GpC Methyltransferase (M.CviPI)

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

M0227S 001120414041

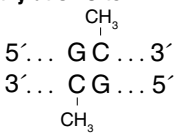
M0227S

RR 37° SAM Yes

200 units **4,000 U/ml** **Lot: 0011204**

RECOMBINANT **Store at –20°C** **Exp: 4/14**

Methylation Site:



Description: The GC Methyltransferase, M.CviPI, methylates all cytosine residues (C^5) within the double-stranded dinucleotide recognition sequence 5´...GC...3´.

Source: The GpC Methyltransferase, M.CviPI, is isolated from a strain of *E. coli* which contains the methyltransferase gene from *Chlorella* virus. This construct is fused to the maltose binding protein (MBP).

Applications:
- Blocking restriction endonuclease cleavage
- Altering the physical properties of DNA
- Uniform [^{3}H]-labeling of DNA

Supplied in: 15 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 0.2 M NaCl, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X GC Reaction Buffer
200X S-adenosylmethionine (32 mM).

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

1X GC Reaction Buffer:
50 mM NaCl
50 mM Tris-HCl
10 mM dithiothreitol
pH 8.5 @ 25°C

Note: $MgCl_2$ is not required as a cofactor.

Protection Assay Conditions: M.CviPI is incubated with 1 µg λ DNA in 20 µl 1X GC Reaction Buffer and 160 µM S-adenosylmethionine, for one hour at 37°C. The extent of protection by M.CviPI is determined by the addition of 30 µl NEBuffer 2 containing 10 units of HaeIII restriction endonuclease. Incubation for 1 hour at 37°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 HaeIII restriction endonuclease.

Quality Assurance: Purified free of contaminating endonucleases and exonucleases.

Quality Control Assays

16-Hour Incubation: Incubation of 60 units of M.CviPI with 1 µg λ DNA in 50 µl of 1X GC Reaction Buffer for 16 hours at 37°C resulted in no detectable endonuclease contamination.

Exonuclease Activity: Incubation of 80 units of M.CviPI with 1 µg sonicated ^{3}H DNA (10^5 cpm/µg) for 4 hours at 37°C in 50 µl GC Reaction Buffer released < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of 40 units of M.CviPI 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.

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.

Requires fresh DTT for optimum activity. For best results, mix assay buffer fresh for each use.

Methylation at cytosine residues has also been shown to affect the physical properties of DNA, including lowering the free energy of Z-DNA formation (1), increasing the helical pitch of DNA (2), and altering the kinetics of cruciform extrusion (3). Positions of 5-methylcytosine can be identified due to decreased reactivity to hydrazine in chemical sequencing protocols (4).

(See other side)

CERTIFICATE OF ANALYSIS

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5´... GC ... 3´
3´... CG ... 5´

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References:

1. Zacharias, W. et al. (1988) *Biochemistry* 27, 2970–2978.
2. Gruenbaum, Y. et al. (1982) *Nature* 295, 620–621.
3. Murchie, A.I. and Lilley, D.M. (1989) *J. Mol. Biol*. 205, 593–602.
4. Ohmori, H. et al. (1978) *Nucl. Acids Res*. 5, 1479–1485.
5. Xu, S. et al. (1998) *Nucl. Acids Res*. 26, 3961–3966.
6. Kladde, M.P. et al. (1991) *Methods Enzymol*. 304, 431–447.

Companion Product:

S-adenosylmethionine (SAM)
#B9003S 0.5 ml

References:

1. Zacharias, W. et al. (1988) *Biochemistry* 27, 2970–2978.
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U.S. Patent Nos. 7,034,116, 6,492,168

Page 2 (M0227)