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Catalog #	Aliquot Size
M343-381G-05	5 µg
M343-381G-10	10 µg

MLL3 (KMT2C), Active

Recombinant human protein expressed in Sf9 cells

Catalog # M343-381G

Lot # L2041-7

Product Description

Recombinant human MLL3 (4689-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The MLL3 (KMT2C) protein accession number is NM_170606.

Gene Aliases

MLL3; KMT2C; HALR

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.

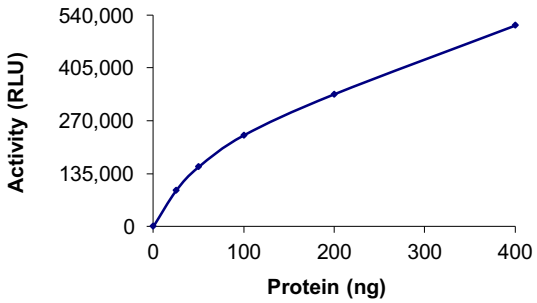
Scientific Background

Histone-lysine N-methyltransferase 2C, also known as mixed-lineage leukemia 2 (MLL3) is the central component of the MLL2/3 complex, a coactivator complex of nuclear receptors involved in transcriptional coactivation. Deletion of the region harboring the MLL3 gene was found to be the most frequently recurrent chromosomal abnormality in acute myeloid leukemia.

References

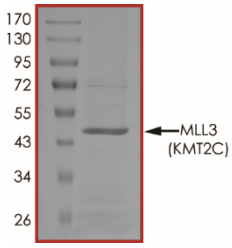
1. Rao RC, et al: Hijacked in cancer: the KMT2 (MLL) family of methyltransferases. Nat Rev Cancer. 15(6): 334-46. 2015.
2. Ford DJ, et al: The cancer COMPASS: navigating the functions of MLL complexes in cancer. Cancer Genet. 208(5):178-91. 2015
3. http://www.uniprot.org/uniprot/Q8NEZ4

Specific Activity



The specific activity of MLL3 (KMT2C) was determined to be **1,000 pmol /min/mg** as per activity assay protocol.

Purity



The purity of MLL3 (KMT2C) was determined to be **>85%** by densitometry, approx. MW **51 kDa**.

MLL3 (KMT2C), Active

Recombinant human protein expressed in Sf9 cells

Catalog #	M343-381G
Specific Activity	1,000 pmol/min/mg
Lot #	L2041-7
Purity	>85%
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 #: M343-381G)

Active MLL3 (KMT2C) (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 MLL3 (KMT2C) 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.

Components of MLL3 (KMT2C) Complex

WDR5 Protein: Catalog #: W325-30H

RBBP5 Protein: Catalog #: R315-30H

ASH2L Protein: Catalog #: A372-30BG

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 20 μM.

Assay Protocol

The MLL3 (KMT2C) assay is performed using the Methyltransferase-Glo™ Assays kit (Promega, Catalog #: V7601).

Step 1. Thaw each active MLL3 (KMT2C) complex component 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 MLL3 (KMT2C) (Catalog # M343-381G) with complex proteins
- 2X Substrate Cocktail: 40 μM of SAM and 20 μM of Histone H3 Peptide (1-21) 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 MLL3 (KMT2C) complex

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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