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Catalog #	Aliquot Size
M323-380G-05	5 µg
M323-380G-10	10 µg

METTL3/METTL14, Active

Recombinant human proteins expressed in Sf9 cells

Catalog # M323-380G
Lot # B2132-12

Product Description

Full-length recombinant human METTL3/METTL14 complex was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The METTL3 and METTL14 gene accession numbers are NM_019852 and NM_020961.

Gene Aliases

METTL3: IME4; M6A; MT-A70; Spo8
METTL14: None

Formulation

Recombinant proteins 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.

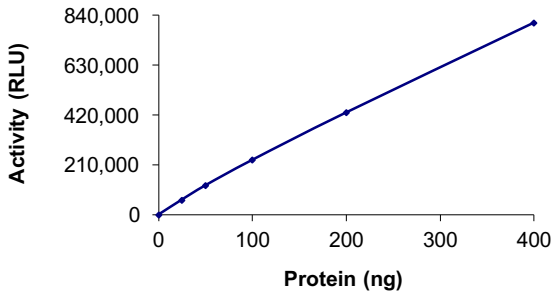
Scientific Background

Methyltransferase-like 3 (METTL3) and methyltransferase-like 14 (METTL14) form a stable heterodimer core complex that catalyzes N6-methyladenosine (m6A) RNA methylation in mammalian cells. m6A is an abundant internal modification in messenger RNA and long non-coding RNA. It functions in multiple aspects of developmental regulation, cell cycle progression, cell fate, and the heat shock stress response by affecting aspects of RNA metabolism such as pre-mRNA processing, translation efficiency, transcript stability and miRNA biogenesis.

References

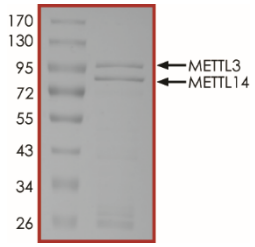
1. Liu J. et al: A METTL3–METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation. Nat. Chem. Biol. 2014; 10: 93–95
2. Wang X. et al: Structural basis of N6-adenosine methylation by the METTL3–METTL14 complex. Nature 2016; 534:575-578
3. Wang P. et al: Structural Basis for Cooperative Function of Mettl3 and Mettl14 Methyltransferases. Mol. Cell 2016; 63(2):306-317

Specific Activity



The specific activity of METTL3/METTL14 was determined to be **750 pmol /min/mg** as per activity assay protocol.

Purity



The purity of METTL3/METTL14 complex was determined to be **>90%** by densitometry, approx. MW **100 kDa (METTL3)** and **84 kDa (METTL14)**.

METTL3/METTL14, Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog #	M323-380G
Specific Activity	750 pmol/min/mg
Lot #	B2132-12
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 #: M323-380G)

Active METTL3/METTL14 complex (0.1μg/μl) diluted with RNA MTase 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 MTase complex for optimal results).

RNA Methyltransferase Reaction Buffer

Buffer components: 20mM Tris-HCl, pH7.5, 0.01% Triton X100. Add 1mM DTT (Signalchem, Catalog #: D86-09B-10) and 1unit/μl RNasin® Plus (Promega, Catalog #: N2611, optional) 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 #: M323-58)

METTL3/METTL14 Substrate, an oligo ssRNA, was reconstituted in 25μl of Methyltransferase Dilution Buffer IV to a final concentration of 100μM.

Assay Protocol

The METTL3/METTL14 assay is performed using the Methyltransferase-Glo™ Assays kit (Promega).

Step 1. Thaw the active MTase complex and all Methyltransferase-Glo™ Assays kit reagents on ice.

Step 2. Prepare the following working solutions with RNA Methyltransferase Reaction Buffer:

- 2X final concentration of Active complex (Catalog # M323-380G)
- 2X Substrate Cocktail: 40μM of SAM + 4μM of METTL3/METTL14 Substrate (Catalog #: M323-58)

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 METTL3/METTL14 complex

Note: A blank control can be set up as outlined in step 3 by replacing the enzyme working solution with an equal volume of RNA Methyltransferase 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)

$$= \frac{[SAH](nM) \times Reaction\ Volume(\mu l)}{Reaction\ Time\ (min) \times Enzyme\ Amount\ (mg)} \times 10^{-6}$$

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