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1-866-9 KINASE (54-6273)

Catalogue #	Aliquot Size
S344-380G-05	5 µg
S344-380G-10	10 µg

SETD7, Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog # S344-380G
Lot # J498-3

Product Description

Recombinant full-length human SETD7 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The SETD7 gene accession number is NM_030648.

Gene Aliases

FLJ21193; KIAA1717; KMT7; SET7; SET7/9; SET9

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.

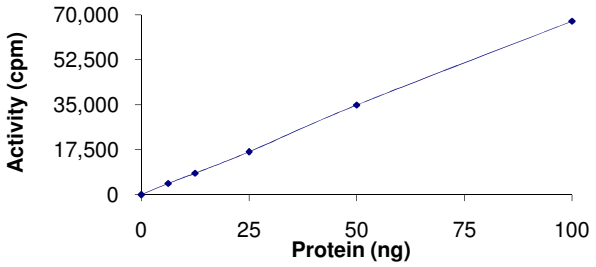
Scientific Background

SETD7 or SET domain containing lysine methyltransferase 7 is a lysine methyltransferase which can methylates lysine-4 (K4) in histone H3 in vitro and in vivo. Methylation of K4 in histone H3 by SETD7 and methylation of K9 in histone H3 by SUV39H1 were found to have differential effects on subsequent histone acetylation by p300 (1). SETD7 can also methylate p53 at lys372 within the C-terminal regulatory region (2). Methylated p53 is restricted to the nucleus and the modification positively affects its stability. SETD7 regulates the expression of p53 target genes in a manner dependent on the p53 methylation site.

References

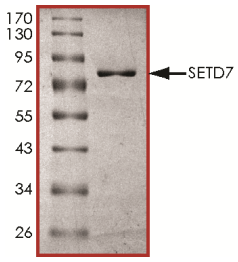
1. Wang, H. et al: Purification and functional characterization of a histone H3-lysine 4-specific methyltransferase. Molec. Cell 8: 1207-1217, 2001.
2. Chuikov, S. et al: Regulation of p53 activity through lysine methylation. Nature 432: 353-360, 2004.

Specific Activity



The specific activity of SETD7 was determined to be **40 nmol /min/mg** as per activity assay protocol.

Purity



The purity of SETD7 was determined to be **>95%** by densitometry, approx. MW **75 kDa**.

SETD7, Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog Number	S344-380G
Specific Activity	40 nmol/min/mg
Specific Lot Number	J498-3
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.

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Activity Assay Protocol

Reaction Components

Active Methyltransferase (Catalog #: S344-380G)

Active SETD7 (0.1µg/µl) diluted with Methyltransferase Dilution Buffer I (Catalog #: M21-09) 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 SETD7 for optimal results).

Methyltransferase Dilution Buffer I (Catalog#: M21-09)

Methyltransferase Assay Buffer I (Catalog #: M01-09) diluted at a 1:4 ratio (5X dilution) with distilled H_2O.

Methyltransferase Assay Buffer I (Catalog#: M01-09)

Buffer components: 250mM Tris-HCl, pH 9.0, 50 ng/µl BSA. Add 2mM DTT to Acetyltransferase Assay Buffer prior to use.

Adenosyl-L-methionine, S-[methyl-^{3}H] solution

The [^{3}H]-Adomet solution (0.54945µCi/µl and 10µCi/nmol) in 10mM H_2SO_4 : Ethenol (9:1) solution was purchased from PerkinElmer (Cat. # NET155250UC). The final concentration of [^{3}H]-Adomet is 54.945 µM or 54.945 pmol/µl.

Substrate (Catalog #: H10-54N)

Histone H1 protein diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [^{3}H]-Adomet solution in shielded container in a designated radioactive working area.

Step 2. Thaw the Active SETD7, Methyltransferase Assay Buffer I, Substrate and Methyltransferase Dilution Buffer I on ice.

Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:

- **Component 1.** 10µl of diluted Active SETD7 (Catalog #S344-380G)
- **Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #H10-54N)
- **Component 3.** 5µl of Methyltransferase Assay Buffer I (Catalog #: M01-09)

Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H_2O.

Step 5. Initiate the reaction by the addition of 5µl [^{3}H]-Adomet solution bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 30 minutes.

Step 6. After the 30 minute incubation period, terminate the reaction by spotting 20µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 10% trichloroacetic acid solution with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the methyltransferase specific activity as outlined below.

Calculation of [^{3}H]-Adomet Specific Activity (SA) (cpm/nmol)

Specific activity (SA) = cpm for 5µl [^{3}H]-Adomet / nmoles of Adomet
5µl of a 54.945 µM Adomet solution gives 165,000cpm
Therefore 165,000cpm / 5µl*54.945 pmol/µl = 600 cpm/pmol

Methyltransferase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of [^{3}H]-Adomet in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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