PRODUCT DATA SHEET

NSD2-T1150A-SET (His)

CATALOG NO.: HMT-11-379 **LOT NO.:**

DESCRIPTION: Mutant human recombinant NSD2 (residues 934-1241) with alanine (A) substituted for threonine-1150 (T1150) and expressed in *E.coli* cells with an N-terminal His-tag. (Otherwise contains wild-type residues 934-1149, 1151-1241 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 review[2]). H4K20 methylation by NSD2 may be linked to double strand breaks and the DNA damage response[3], 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) translocation[6]. NSD2 knockdown in such cells induces apoptosis[4], while overexpression of catalytically active NSD2 promotes oncogenic transformation and tumor formation even in the absence of the translocation[5]. In addition to t(4;14)+ multiple myelomas, NSD2 expression is elevated in a variety of cancers (bladder[7,8], breast[7], prostate[7], kidney[7], lung[7,8], pancreas[7], colon[8], stomach[8], anal canal[8], female genitals[8], skin[8], neuroblastoma[9]) and its carcinogenic effects may be mediated by interaction with ß-catenin and effects on the WNT pathway[7]. The T1150A mutation, along with E1099K, a prevalent mutation in pediatric acute lymphoblastic leukemia (ALL)[10,11], are found recurrently in mantle cell lymphomas (MCL)[12]. A truncated E1099K construct (residues 955-1365) has been reported to exhibit elevated methylation activity toward nucleosomes *in vitro*[11] and the same appears to be the case with full-length NSD2-T1150A (see figure below).

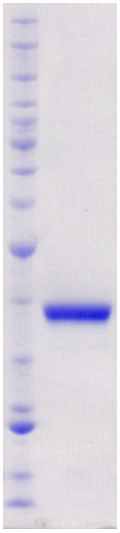
PURITY: >90% by SDS-PAGE

ASSAY CONDITIONS: RBC's NSD2-T1150A-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 nucleosomes (0.05 mg/mL as [DNA]; see Figure)). Reaction conditions are: 50 mM Tris-HCl, pH 8.5, 50 mM NaCl, 5 mM $MgCl_2$, 2 mM TCEP, 1 mM PMSF, substrates at concentrations indicated above.

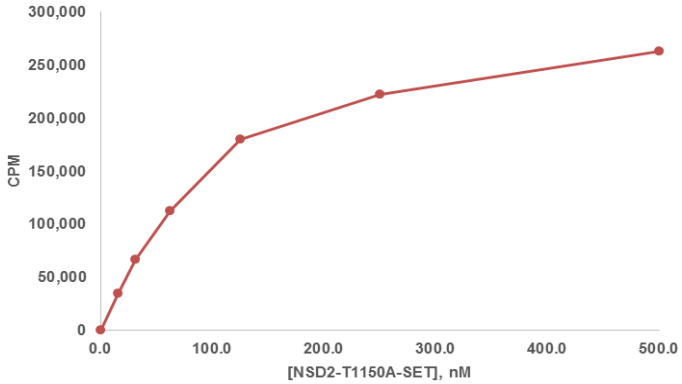
SUPPLIED AS: __ µM NSD2-T1150A-SET (__ µg/µl total protein) in 50 mM Tris/HCl pH 7.5, 500 mM NaCl, 1 mM TCEP, 10% glycerol (v/v) as determined by OD_{280}.

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) 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; 12) Bea *et al. Proc. Natl. Acad. Sci. USA* 2013 **110** 18250



Coomassie blue stained SDS-PAGE (4-12% acrylamide) of 4 µg of purified NSD2-T1150A-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-T1150A-SET with HeLa Oligonucleosomes. Assays were performed with a scintillation/filter plate assay. Incubations were 60 min., 30°C with HeLa oligonucleosomes (RBC Cat. # HMT-35-130), both 0.05 mg/mL as [DNA], and 1 µM [^{3}H]-SAM.

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