

## EHMT2, Active

Recombinant human protein expressed in Sf9 cells

**Catalog # E318-381G**

Lot # X478-2

### Product Description

Recombinant human EHMT2 (807-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The EHMT2 gene accession number is [NM\\_006709](#).

### Gene Aliases

G9A, NG36, NG36/G9a

### 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  $-70^{\circ}\text{C}$ . 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.

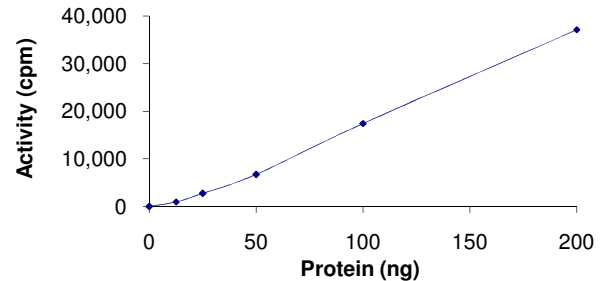
### Scientific Background

EHMT2 is a lysine-preferring histone methyltransferase. EHMT2 can methylate itself at K165 and this automethylation is necessary and sufficient to mediate in vivo interaction of EHMT2 with heterochromatin protein-1 (HP1) (1). EHMT2 is responsible for the majority of dimethylation of histone H3 at lysine 9 (H3K9me2) and is required for the efficient repression of developmentally regulated genes during embryonic stem cell differentiation (2). EHMT2 is overexpressed in various cancers and can specifically methylate p53 at Lys(373). EHMT2 polymorphisms have been associated with colorectal cancer.

### References

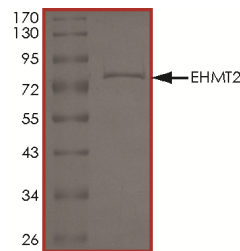
1. Sampath, S C. et al: Methylation of a histone mimic within the histone methyltransferase G9a regulates protein complex assembly. *Molec. Cell* 27: 596-608, 2007.
2. Lehnertz B. et al: Activating and inhibitory functions for the histone lysine methyltransferase G9a in T helper cell differentiation and function. *J Exp Med.* 2010 May 10;207(5):915-22.

### Specific Activity



The specific activity of EHMT2 was determined to be **15 nmol /min/mg** as per activity assay protocol.

### Purity



The purity of EHMT2 was determined to be **>95%** by densitometry, approx. MW **75 kDa**.

## EHMT2, Active

Recombinant human protein expressed in Sf9 cells

Catalog Number	E318-381G
Specific Activity	15 nmol/min/mg
Specific Lot Number	X478-2
Purity	>95%
Concentration	0.1 µg/µl
Stability	1yr at $-70^{\circ}\text{C}$ from date of shipment
Storage & Shipping	Store product at $-70^{\circ}\text{C}$ . 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 #: E318-381G)

Active EHMT2 (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 EHMT2 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<sub>2</sub>O.

### 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-<sup>3</sup>H] solution

The [<sup>3</sup>H]-Adomet solution (0.54945µCi/µl and 10µCi/nmol) in 10mM H<sub>2</sub>SO<sub>4</sub> : Ethenol (9:1) solution was purchased from PerkinElmer (Cat. # NET155250UC). The final concentration of [<sup>3</sup>H]-Adomet is 54.945 µM or 54.945 pmol/µl.

### Substrate (Catalog #: H12-58)

Histone H3 Peptide (1-21) substrate (ARTKQTARKSTGGKAPRK-QLAGGKCC) diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml.

## Assay Protocol

- Step 1.** Thaw [<sup>3</sup>H]-Adomet solution in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active EHMT2, 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 EHMT2 (Catalog #E318-381G)
  - Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #H12-58)
  - 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<sub>2</sub>O.
- Step 5.** Initiate the reaction by the addition of 5µl [<sup>3</sup>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 [<sup>3</sup>H]-Adomet Specific Activity (SA) (cpm/nmol)

Specific activity (SA) = cpm for 5µl [<sup>3</sup>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 [<sup>3</sup>H]-Adomet in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg)]\*[(Reaction Volume) / (Spot Volume)]

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