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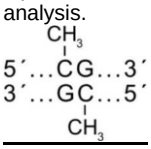
Product Specification Sheet

□ Cat. #ZE-2010 CpG Methylase (M.SssI) SIZE: 200 units

The CpG Methylase (EC 2.1.1.37)1 completely methylates all cytosine residues (C5) in double-stranded, nonmethylated and hemi-methylated DNA having the dinucleotide sequence 5′…CpG…3′. The recombinant CpG Methylase is isolated from an *E. coli* strain that expresses the methyltransferase gene from *Spiroplasma* sp. strain MQ1. The reaction conditions are optimized to maximize the processivity of the enzyme to ensure *rapid*, *complete,* and *reproducible* methylation of DNA for accurate DNA methylation analysis.



Applications:
· For complete *in vitro* methylation of DNA for methylation analysis.
· Methylation of chromatin DNA for DNA accessibility studies.
· Inhibition of endonucleases with overlapping CpG sequence recognition.
· [3H]-labeling of DNA.

Product contents:
CpG Methylase; 200 units, 10X CpG Reaction Buffer (ZE2010-2) ; 1ml, 20X SAM (S-adenosylmethionine),12 mM (ZE2010-3); 0.2ml

Storage: Store reagents at -20 °C for up to 12 months. Avoid repeated freeze/thawing. Prolonged storage should be ≤ -70 °C.

Enzyme Concentration: 4 units/µl

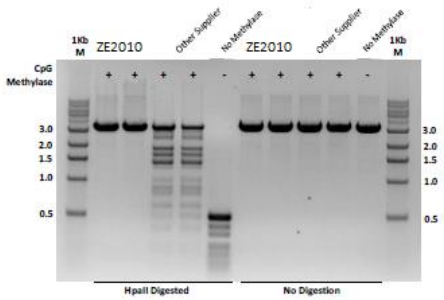
Unit Definition: One unit is defined as the amount of enzyme required to "protect" 1 µg of λ DNA against cleavage by BstUI restriction endonuclease in a total reaction volume of 20 µl for 1 hour at 37 °C.

Reaction Conditions: CpG Methylase in 1X CpG Reaction Buffer w/ 600 µM SAM. Incubate reaction mixtures at 30 °C (Performing the Methylase reaction at 30 °C rather than 37 °C promotes optimal reaction kinetics).

Inactivation: Heat-inactivate the enzyme at 65 °C for 20 minutes.

Standard Reaction Setup: The setup (below) is an example of a typical CpG methylase reaction in a 20 µl final reaction volume. The reaction volumes can be adjusted accordingly depending on experimental requirements (see Notes 1 & 2).
2 µl 10X CpG Reaction Buffer
1 µl 20X SAM (S-adenosylmethionine), 12 mM
4 µl DNA at 100-250 ng/µl
1 µl CpG Methylase (4 units/µl)
12 µl Water
Incubate at 30 °C for 2 hours.

Notes:
1. SAM Concentration
SAM is supplied as a 20X stock solution (12 mM SAM in a low pH buffer) and is 600 µM at 1X. Although we recommend using SAM at 600 µM, the concentration can be adjusted from 150 µM to 800 µM depending on experimental requirements. The recommended SAM concentration of 600 µM will be adequate for most reactions with DNA concentrations up to 0.4 µg/µl) containing high CpG content. SAM is sensitive to degradation at elevated pH. It should be thawed on ice prior to use and stored at -20 °C.
2. Complete Methylation of All CpG Dinucleotides.
For complete methylation of all CpG sites in DNA, we recommend increasing reaction times to 4 hours to overnight at 30 °C. Re-addition of CpG Methylase after 2 to 4 hours of initial incubation is helpful to drive the methylation of DNA to completion. Also, supercoiled, circular DNA is slightly more resistant to methylation than linearized DNA. Therefore, linearization of circular DNA is recommended whenever possible for complete CpG methylation. Finally, the methylation reaction can be sensitive to contaminants (i.e.,salts) from the DNA sample. It is recommended that impure preparations of DNA be "cleaned" prior to manipulation . The unique formulation of the 10X CpG Reaction Buffer ensures optimal activity of the CpG Methylase. A comparison of the methylase activity of the CpG Methylase from GSI versus that of another supplier is demonstrated in the figure below.
3. Factors That Influence Methylase Activity
a. *Reaction Buffers*: Although supplied with a 10X CpG Reaction Buffer for maximal activity, the CpG Methylase is compatible, with limited activity (i.e., 10 to 70%), with most restriction enzyme digestion buffers. When using these buffers, increasing the enzyme concentration and incubation time at 30 °C may be necessary for complete methylation of all CpG sites in DNA.
b. *Magnesium and EDTA Concentration*: The CpG Methylase does not require magnesium as a cofactor for its activity and works well in the presence of EDTA. The enzyme is more distributive and less processive in the presence of magnesium.
c. The presence of free nucleotides, oligonucleotide primers and/or small amounts of RNA has no significant effect on CpG Methylase activity.



The CpG Methylase catalyzes complete methylation of the CpG sites in DNA. Methylase activities of CpG Methylase from GSI versus that of another supplier were tested for complete methylation of equivalent amounts of a linearized plasmid DNA using reaction conditions recommended by the supplier. "Completion" of CpG methylation was assessed by resistance to digestion with a methylation-specific endonuclease (HpaII) and subsequently analyzed in an agarose gel. As shown in the figure above, the CpG Methylase from GSI completely methylated the CpG sites in the DNA whereas that of the other supplier did not. Samples were assayed in duplicate.

References: Nur, I. *et al.* J. Bacteriol., 164, 19-24 (1985). Renbaum, P. *et al.* Nucl. Acids Res., 18, 1145-1152 (1990).