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Catalog #	Aliquot Size
D344-381G-05	5 µg
D344-381G-10	10 µg

DOT1L (KMT4), Active

Recombinant human protein expressed in Sf9 cells

Catalog # D344-381G

Lot # L1956-7

Product Description

Recombinant human DOT1L (KMT4) (1-435) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The DOT1L (KMT4) gene accession number is NM_032482.

Gene Aliases

DOT1; KMT4

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.

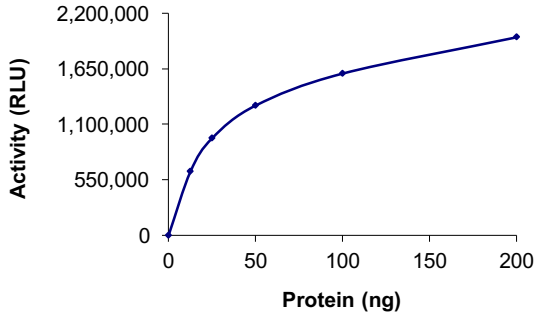
Scientific Background

DOT1-like protein (DOT1L) specifically methylates lysine-79 of histone H3 in the globular domain. DOT1L lacks a SET-domain, which is commonly considered to mediate lysine methylation of histones (1, 2). DOT1 homologs exist in a variety of eukaryotic organisms. The activity of this methyltransferase is involved in cell cycle regulation and leukemogenesis (3).

References

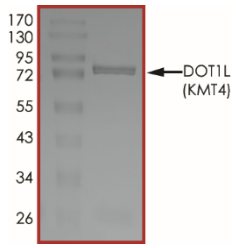
1. Feng, Q., et al. Methylation of H3-lysine 79 is mediated by a new family of HMTases without a SET domain. Curr Biol. 12(12):1052-8, 2002.
2. Min, J., et al. Structure of the catalytic domain of human DOT1L, a non-SET domain nucleosomal histone methyltransferase. Cell. 112(5):711-23, 2003.
3. Okada, Y., et al. hDOT1L links histone methylation to leukemo-genesis. Cell. 121(2):167-78, 2005.

Specific Activity



The specific activity of DOT1L (KMT4) was determined to be **14 nmol /min/mg** as per activity assay protocol.

Purity



The purity of DOT1L (KMT4) was determined to be **>90%** by densitometry, approx. MW **78 kDa**.

DOT1L (KMT4), Active

Recombinant human protein expressed in Sf9 cells

Catalog #	D344-381G
Specific Activity	14 nmol/min/mg
Lot #	L1956-7
Purity	>90%
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 #: D344-381G)

Active DOT1L (KMT4) (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 DOT1L (KMT4) 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 (Reaction Biology Cat.# HMT-35-130)

Nucleosomes (Hela Oligo) diluted in Reaction Buffer to a final concentration of 4 μM.

Assay Protocol

The DOT1L (KMT4) assay is performed using the Methyltransferase-Glo™ Assays kit (Promega, Catalog #: V7601).

Step 1. Thaw the active DOT1L (KMT4) 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 DOT1L (KMT4) (Catalog # D344-381G)
- 2X Substrate Cocktail: 40 μM of SAM and 4 μM of Nucleosomes (Hela Oligo) 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 DOT1L (KMT4)

Note: A blank control can be set up as outlined in step 3 by replacing the enzyme 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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