

SMYD2 (KMT3C), Active

Full length recombinant protein expressed in Sf9 cells

Catalog # S348-380BG

Lot # 1085-2

Product Description

Recombinant full-length human SMYD2 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [BC049367](#).

Gene Aliases

KMT3C; HSKM-B; ZMYND14

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 50mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

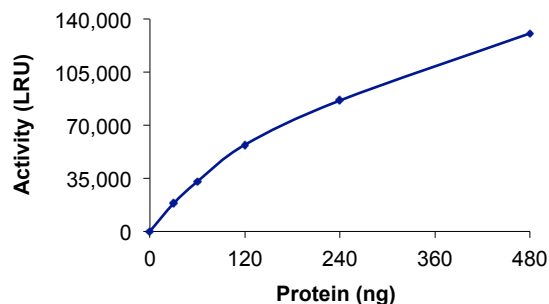
Scientific Background

SMYD2 (also known as SET and MYND domain-containing protein 2) is a member of the SMYD family of protein methyltransferases. SMYD2 is also known as lysine methyltransferase protein 3C (KMT3C). SMYD2 functions to repress transcription by interacting with the Sin3A histone deacetylase complex and performs methylation of Lys36 in histone H3 (1). The exogenous expression of SMYD2 in mouse fibroblasts decreases cell growth. SMYD2 also function as a putative oncogene by methylating p53 and repressing its tumor suppressive function (2).

References

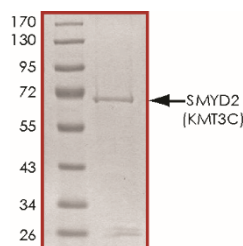
1. Brown, M. A. et.al: Identification and characterization of Smyd2: a split SET/MYND domain-containing histone H3 lysine 36-specific methyltransferase that interacts with the Sin3 histone deacetylase complex. *Molec. Cancer* 5: 26, 2006.
2. Huang, J. et.al: Repression of p53 activity by Smyd2-mediated methylation. *Nature* 444: 629-632, 2006.

Specific Activity



The specific activity of SMYD2 (KMT3C) was determined to be **180 pmol /min/mg** as per activity assay protocol.

Purity



The purity of SMYD2 (KMT3C) was determined to be **>80%** by densitometry, approx. MW **70 kDa**.

SMYD2 (KMT3C), Active

Full length recombinant protein expressed in Sf9 cells

Catalog #	S348-380BG
Specific Activity	180 pmol/min/mg
Lot #	1085-2
Purity	>80%
Concentration	0.1 µg/µl
Stability	1yr at -70°C from date of shipment
Storage & Shipping	Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

Activity Assay Protocol

Reaction Components

Active Methyltransferase (Catalog #: S348-380BG)

Active SMYD2 (KMT3C) (0.1 µg/µl) diluted with Methyltransferase Reaction Buffer and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active SMYD2 (KMT3C) for optimal results).

Methyltransferase Reaction Buffer

Buffer components: 20mM Tris-HCl, pH 8.0, 50 mM NaCl, 1 mM EDTA, 3 mM MgCl₂, 0.1 mg/ml BSA. Add 1mM DTT prior to use.

MTase-Glo™ Methyltransferase Assay (Promega, Catalog #: V7601)

S-Adenosyl-Methionine (SAM), 1mM
S-Adenosyl-Homocysteine (SAH), 15 µM
Methyltransferase-Glo™ Reagent, 10X
MTase-Glo™ Detection Solution, 1 bottle

Substrate (Catalog #: H12-58)

Histone H3 Peptide (1-21) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

The SMYD2 (KMT3C) assay is performed using the Methyltransferase-Glo™ Assays kit (Promega, Catalog #: V7601).

- Step 1.** Thaw the active SMYD2 (KMT3C) and all Methyltransferase-Glo™ Assays kit reagents on ice.
- Step 2.** Prepare the following working solutions with Methyltransferase Reaction Buffer on ice:
 - o 2X final concentration of Active SMYD2 (KMT3C) (Catalog # S348-380BG)
 - o 2X Substrate Cocktail: 40 µM of SAM and 100ng/µl of Histone H3 Peptide (1-21) (Catalog # H12-58) in water
- Step 3.** In a polystyrene 96-well plate, add the following components to bring the initial reaction volume to 20 µl:
 - Component 1.** 10 µl of 2X Substrate Cocktail
 - Component 2.** 10 µl of 2X Active SMYD2 (KMT3C)

Note: A blank control can be set up as outlined in step 3 by replacing the substrate working solution with an equal volume of Reaction Buffer.

- Step 4.** Mix the reaction on an orbital shaker for 2 minutes. Seal the plate with a plate seal and incubate at 37°C for 60 minutes
- Step 5.** Dilute 10X Methyltransferase-Glo™ Reagent with equal volume of nanopure water, and add 5 µl of the 5X Methyltransferase-Glo™ Reagent to all reaction wells
- Step 6.** Mix on an orbital shaker for 2 minutes and then incubate at room temperature for 30 minutes.
- Step 7.** Add 25 µl of MTase-Glo™ Detection Solution to all reaction wells. Mix for 2 minutes and then incubate at room temperature for 30 minutes
- Step 8.** Read the plate using the KinaseGlo Luminescence Protocol on a GloMax plate reader (Promega; Cat# E7031)
- Step 9.** Using the SAH standard curve, determine the concentration of SAH produced (nM) and calculate the methyltransferase specific activity as outlined below. For a detailed protocol of how to determine SAH amount from RLUs, see MTase-Glo™ Methyltransferase Assay protocol at Promega's website: www.promega.com/protocols

Methyltransferase Specific Activity (SA) (nmol/min/mg)

$$= \frac{[SAH](nM) \times Reaction Volume(\mu l)}{Reaction Time (min) \times Enzyme Amount (mg)} \times 10^{-6}$$

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