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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
S350-380G-05	5 µg
S350-380G-10	10 µg

SUV39H1 (KMT1A), Active

Full length recombinant protein expressed in Sf9 cells

Catalog # S350-380G
Lot # N330-5

Product Description

Recombinant full-length human SUV39H1 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The SUV39H1 gene accession number is NM_003173.

Gene Aliases

KMT1A; MG44; SUV39H

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM 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.

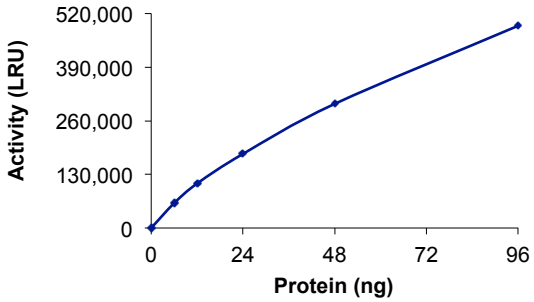
Scientific Background

SUV39H1 is a histone H3-specific methyltransferases that selectively methylates lysine-9 of the N terminus of histone H3 in vitro (1). SUV39H1 is also a member of the suppressor of variegation 3-9 homolog family and encodes a protein with a chromodomain and a C-terminal SET domain. SUV39H1 moves to the centromeres during mitosis where it functions as a histone methyltransferase, methylating Lys-9 of histone H3. SUV39H1 activity is regulated by acetylation at lysine residue 266 in its catalytic SET domain by SIRT1. SIRT1 interacts directly with, recruits, and deacetylates SUV39H1, and these activities independently contribute to elevated levels of SUV39H1 activity resulting in increased levels of the H3K9me3 modification (2).

References

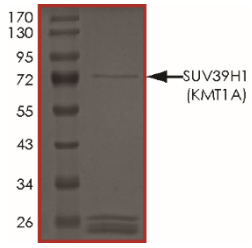
1. Rea, S.et al: Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature 406: 593-599, 2000.
2. Vaquero, A. et al: SIRT1 regulates the histone methyl-transferase SUV39H1 during heterochromatin formation. Nature 450: 440-444, 2007.

Specific Activity



The specific activity of SUV39H1 (KMT1A) was determined to be **2.6 nmol /min/mg** as per activity assay protocol.

Purity



The purity of SUV39H1 (KMT1A) was determined to be **>70%** by densitometry, approx. MW **73 kDa**.

SUV39H1 (KMT1A), Active

Full length recombinant protein expressed in Sf9 cells

Catalog #	S350-380G
Specific Activity	2.6 nmol/min/mg
Lot #	N330-5
Purity	>70%
Concentration	0.05 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.

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Activity Assay Protocol

Reaction Components

Active Methyltransferase (Catalog #: S350-380G)

Active SUV39H1 (KMT1A) (0.05 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 SUV39H1 (KMT1A) for optimal results).

Methyltransferase Reaction Buffer

Buffer components: 20mM Tris-HCl, pH 8.0, 50 mM NaCl, 1 mM EDTA, 3 mM $MgCl_2$, 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 H_2O to a final concentration of 1mg/ml.

Assay Protocol

The SUV39H1 (KMT1A) assay is performed using the Methyltransferase-Glo™ Assays kit (Promega, Catalog #: V7601).

Step 1. Thaw the active SUV39H1 (KMT1A) and all Methyltransferase-Glo™ Assays kit reagents on ice.

Step 2. Prepare the following working solutions with Methyltransferase Reaction Buffer on ice:

- 2X final concentration of Active SUV39H1 (KMT1A) (Catalog # S350-380G)
- 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 SUV39H1 (KMT1A)

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-GloTM 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)

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

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