

CpG Methyltransferase (M.SssI)



1-800-632-7799
info@neb.com
www.neb.com



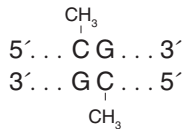
M0226S 031170218021

M0226S RR NEB2 37° SAM Yes

100 units 4,000 U/ml Lot: 0311702

RECOMBINANT Store at -20°C Exp: 2/18

Methylation Site:



Description: The CpG Methyltransferase, M.SssI, methylates all cytosine residues (C⁵) within the double-stranded dinucleotide recognition sequence 5'...CG...3' (1).

Source: The CpG Methyltransferase, M.SssI, is isolated from a strain of E. coli which contains the Methyltransferase gene from Spiroplasma sp. strain MQ1 (2,3).

Applications:

- Blocking restriction endonuclease cleavage
- Studying of CpG methylation-dependent gene expression
- Probing sequence-specific contacts within the major groove of DNA
- Altering the physical properties of DNA
- Uniform [³H]-labeling of DNA
- Decreasing the number of sites cut by restriction endonucleases, yielding an apparent increase in specificity.

Supplied in: 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:

10X NEBuffer 2, 200X S-adenosylmethionine (32 mM).

Reaction Conditions: 1X NEBuffer 2, supplemented with 160 µM S-adenosylmethionine (supplied). Incubate at 37°C.

1X NEBuffer 2:

50 mM NaCl
10 mM Tris-HCl
10 mM MgCl₂
1 mM dithiothreitol
pH 7.9 @ 25°C

Note: MgCl₂ is not required as a cofactor. In the presence of Mg²⁺, methylation by M.SssI becomes distributive rather than processive and also exhibits topoisomerase activity (4).

A buffer containing 50 mM NaCl, 10 mM Tris-HCl (pH 7.9 @ 25°C), 10 mM EDTA, 160 µM S-adenosylmethionine may be substituted for NEBuffer 2.

Protection Assay Conditions: M.SssI is incubated with 1 µg λ DNA in 20 µl 1X NEBuffer 2 [50 mM NaCl, 10 mM Tris-HCl (pH 7.9 @ 25°C), 10 mM MgCl₂, 1 mM dithiothreitol], 160 µM S-adenosylmethionine, for one hour at 37°C. The extent of protection by M.SssI is determined by the addition of 30 µl NEBuffer 2 containing 10 units of BstUI restriction endonuclease. Incubation for 1 hour at 60°C is followed by analysis on an agarose gel.

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

Quality Assurance: Purified free of contaminating endonucleases and exonucleases.

Quality Control Assays

16-Hour Incubation: Incubation of 100 units of M.SssI with 1 µg λ DNA in 50 µl of 1X NEBuffer 2 for 16 hours at 37°C resulted in no detectable endonuclease contamination.

Exonuclease Activity: Incubation of 100 units of M.SssI with 1 µg sonicated ³H DNA (10⁵ cpm/µg) for 4 hours at 37°C in 50 µl NEBuffer 2 [50 mM NaCl, 10 mM Tris-HCl (pH 7.9 @ 25°C), 10 mM MgCl₂, 1 mM dithiothreitol] released < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of 50 units of M.SssI with 1 µg of φX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 5% conversion to RF II.

Heat Inactivation: 65°C for 20 minutes.

(See other side)

CERTIFICATE OF ANALYSIS

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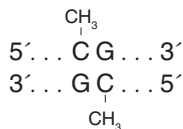
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Notes: S-adenosylmethionine (SAM) is supplied as a 32 mM solution in 0.005 M sulfuric acid and 10% ethanol. Under these conditions SAM is stable for up to 6 months when stored at -20°C.

SAM is unstable at (pH 7.5), 37°C, (1) and should be replenished in reactions incubated longer than 4 hours.

Methylation can be optimized by using fresh SAM.

This CPG Methyltransferase may be useful for studying the function of cytosine methylation in higher eukaryotes as its specificity mimics the pattern of modification found in their genomes (5). In contrast to the mammalian enzyme (6,7), both unmethylated and hemi-methylated DNA substrates are methylated with equal efficiency by the CpG Methyltransferase (2), making it a more useful tool for modifying DNA.

The CpG Methyltransferase can be used to block cleavage by a variety of restriction endonucleases whose recognition sites either contain the sequence CG, or overlap the dinucleotide. It should be noted that DNAs methylated by the CpG Methyltransferase are subject to Mcr and

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Mrr restriction in *E. coli*, and thus should be transformed into Mcr⁻ Mrr⁻ *E. coli* strains.

Methylation at cytosine residues has also been shown to affect the physical properties of DNA, including lowering the free energy of Z-DNA formation (8), increasing the helical pitch of DNA (6), and altering the kinetics of cruciform extrusion (9). Positions of 5-methylcytosine can be identified due to decreased reactivity to hydrazine in chemical sequencing protocols (10).

The high density of CpG dinucleotides in DNA substrates should be taken into account when methylating DNAs *in vitro*. For example, lambda DNA (48,502 bp) contains 3112 CpG sites, and thus a 0.1 mg DNA/ml solution is 19 µM with respect to methyl acceptor sites for the Methyltransferase. This is significant because the recommended concentration of methyl donor, S-adenosylmethionine (SAM), is 160 µM, an 8-fold excess over acceptor sites. Reducing the DNA concentration (< 0.02 mg/ml) gives two advantages. First, the SAM concentration remains high enough to drive the reaction. Second, potential end product inhibition arising from S-adenosyl-L-homocysteine (AdoHcy) generated during the reaction is limited.

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References:

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3. Forney, J.A. and Jack, W. E. (1991) *The NEB Transcript* 3:1, 5.; Benoit, C.C. and Robinson, D.P., unpublished observations.
4. Matsuo, K. et al. (1994) *Nucl. Acids Res.* 22, 5354–5359.
5. Doerfler, W. (1983) *Ann. Rev. Biochem.* 52, 93–124.
6. Gruenbaum, Y. et al. (1982) *Nature* 295, 620–621.
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8. Zacharias, W. et al. (1988) *Biochemistry* 27, 2970–2978.
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Companion Product:

S-adenosylmethionine (SAM)
#B9003S 0.5 ml



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