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Catalog #	Aliquot Size
L321-380G-05	5 µg
L321-380G-10	10 µg

LCMT1, Active

Full-length recombinant human protein expressed in Sf9 cells

Catalog # L321-380G

Lot # B2229-4

Product Description

Recombinant full-length human LCMT1 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The LCMT1 protein accession number is NM_016309.

Gene Aliases

CGI-68; LCMT; PPMT1

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

Leucine carboxyl methyltransferase 1 (LCMT1) exclusively methylates the carboxyl group of leucine-309 in the catalytic subunit of protein phosphatase 2A (1). LCMT1 is essential for normal progression through mitosis and cell survival. Knocking-down LCMT1 leads to reduced formation of PP2A heterodimers and increased apoptosis (2). LCMT1 also regulates the association of PP2A and its substrate, Tau protein, with plasma membrane in neuroblastoma cells. Decreased level of PP2A methylation is found in Alzheimer disease (3).

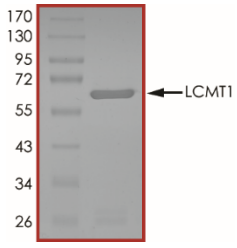
References

1. De Baere, I., et al. Purification of porcine brain protein phosphatase 2A leucine carboxyl methyltransferase and cloning of the human homologue. Biochemistry. 38(50):16539-47, 1999.
2. Lee, J.A., et al. Leucine carboxyl methyltransferase-1 is necessary for normal progression through mitosis in mammalian cells. J Biol Chem. 282(42):30974-84, 2007.
3. Sontag, J.M., et al. Leucine carboxyl methyltransferase 1 (LCMT1)-dependent methylation regulates the association of protein phosphatase 2A and Tau protein with plasma membrane microdomains in neuroblastoma cells. J Biol Chem. 288(38):27396-405, 2013.

Specific Activity

The specific activity of LCMT1 was determined to be **480 pmol /min/mg** as per activity assay protocol.

Purity



The purity of LCMT1 was determined to be **>95%** by densitometry, approx. MW **63 kDa**.

LCMT1, Active

Full-length recombinant human protein expressed in Sf9 cells

Catalog #	L321-380G
Specific Activity	480 pmol/min/mg
Lot #	B2229-4
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.

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com
www.signalchem.com

FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.

Activity Assay Protocol

Reaction Components

Active Methyltransferase (Catalog #: L321-380G)

Active LCMT1 (0.1μg/μl) diluted with Methyl-transferase 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 LCMT1 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 #: P17-34G)

PP2Aβ protein with a final concentration of 0.1μg/ml.

Assay Protocol

The LCMT1 assay is performed using the Methyltransferase-Glo™ Assays kit (Promega, Catalog #: V7601).

Step 1. Thaw the active LCMT1 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 LCMT1 (Catalog # L321-380G)
- 2X Substrate Cocktail: 40 μM of SAM and 100 ng/μl of PP2Aβ protein (Catalog # P17-34G) 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 LCMT1

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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