REACTION BIOLOGY

PRODUCT DATASHEET

SMYD3 (SET and MYND Domain-Containing Protein-3; Lysine N-Methyltransferase Smyd3)

CATALOG NO.: HMT-11-477 **LOT NO.:**

DESCRIPTION: Human recombinant SMYD3 (residues 2-428 (C-terminus); Genbank Accession # NM_001167740) expressed with an N-terminal His-tag, in *E. coli.* MW = 51.4 kDa. Catalyzes the transfer of methyl groups from S-adenosyl-L-methionine (SAM) to the ε-amino function of protein L-lysine residues, particularly in histones H4 and H3, although other activities have been reported (see below). Lysine methyltransferases of the SMYD family are unusual in that their SET domains are split in two by insertion of a myeloid-Nervy-DEAF-1 (MYND) domain in the primary amino acid sequence[1-3]. SMYD3 (SET and MYND domain containing protein 3) has been described as a histone H3 lysine-4 (H3K4) and histone H4 lysine-20 (H4K20) methyltransferase (HMT), although more recent evidence suggests that it may be a histone H4 lysine-5 (H4K5) HMT. Smyd3 has also been shown to methylate K260 of MAP3K2[4,5]. SMYD3 is overexpressed in a number of cancers, including breast and liver.

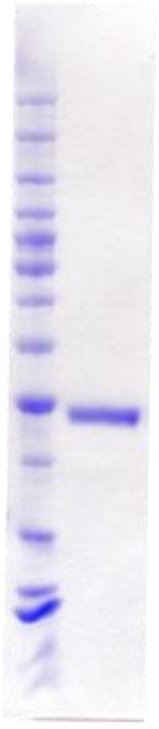
PURITY: >90% by SDS-PAGE.

ASSAY CONDITIONS: RBC's SMYD3 displays methyltransferase activity with recombinant human MAP3K2 (MEKK2) protein substrate in the HMT HotSpot^{SM} Assay format. Reaction conditions are: 50 mM Tris-HCl, pH 8.5, 50 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 0.01% Brij35, 1% DMSO, 0.25µM MEKK2 and and 0.25µM [^{3}H]-SAM.

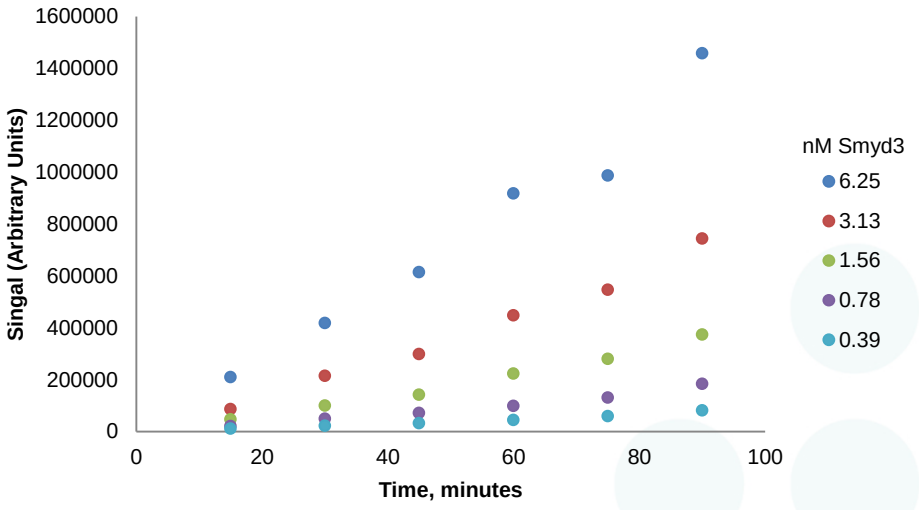
SUPPLIED AS: __ µg/µl in 20 mM Tris/HCl, pH 8.0, 250 mM NaCl, 1 mM TCEP, 10% (w/v) glycerol as determined by OD_{280}.

STORAGE: -70°C. Thaw quickly and store on ice before use. The remaining, unused, undiluted enzyme should be refrozen quickly by, for example, snap freezing in a dry/ice ethanol bath or liquid nitrogen. Freezing and storage of diluted enzyme is not recommended.

REFERENCES: 1) P.D. Gottlieb *et al. Nat. Genet.* 2002 **31** 25; 2) R. Hamamoto *et al. Nat. Cell Biol.* 2004 **6** 731; 3) M.A. Brown *et al. Mol. Cancer* 2006 **5** 26; 4) T.V. Riera, *et al.* Kinetic Mechanism of the Lysine Methyltransferase Smyd3 Using MAP3K2 Protein Substrate. Poster session presented at: AACR 106th Annual Meeting 2015; April 18-22, 2015; Philadelphia, PA; 5) Mazur *et al. Nature* 2014 **510(7504)**: 283-7.



Coomassie blue stained SDS-PAGE (4-12% acrylamide) of 2 µg of purified RBC SMYD3. MW markers (left) are, from top, 220, 160, 120, 90, 70, 60, **50**, 40, 30, 25, **20**, 15, 10 kDa.



Time courses of SMYD3 methyltransferase reactions in the HotSpot^{SM} assay format. SMYD3, at variable concentrations, was assayed with 0.25µM MEKK2 and 0.25µM [^{3}H]-SAM for indicated timepoints as above.

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