nM, 60 min. reactions, 30°C, as TCA-precipitated counts in a scintillation/filter plate assay (Multiscreen FB, Topcount), with HeLa oligonucleosomes (0.05 mg/mL as [DNA]; see Figure)). Reaction conditions are: 50 mM Tris-HCl, pH 8.5, 50 mM NaCl, 5 mM MgCl₂, 1 mM DTT, 1 mM PMSF, substrates at concentrations indicated above.

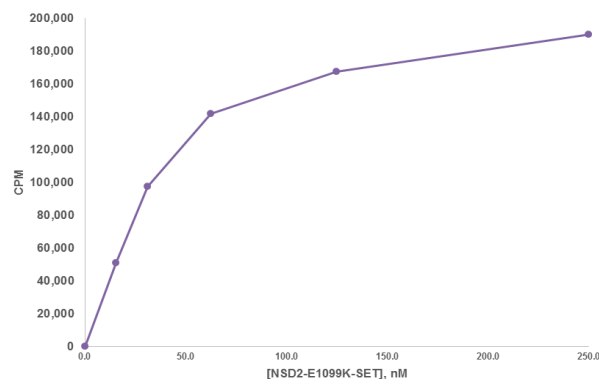
SUPPLIED AS: ___ μ M NSD2-E1099K-SET (___ μ g/ μ l total protein) in 50 mM Tris/HCl pH 7.5, 500 mM NaCl, 1 mM TCEP, 10% glycerol (v/v) as determined by OD₂₈₀.

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 μ l) or storage of diluted enzyme is not recommended.

REFERENCES: 1) Y Li *et al. J. Biol. Chem.* 2009 **284** 34283; 2) M. Morishita & E. di Luccio *Biochim. Biophys. Acta* 2011 **1816** 158; 3) H. Pei *et al. Nature* 2011 **470** 124; 4) E. Martinez-Garcia *et al. Blood* 2011 **117** 211; 5) A.J. Kuo *et al. Mol. Cell* 2011 **44** 609; 6) J.J. Keats *et al. Blood* 2005 **105** 4060; 7) G. Toyokawa *et al. Neoplasia* 2011 **13** 887; 8) H.R. Hudlebusch *et al. Clin. Cancer Res.* 2011 **17** 2919; 9) H.R. Hudlebusch *et al. Cancer Res.* 2011 **71** 4226; 10) J.A. Oyer *et al. Leukemia* 2013 **28** 198; 11) J.D. Jaffe *et al. Nat. Genet.* 2013 **45** 1386



Coomassie blue stained SDS-PAGE (4-12% acrylamide) of 4 μ g of purified NSD2-E1099K-SET (His). MW markers at left, from top: 220, 160, 120, 100, 90, 80, 70, 60, 50, 40, 30, 25, 20, 15, & 10 kDa.



Methylation Activity of NSD2-E1099K-SET with HeLa Nucleosomes. Assays were performed with a scintillation/filter plate assay. Incubations were 60 min., 30°C with HeLa oligonucleosomes (RBC Cat. # HMT-35-130) at 0.05 mg/mL as [DNA], and 1 μ M [³H]-SAM.

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