

SMYD3

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

CATALOG NO.: HMT-21-478

LOT NO.:

DESCRIPTION: Human recombinant SMYD3 (residues 2-428 (C-terminus); Genbank Accession # NM_001167740) expressed with an C-terminal His-tag in *Sf9* insect cells; MW = 50.1 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 sequence¹⁻³. 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.

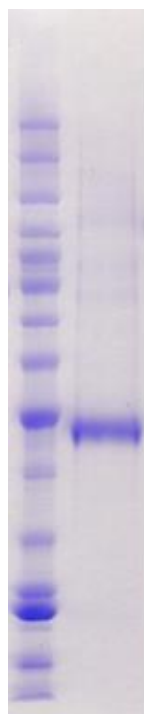
PURITY: >90% by SDS-PAGE.

ASSAY CONDITIONS: RBC's SMYD3 displays methyltransferase activity at enzyme concentrations of 6.25 nM and above, 30°C, with recombinant human MAP3K2 (MEKK2) protein substrate as TCA-precipitated counts in a scintillation/filter plate assay (Multiscreen FB, TopCount), Reaction conditions are: 25 mM Tris-HCl, pH 8.0, 1 mM TCEP, 0.005% BSA, 0.005% Tween-20, 1% DMSO with 150nM MEKK2 and 100nM [³H]-SAM as substrates.

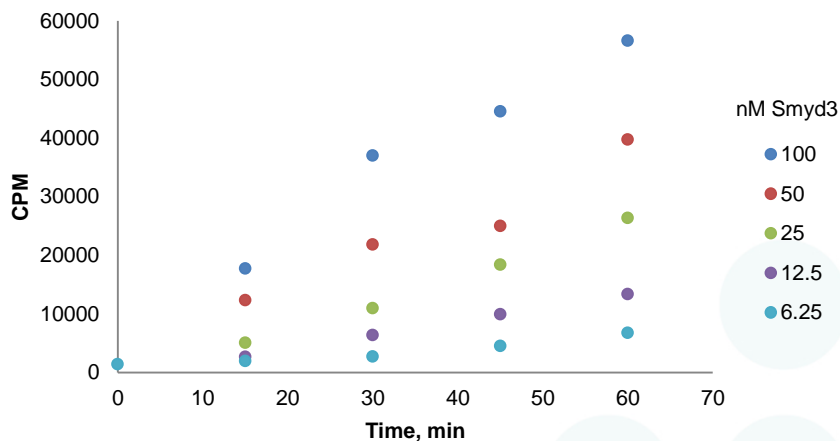
SUPPLIED AS: ___ $\mu\text{g}/\mu\text{l}$ in 20 mM Tris/HCl, pH 8.0, 250 mM NaCl, 1 mM TCEP, 10% (w/v) glycerol as determined by OD₂₈₀.

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 4 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.



Methylation Activity of Smyd3 with MEKK2. Assays were performed with a scintillation/filter plate assay. The 20 μL reaction contained 150nM MAP3K2 (MEKK2), 100nM SAM (100nM ³H-SAM) and variable concentrations of Smyd3. Reactions were quenched at various timepoints and quantified in a scintillation/filter plate assay.

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

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