

HaeIII Methyltransferase

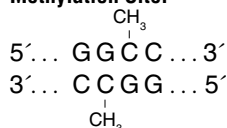


M0224S



500 units 10,000 U/ml Lot: 0061312
RECOMBINANT Store at -20°C Exp: 12/14

Methylation Site:



Description: HaeIII Methyltransferase modifies the internal cytosine residue (C⁵) in the sequence above.

Source: An *E. coli* strain that carries the cloned HaeIII modification gene from *Haemophilus aegyptius* (ATCC 11116)

Supplied in: 50 mM KCl, 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 HaeIII Methyltransferase Reaction Buffer, 400X S-adenosylmethionine (32 mM).

Reaction Conditions:

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

1X HaeIII Methyltransferase Reaction Buffer:

50 mM NaCl
50 mM Tris-HCl
10 mM dithiothreitol
pH 8.5 @ 25°C

Protection Assay Conditions:

HaeIII Methyltransferase is incubated with 1 µg of λ DNA in 10 µl of 1X HaeIII 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 is determined by the addition of 40 µl NEBuffer 2 and 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 λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by HaeIII restriction endonuclease.

Quality Control Assays

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

Exonuclease Activity: Incubation of 100 units of HaeIII 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 dithiothreitol] released < 0.05% of the total radioactivity.

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Endonuclease Contamination: Incubation of 100 units with 1 µg φX174 RF I DNA (4 hours, 37°C in 50 µl of NEBuffer 2) gave < 5% conversion to RF II.

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.

Note: HaeIII Methyltransferase protects DNA against cleavage by NotI.

Reference:

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

Companion Product:

S-adenosylmethionine (SAM)
#B9003S 0.5 ml

CERTIFICATE OF ANALYSIS

HaeIII Methyltransferase

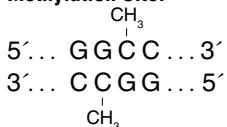


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