

Product Data Sheet

Product Name: N6-Methyl-dATP

Cat. No.: GB20075

Chemical Properties

Cas. No. 5085-65-4(free acid)

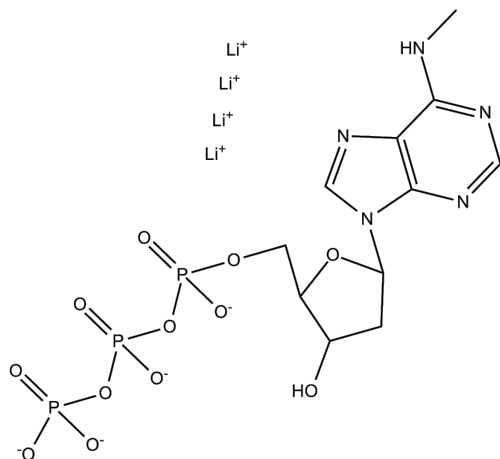
Formula $C_{11}H_{18}N_5O_{12}P_3$ (free acid) M.Wt 505.2 (free acid)

Solubility Storage Store at -20°C or below

General tips For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.

Structure



Protocol

Cell experiment [1]:

Cell lines HeLa cells

Caution: Product has not been fully validated for medical applications. For research use only.

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

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HeLa cells were collected, trypsin-digested, washed and lysed. The protein concentration of the cell lysate was determined by the Bradford assay. In a reaction system with components like 40mM HEPES/KOH pH 7.8, 70mM KCl, 5m MMgCl₂, 10μM ZnCl₂ and 500μM DTT, experimental groups with 20μM dATP or 16μM dATP plus 4μm N6-Methyl-dATP were set up. Template DNA, primers and HeLa cell lysate were added and incubated at 37°C for 45 minutes. After reaction, nucleic acids were purified by phenol-chloroform extraction and ethanol precipitation. Nucleotides and primers were removed by ultrafiltration and concentration. Amplification products were PAGE-purified and ethanol-precipitated. The DNA precipitate was dissolved and digested into nucleosides, and then N6-mA level was measured by LC-MS.

Reaction Conditions 4μM; 37°C for 45min

Applications In mammalian cells, DNA polymerase can utilize N6-Methyl-dATP and incorporate N6-mA into DNA.

Animal experiment [2]:

Animal models MTH1KO zebrafish

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Preparation Method	The fertilized MTH1WT and MTH1KO zebrafish eggs were grouped. One group was microinjected with N6-methyl-dATP solution. Approximately 2nl volume of 0.3mM N6-methyl-dATP was injected into the injection buffer (9μM spermidine, 0.21μM spermidine, 0.3% phenol red) , while the other group was not injected as a control. And the eggs were distributed onto the plates of E3 medium (5mM NaCl, 0.17mM KCl, 0.33mM CaCl₂·2H₂O, 0.33mM MgSO₄). The viability of zebrafish embryos was evaluated 24 hours after injection, and the zebrafish were harvested 32 hours later. Zebrafish DNA from different treatment groups was isolated using the DNeasy Blood and Tissue Kit. The processed DNA samples were hydrolyzed, and various enzymes (nuclease P1, Benzonase nuclease, Escherichia coli alkaline phosphatase) were added and incubated at 37°C for 1hour. Precipitate the protein, take the supernatant and redissolve it in water. The content of N6-methyl-dATP in zebrafish embryo DNA of different treatment groups was analyzed by LC-MS/MS using the Agilent 6495 triple quadrupole LC/MS/MS system and specific chromatographic columns and mobile phase conditions.
Dosage form	0.3mM; 24h
Applications	N6-methyl-dATP is incorporated into DNA in an MTH1-dependent manner.

References:

- [1] Musheev MU, Baumgärtner A, Krebs L, et al. The origin of genomic N6-methyl-deoxyadenosine in mammalian cells. *Nature Chemical Biology* 2020, 16(6):630-634.
- [2] Scaletti ER, Vallin KS, Bräutigam L, et al. MutT homologue 1 (MTH1) removes N6-methyl-dATP from the dNTP pool. *J Biol Chem* 2020, 295(15):4761-4772.

Background

N6-Methyl-dATP, as a base-modified ribonucleoside triphosphate, can act as an effective agonist for

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P2Y purinergic receptors in organisms such as guinea pigs and tapeworms^[1]. N6-Methyl-dATP has also been demonstrated to play a critical role in carcinogenesis and stem cell fate determination^[2].

In vitro, when HeLa cells are treated with 4µM N6-Methyl-dATP for 45 minutes, N6-methyladenine (N6-mA) must first be taken up by the cells and metabolized into the active form N6-Methyl-dATP to serve as a substrate for DNA polymerase and be integrated into DNA. This process falls under the category of RNA methylation products erroneously intervening in DNA synthesis through the nucleotide salvage pathway, and its integration mechanism relies on the non-specific recognition of modified nucleotides by DNA polymerase^[3].

In vivo, microinjection of 0.3 mM N6-Methyl-dATP into homozygous MTH1KO zebrafish with knockout of the mth1 gene reveals that N6-Methyl-dATP present in the nucleotide pool can be incorporated into DNA, and this process can be blocked by the MTH1 protein^[4]. In Arabidopsis MAPDA mutants, a high ratio of N6-Methyl-dATP/ATP (1.49%) induces root growth defects due to erroneous integration by RNA polymerase II or interference with energy metabolism^[5].

References:

- [1] He ML, Koshimizu TA, Tomić M, et al. Purinergic P2X(2) receptor desensitization depends on coupling between ectodomain and C-terminal domain. *Mol Pharmacol* 2002, 62(5):1187-1197.
- [2] Pacini CE, Bradshaw CR, Garrett NJ, et al. Characteristics and homogeneity of N6-methylation in human genomes. *Sci Rep* 2019, 9(1):5185.
- [3] Musheev MU, Baumgärtner A, Krebs L, et al. The origin of genomic N6-methyl-deoxyadenosine in mammalian cells. *Nature Chemical Biology* 2020, 16(6):630-634.
- [4] Scaletti ER, Vallin KS, Bräutigam L, et al. MutT homologue 1 (MTH1) removes N6-methyl-dATP from the dNTP pool. *J Biol Chem* 2020, 295(15):4761-4772.
- [5] Chen M, Urs MJ, Sánchez-González I, et al. m(6)A RNA Degradation Products Are Catabolized by an Evolutionarily Conserved N(6)-Methyl-AMP Deaminase in Plant and Mammalian Cells. *Plant Cell* 2018, 30(7):1511-1522.

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