CpG Methyltransferase (M.SssI)

REF: EG26204S

Storage Condition

Store at -20℃ for 2 years.

Components

Component	Amount
CpG Methyltransferase (M.SssI) (4 U/μl)	25 μl
10× M.SssI Buffer	1 ml
SAM (32 mM)	100 μl

Description

CpG Methyltransferase (M.SssI) is derived from the methyltransferase gene of the *Spiroplasma* MQ1 strain, recombinantly expressed and purified from *E. coli*. M.SssI methylates the cytosine residue (C^5) within the 5'-CG-3' sequence of unmethylated or hemimethylated double-stranded DNA. It is one of the key epigenetic tool enzymes and can be used to mimic methylation modifications in eukaryotic genomes and to explore the functions of cytosine methylation.

Applications

1. Epigenetic studies.
2. In vitro DNA methylation for methylation analysis.
3. Blocking endonuclease cleavage at overlapping CpG sequences.
4. Uniform [^{3}H]-labeling of DNA.

Quality Control Assays

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

Endonuclease Activity

A 20 μl reaction containing 200 ng of supercoiled plasmid and 4 U of CpG Methyltransferase incubated for 4 hours at 37℃ results in <20% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

DNase Activity

A 20 μl reaction containing 15 ng of dsDNA fragments and 4 U of CpG Methyltransferase incubated for 16 hours at 37 ℃ results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

Protocol

① Prepare the following reaction mixture on ice:

Reagent	Amount
DNA	X μl (≤1 μg)
10× M.SssI Buffer	2 μl
SAM (3.2 mM)[a]	1 μl
CpG Methyltransferase (M.SssI) (4 U/μl)	1 μl
ddH_2O	Up to 20 μl

a: Dilute the supplied SAM solution (32 mM) 10-fold with ultrapure water to obtain a final concentration of 3.2 mM. The diluted SAM solution should not be stored for extended periods. To ensure SAM stability, add SAM last to avoid degradation in aqueous solution.

② Mix gently and spin down;

③ Incubate at 37℃ for 15~60 min. For substrates with many methylation sites, the enzyme amount or reaction time may be increased accordingly.

④ Terminate the reaction by incubation at 65℃ for 20 minutes.

Notice

1. Freshly prepared SAM optimizes the methylation reaction. If possible, store SAM at -80℃ to extend its shelf life.

2. M.SssI does not require $MgCl_2$ as a cofactor. In the presence of Mg^{2+}, methylation by M.SssI becomes discontinuous and exhibits topoisomerase activity.

3. For CpG-methylation-sensitive restriction enzymes whose recognition sites contain CG or overlap with it, CpG methyltransferase can block cleavage. In addition, DNA methylated by CpG methyltransferase is subject to Mcr and Mrr restriction systems in *E. coli*; therefore, Mcr^- Mrr^- *E. coli* strains should be used for transformation.

4. Methylation of cytosine residues affects the physical properties of DNA, including lowering the free energy of Z-DNA formation, increasing DNA winding, and altering the kinetics of cruciform extrusion. In chemical sequencing protocols, 5-methylcytosine can be identified by its reduced reactivity to hydrazine.

5. The density of CpG dinucleotides in DNA substrates should be considered during in vitro methylation. For example, λDNA (48,502 bp) contains 3,112 CpG sites. Therefore, a 0.1 mg/ml λDNA solution contains approximately 19 μM methyl acceptor sites for methyltransferase. The recommended SAM concentration is 160 μM, which is more than eight-fold higher than the acceptor site concentration, allowing efficient methylation. However, when λDNA concentration reaches 1 mg/ml, the concentration of methyl acceptor sites exceeds the SAM concentration, preventing complete methylation. To ensure complete methylation, the DNA concentration in the reaction mixture should not exceed 0.02 mg/ml.

6. CpG Methyltransferase (M.SssI) cannot methylate single-stranded DNA or RNA.

7. This product is for research use only and not intended for any other purpose.

8. For your safety and health, please wear a lab coat, disposable gloves, and a mask while conducting the experiment.

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