

HpaII Methyltransferase



1-800-632-7799
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M0214S 017121013101

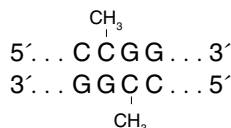
M0214S



100 units 4,000 U/ml Lot: 0171210

RECOMBINANT Store at -20°C Exp: 10/13

Methylation Site:



Description: HpaII Methyltransferase recognizes the same sequence as the MspI Methyltransferase, but modifies the internal cytosine residue (C⁵) in the sequence above.

Source: An *E. coli* strain that carries the cloned HpaII modification gene from *Haemophilus parainfluenzae* (ATCC 49669).

Supplied in: 100 mM NaCl, 50 mM Tris-HCl (pH 7.5), 10 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:

10X HpaII Methyltransferase Reaction Buffer, 400X S-adenosylmethionine (32 mM).

Reaction Conditions: 1X HpaII Methyltransferase Reaction Buffer, supplemented with 80 µM S-adenosylmethionine (supplied). Incubate at 37°C.

1X HpaII Methyltransferase Reaction Buffer:

50 mM Tris-HCl
10 mM EDTA
5 mM βME
pH 7.5 @ 25°C

Protection Assay Conditions: HpaII Methyltransferase is incubated with 1 µg of λ DNA in 10 µl 1X HpaII Methyltransferase Buffer, supplemented with 80 µM S-adenosylmethionine, for one hour at 37°C followed by 15 minutes at 65°C.

The extent of protection by HpaII Methyltransferase is determined by the addition of 40 µl NEBuffer 1 supplemented with 10 mM MgCl₂ and 10 units of HpaII restriction endonuclease. Incubation at 37°C for 30 minutes is followed by analysis on agarose gels.

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by HpaII restriction endonuclease.

Quality Control Assays

16-Hour Incubation: Incubation of 200 units of HpaII Methyltransferase with 1 µg of λ DNA in 50 µl 1X NEBuffer 2 for 16 hours at 37°C resulted in no detectable endonuclease contamination.

Exonuclease Activity: Incubation of 150 units of HpaII Methyltransferase 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 DTT] released < 0.1% of the total radioactivity.

Other Methylase Activity: Incubation of 100 units of HpaII Methyltransferase with 1 µg λ DNA for 4 hours under standard Protection Assay Conditions did not protect the DNA from digestion by HpaI or MboI endonuclease.

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

Reference:

1. Hoffman, J.L. (1986) *Biochemistry* 25, 4444-4449.

Companion Product:

S-adenosylmethionine (SAM)
#B9003S 0.5 ml

CERTIFICATE OF ANALYSIS

HpaII Methyltransferase



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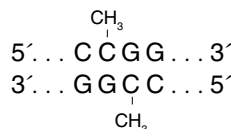
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#B9003S 0.5 ml

CERTIFICATE OF ANALYSIS