

DNMT3B, Active

Full length recombinant protein expressed in Sf9 cells

Catalog # D353-380BG

Lot # O1059-7

Product Description

Recombinant full-length human DNMT3B was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [BC111933](#).

Gene Aliases

ICF; ICF1; M.HsaIIIB

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.

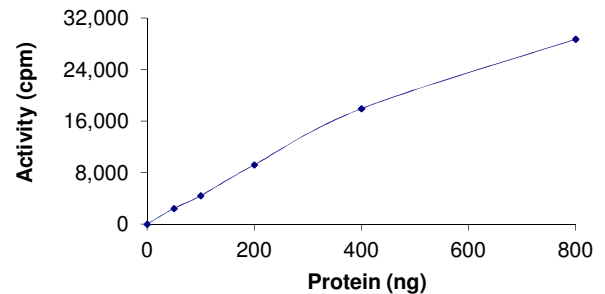
Scientific Background

DNMT3B or DNA (cytosine-5-)-methyltransferase 3 beta encodes a DNA methyltransferase which is thought to function in de novo methylation, rather than maintenance methylation. DNMT3B can methylate unmethylated and hemimethylated DNA with equal efficiencies (1). DNMT3B localizes primarily to the nucleus and its expression is developmentally regulated and is required for genome wide de novo methylation and is essential for mammalian development (2). Mutations in DNMT3B cause the immunodeficiency-centromeric instability-facial anomalies (ICF) syndrome.

References

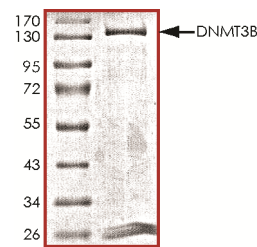
1. Yanagisawa, Y. et al: The human DNA methyltransferases DNMT3A and DNMT3B have two types of promoters with different CpG contents. *Biochim. Biophys. Acta* 1577: 457-465, 2002.
2. Okano, M. et al: DNA methyltransferase Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* 99: 247-257, 1999.

Specific Activity



The specific activity of DNMT3B was determined to be **525 pmol /min/mg** as per activity assay protocol.

Purity



The purity of DNMT3B was determined to be **>75%** by densitometry. Approx. MW **138 kDa**.

DNMT3B, Active

Recombinant human protein expressed in Sf9 cells

Catalog Number	D353-380BG
Specific Activity	525 pmol/min/mg
Specific Lot Number	O1059-7
Purity	>75%
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 #: D353-380BG)

Active DNMT3B (0.1µg/µl) diluted with Methyltransferase Dilution Buffer (Catalog #: M23-09) 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 DNMT3B 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: Ethenol (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 (Catalog #: P62-58)

Poly (1:1 dl, dC) Acid substrate reconstituted in 500µl distilled H₂O to a final concentration of 50ng/µl.

Assay Protocol

- Step 1.** Thaw [³H]-Adomet solution in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active DNMT3B, 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.** 10µl of diluted Active DNMT3B (Catalog # D353-380BG)
 - Component 2.** 5µl of 50ng/µl of stock solution of substrate (Catalog # P62-58)
 - Component 3.** 5µl of Methyltransferase Assay Buffer III (5X) (Catalog #: M03-09)
- 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 H₂O.
- Step 5.** Initiate the reaction by the addition of 5µl [³H]-Adomet solution bringing the final volume up to 25µl and incubate the mixture in a water bath at 37°C for 60 minutes.
- Step 6.** After the 60-minute incubation period, terminate the reaction by spotting 20µl of the reaction mixture onto individual pre-cut strips of DE81 paper.
- Step 7.** Air dry the pre-cut DE81 strip 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 8.** Wash DE81 strip with distilled H₂O twice with constant gentle stirring.
- Step 9.** Count the radioactivity on the DE81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 10.** 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 [³H]-Adomet Specific Activity (SA) (cpm/nmol)

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

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