GpC Methylase (M.CviPI)

Cat. Nos. E2014 (200 Units)
E2015 (1000 Units)

Storage: -20 °C

ZYMO RESEARCH
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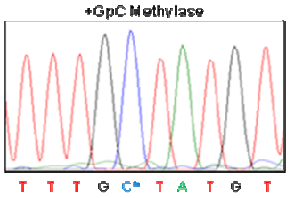
Product Information

Description:

The GpC Methylase (EC 2.1.1.37)[1] from Zymo Research completely methylates all cytosine residues (C^5) in double-stranded, non-methylated and hemi-methylated DNA having the dinucleotide sequence 5′…GpC…3′. The recombinant GpC Methylase is isolated from an *E. coli* strain that expresses the methyltransferase gene from a *Chlorella* virus. 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.

Recognition Site: Methylation of cytosines when found in a 5'…GpC…3' context.

	Original Sequence	+GpC Methylase
Non-Converted →	CTCGCCATGT	CTCGC^{m}CATGT
Bisulfite Converted →	TTTGTTATGT	TTTGC^{m}TATGT



DNA sequence after bisulfite treatment. Bisulfite-treated DNA converts cytosine to uracil, which reads as thymine upon sequencing. But methylated cytosines remain unconverted. As shown above, treatment of DNA with GpC methylase shows methylation of cytosines in a GpC context.

Applications:

- For complete *in vitro* methylation of DNA for methylation analysis.
- Methylation of chromatin DNA for DNA accessibility studies.
- Inhibition of endonucleases with overlapping GpC sequence recognition.
- [^{3}H]-labeling of DNA.

Product Contents:

	Cat. # E2014	Cat. # E2015	Storage
GpC Methylase	200 units	1000 units	-20 °C
10X GpC Reaction Buffer (E2014-2)	1 ml	1 ml	-20 °C
20X SAM (S-adenosylmethionine), 12 mM (E2010-3)	200 µl	200 µl	-20 °C

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 HaeIII restriction endonuclease in a total reaction volume of 20 µl for 1 hour at 37 °C.

Reaction Conditions: GpC Methylase in 1X GpC Reaction Buffer w/ 600 µM SAM. Incubate reaction mixtures at 37 °C.

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

Standard Reaction Setup: The setup (below) is an example of a typical GpC 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 GpC Reaction Buffer
1 µl	20X SAM (S-adenosylmethionine) [12 mM]
4 µl	DNA at 100-250 ng/µl
1 µl	GpC Methylase (4 units/µl)
12 µl	Water

Incubate at 37 °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 GpC content.

SAM is sensitive to degradation at elevated pH. It should be thawed on ice prior to use and stored at -20 °C.

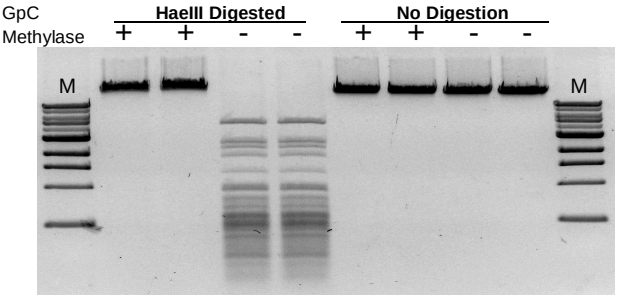
2. Complete Methylation of All GpC Dinucleotides.

For complete methylation of all GpC sites in DNA, we recommend increasing reaction times to 4 hours to overnight at 37 °C. Re-addition of GpC 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 GpC 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 (e.g., DNA Clean and Concentrator™ from Zymo Research).

The unique formulation of the 10X GpC Reaction Buffer ensures optimal activity of the GpC Methylase.



The GpC Methylase from Zymo Research catalyzes *complete* methylation of the GpC sites in DNA. Methylase activity of GpC Methylase from Zymo Research was tested for complete methylation of λ DNA using recommended reaction conditions. "Completion" of GpC methylation was assessed by resistance to digestion with a methylation-sensitive endonuclease (HaeIII) and subsequently analyzed in an agarose gel. "M" is a 1kb DNA ladder (Zymo Research).

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References:

1. Xu, M. *et al.* Nucleic Acids Res. 1998 Sept; 26(17): 3961–3966.

Also Available:

Product Name	Size	Catalog number
CpG Methylase	200U 400U	E2010 E2011
EZ DNA Methylation™ Kit	50 200 2 x 96 2 x 96	D5001 D5002 D5003 D5004
EZ DNA Methylation-Gold™ Kit	50 200 2 x 96 2 x 96	D5005 D5006 D5007 D5008
EZ DNA Methylation-Direct™ Kit	50 200 2 x 96 2 x 96	D5020 D5021 D5022 D5023
EZ DNA Methylation-Startup™ Kit	1 Kit	D5024
EZ Bisulfite DNA Clean-up Kit™	50 200 2 x 96 2 x 96	D5025 D5026 D5027 D5028
Universal Methylated DNA Standard	1 set	D5010
Universal Methylated Human DNA Standard	1 set	D5011
Universal Methylated Mouse DNA Standard	1 set	D5012
Human HCT116 DKO Methylation Standards	1 set	D5014
Human HCT116 DKO Non-methylated DNA Standard	5 µg	D5014-1
Human HCT116 DKO Methylated DNA Standard	5 µg	D5014-2
Bisulfite Converted Universal Methylated Human DNA Standard	1 set	D5015
E. coli Non-methylated Genomic DNA	5 µg	D5016
Methylated-DNA IP Kit	10	D5101
ChIP DNA Clean & Concentrator™	50 50	D5201 D5205
Anti-5-Methylcytosine Monoclonal Antibody (clone 10G4)	50 µg 200 µg	A3001-50 A3001-200
Zymo*Taq*™ DNA Polymerase	50 200	E2001 E2002
Zymo*Taq*™ PreMix (2X concentrated)	50 200	E2003 E2004

Trademarks and Disclaimers:

This product is for research use only and should only be used by trained professionals. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility. Products are protected by U.S. Patent Nos. 7,034,116 6,492,168.

Version 1.0.0

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