

DNMT3L, Active

Full length recombinant protein expressed in Sf9 cells

Catalog # D353-380CG

Lot # O1059-8

Product Description

Recombinant full-length human DNMT3L was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [BC002560](#).

Gene Aliases

MGC1090

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 50mM 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.

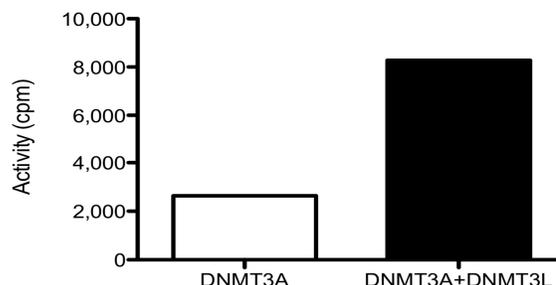
Scientific Background

DNMT3L is an enzymatically inactive regulatory factor that shares sequence homology with the DNA methyltransferase DNMT3A and DNMT3B. DNMT3L is involved in the establishment of DNA methylation patterns during gametogenesis. In mammals, DNA methylation is used for heritable silencing of retrotransposons and imprinted genes and for inactivation of the X chromosome in females (1). DNMT3L has a genomewide role in CpG island methylation beyond genomic imprinting (2). DNMT3L functions as a regulators of methylation at imprinted loci rather than a DNA cytosine methyltransferase because of a lack of catalytic motifs in its sequence.

References

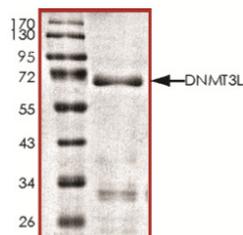
1. Ooi, S.K.T. et al: DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. *Nature* 448: 714-717, 2007.
2. Smallwood, S. A. et al: Dynamic CpG island methylation landscape in oocytes and preimplantation embryos. *Nature Genet.* 43: 811-814, 2011.

Specific Activity



The specific activity of DNMT3A (0.25µg) was increased 3-fold co-incubated with DNMT3L (0.25µg).

Purity



The purity of DNMT3L was determined to be **>80%** by densitometry. Approx. MW **70 kDa**.

DNMT3L, Active

Recombinant human protein expressed in Sf9 cells

Catalog Number	D353-380CG
Specific Lot Number	O1059-8
Purity	>80%
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 #: D353-380CG)

Active DNMT3L (0.1µg/µl) diluted with Methyltransferase Dilution Buffer III (Catalog #: M23-09) and assayed with DNMT3A as 1:1 ratio (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active DNMT3L for optimal results).

Methyltransferase Dilution Buffer III (Catalog #: M23-09)

Methyltransferase Assay Buffer (Catalog #: M03-09) diluted at a 1:4 ratio (5X dilution) with distilled H₂O.

Methyltransferase Assay Buffer III (Catalog #: M03-09)

Buffer components: 250mM Tris-HCl, pH 7.5, 5mM EDTA, 25% Glycerol, 50ng/µl BSA. Add 2.5 mM DTT to Methyltransferase Assay Buffer prior to use.

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

The [³H]-Adomet solution (0.54945µCi/µl and 10µCi/nmol) in 10mM H₂SO₄: Ethanol (9:1) solution was purchased from PerkinElmer (Cat. # NET155250UC). The final concentration of [³H]-Adomet is 54.945 µM or 54.945 pmol/µl.

Substrate for DNMT3A (Catalog #: D53-58)

5nmol of DNMT1 Substrate-1 oligonucleotide reconstituted in 500µl distilled H₂O to a final concentration of 10µM.

Assay Protocol

- Step 1.** Thaw [³H]-Adomet solution in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active DNMT3A, DNMT3L, Methyltransferase Assay Buffer III, Substrate and Methyltransferase Dilution Buffer III 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.** 5µl of diluted Active DNMT3A (Catalog # D353-380G)
 - Component 2.** 5µl of diluted Active DNMT3L (Catalog # D353-380CG)
 - Component 3.** 5µl of Methyltransferase Assay Buffer III (Catalog #: M03-09)
 - Component 4.** 5µl [³H]-Adomet solution
- Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the DNMT3L. Replace the substrate with an equal volume of Methyltransferase Dilution Buffer III.
- Step 5.** Pre-incubate the above mixtures from step 3 and step 4 at 37°C for 30 minutes.
- Step 6.** After 30-minute pre-incubation, then add 5µl of substrate (Catalog # D53-58) into the mixtures and incubate the mixtures in a water bath at 37°C for 60 minutes.
- Step 7.** After the 60-minute incubation period, terminate the reactions by spotting 20µl of the reaction mixtures onto individual pre-cut strips of DE81 papers.
- Step 8.** Air dry the pre-cut DE81 strips and sequentially wash in a 0.2M NH₄HCO₃ solution with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 9.** Wash DE81 strips with distilled H₂O twice with constant gentle stirring.
- Step 10.** Count the radioactivity on the DE81 paper in the presence of scintillation fluid in a scintillation counter.

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