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Catalog #	Aliquot Size
M345-381G-05	5 µg
M345-381G-10	10 µg

MLL5 (KMT2E), Active

Recombinant human protein expressed in Sf9 cells

Catalog # M345-381G
Lot # B2145-8

Product Description

Recombinant human MLL5 (1-548) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The MLL5 protein accession number is NM_182931.

Gene Aliases

HDCMC04P; MLL5; NKp44L

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.

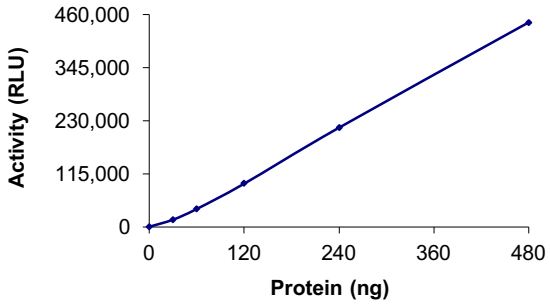
Scientific Background

Mixed-lineage leukemia protein 5 (MLL5) plays a key regulatory role in hematopoiesis by mediating mono- or demethylation of lysine-4 of histone H3 (1). MLL5 can associate with host cell factor-1 (HCF-1) and be recruited to E2F1-responsive promoters, and thus promotes cell cycle progression (2). Isoform NKp44L acts as cellular ligand for a natural cytotoxicity receptor, which is critical for NK cell-mediated innate immunity (3).

References

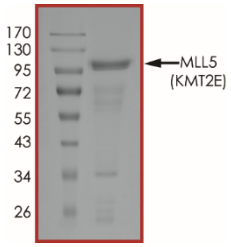
1. Emerling, B.M., et al. MLL5, a homolog of Drosophila trithorax located within a segment of chromosome band 7q22 implicated in myeloid leukemia. Oncogene. 21(31):4849-54, 2002.
2. Zhou, P., et al. Mixed lineage leukemia 5 (MLL5) protein regulates cell cycle progression and E2F1-responsive gene expression via association with host cell factor-1 (HCF-1). J Biol Chem. 288(24):17532-43, 2013.
3. Baychelier, F., et al. Identification of a cellular ligand for the natural cytotoxicity receptor NKp44. Blood. 122(17):2935-42, 2013.

Specific Activity



The specific activity of MLL5 (KMT2E) was determined to be **250 pmol /min/mg** as per activity assay protocol.

Purity



The purity of MLL5 (KMT2E) was determined to be **>90%** by densitometry, approx. MW **100 kDa**.

MLL5 (KMT2E), Active

Recombinant human protein expressed in Sf9 cells

Catalog #	M345-381G
Specific Activity	250 pmol/min/mg
Lot #	B2145-8
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 #: M345-381G)

Active MLL5 (KMT2E) (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 MLL5 (KMT2E) 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 MLL5 (KMT2E) 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 MLL5 (KMT2E) assay is performed using the Methyltransferase-Glo™ Assays kit (Promega, Catalog #: V7601).

Step 1. Thaw each active MLL5 (KMT2E) 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 MLL5 (KMT2E) (Catalog # M345-381G) 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 MLL5 (KMT2E) 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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