HhaI Methyltransferase

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

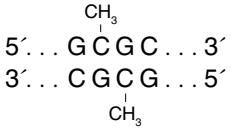
M0217S 006130114011

M0217S

RR SAM

1,000 units **25,000 U/ml** **Lot: 0061301**
RECOMBINANT **Store at –20°C** **Exp: 1/14**

Methylation Site:



Description: HhaI Methyltransferase modifies the internal cytosine residue (C^5) in the sequence above.

Source: An *E. coli* strain that carries the cloned HhaI modification gene from *Haemophilus haemolyticus* (ATCC 10014)

Supplied in: 150 mM NaCl, 50 mM Tris-HCl (pH 7.5), 10 mM EDTA, 5 mM 2-mercapto-ethanol, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X HhaI Methyltransferase Reaction Buffer, 400X S-adenosylmethionine (32 mM).

Reaction Conditions: 1X HhaI Methyltransferase Reaction Buffer, 80 µM S-adenosylmethionine. Incubate at 37°C.

1X HhaI Methyltransferase Reaction Buffer:
50 mM Tris-HCl
10 mM EDTA
5 mM 2-mercaptoethanol
pH 7.5 @ 25°C

Protection Assay Conditions: HhaI Methyltransferase is incubated with 1 µg of λ DNA in 10 µl 1X HhaI Methyltransferase Reaction 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 HhaI Methyltransferase is determined by the addition of 40 µl NEBuffer 4 supplemented with 100 µg/ml BSA, 10 mM $MgCl_2$ and 10 units of Hha I restriction endonuclease. Incubation at 37°C for 30 minutes is followed by analysis on an agarose gel.

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 Hha I restriction endonuclease.

Quality Control Assays

16-Hour Incubation: Incubation of 125 units of HhaI Methyltransferase with 1 µg of HindIII-digested DNA in 50 µl 1X NEBuffer 2 for 16 hours at 37°C resulted in no detectable endonuclease contamination.

Exonuclease Activity: Incubation of 250 units of HhaI Methyltransferase with 1 µg sonicated ^3H DNA (10^5 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 $MgCl_2$, 1 mM DTT] released 0.39% of the total radioactivity.

Storage of SAM: S-adenosylmethionine or 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

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M0217S
RR SAM
1,000 units 25,000 U/ml Lot: 0061301
RECOMBINANT Store at –20°C Exp: 1/14
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5′. . . G C G C . . . 3′
3′. . . C G C G . . . 5′
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