

EHMT1 (KMT1D), Active

Recombinant human protein expressed in Sf9 cells

Catalog # E317-381G

Lot # L2207-6

Product Description

Recombinant human EHMT1 (895-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The EHMT1 (KMT1D) protein accession number is [NM_024757](#).

Gene Aliases

GLP; GLP1; Eu-HMTase1; EUHMTASE1; FP13812; KMT1D; bA188C12.1

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.

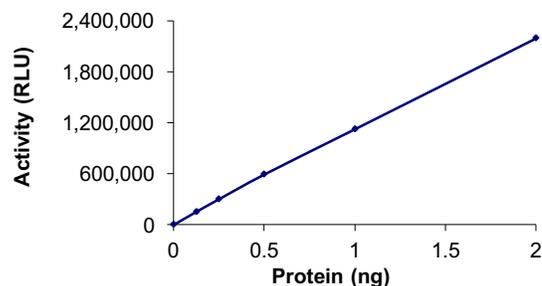
Scientific Background

Euchromatic histone-lysine N-methyltransferase 1 (EHMT1) is in charge of the mono- and demethylation of lysine-9 of histone H3. EHMT1 is found in a multimeric protein complex containing chromatin modifiers, which contributes to silencing of MYC- and E2F-responsive genes during G0 phase of cell cycle (1). EHMT1 plays a role in epithelial-to-mesenchymal transition and tumor invasion by repressing expression of tumour suppressor genes (2). Additionally, EHMT1 also methylates non-histone proteins such as lysine-373 of p53 (3).

References

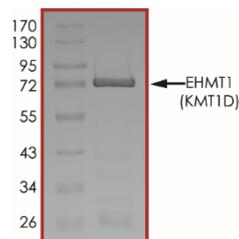
- Ogawa, H., et al. A complex with chromatin modifiers that occupies E2F- and Myc-responsive genes in G0 cells. *Science*. 296(5570):1132-6, 2002.
- Kokura, K., et al. Methyl-H3K9-binding protein MPP8 mediates E-cadherin gene silencing and promotes tumour cell motility and invasion. *EMBO J*. 29(21):3673-87, 2010.
- Huang, J., et al. G9a and Glp methylate lysine 373 in the tumor suppressor p53. *J Biol Chem*. 285(13):9636-41, 2010.

Specific Activity



The specific activity of EHMT1 (KMT1D) was determined to be **380 nmol /min/mg** as per activity assay protocol.

Purity



The purity of EHMT1 (KMT1D) was determined to be **>95%** by densitometry, approx. MW **74 kDa**.

EHMT1 (KMT1D), Active

Recombinant human protein expressed in Sf9 cells

Catalog #	E317-381G
Specific Activity	380 nmol/min/mg
Lot #	L2207-6
Purity	>95%
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.

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Activity Assay Protocol

Reaction Components

Active Methyltransferase (Catalog #: E317-381G)

Active EHMT1 (KMT1D) (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 EHMT1 (KMT1D) 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 Reaction Buffer to a final concentration of 40 µM.

Assay Protocol

The EHMT1 (KMT1D) assay is performed using the Methyltransferase-Glo™ Assays kit (Promega, Catalog #: V7601).

- Step 1.** Thaw the active EHMT1 (KMT1D) 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 EHMT1 (KMT1D) (Catalog # E317-381G)
 - o 2X Substrate Cocktail: 40 µM of SAM and 40 µM of Histone H3 Peptide (1-21) (Catalog # H12-58) in Reaction Buffer
- 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 EHMT1 (KMT1D)

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 RLU, 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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