BamHI Methyltransferase

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

M0223S 008151016101

M0223S

RR SAM

100 units **4,000 U/ml** **Lot: 0081510**
RECOMBINANT **Store at –20°C** **Exp: 10/16**

Methylation Site:

5′. . . G G A T $\overset{CH_3}{C}$ C . . . 3′
3′. . . C C T A G G . . . 5′
CH_3

Description: BamHI Methyltransferase modifies the internal cytosine residue (N^4) in the sequence above.

Source: An *E. coli* strain that carries the cloned BamHI modification gene from *Bacillus amyloliquefaciens* H (ATCC 49763)

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

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

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

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

Protection Assay Conditions: BamHI Methyltransferase is incubated with 1 µg λ DNA in 10 µl 1X BamHI 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 BamHI Methyltransferase is determined by the addition of 40 µl NEBuffer 1 supplemented with 10 mM $MgCl_2$ and 10 units of BamHI 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 BamHI restriction endonuclease.

Quality Control Assays

Exonuclease Activity: Incubation of 30 units of BamHI 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 < 0.3% of the total radioactivity.

Endonuclease Activity: Incubation of 15 units with 1 µg of λ HindIII DNA fragments in a 50 µl NEBuffer 2 for 16 hours at 37°C resulted in no degradation of the DNA as determined by visualization of clear and sharp bands following gel electrophoresis.

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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3′. . . C C T A G G . . . 5′
CH3
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Reaction Conditions: 1X BamHI Methyltransferase Reaction Buffer, 80 µM S-adenosylmethionine. Incubate at 37°C.
1X BamHI Methyltransferase Reaction Buffer:
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10 mM EDTA
5 mM DTT
pH 7.5 @ 25°C
Protection Assay Conditions: BamHI Methyltransferase is incubated with 1 µg λ DNA in 10 µl 1X BamHI 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 BamHI Methyltransferase is determined by the addition of 40 µl NEBuffer 1 supplemented with 10 mM MgCl2 and 10 units of BamHI 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 BamHI restriction endonuclease.
Quality Control Assays
Exonuclease Activity: Incubation of 30 units of BamHI 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 MgCl2, 1 mM DTT] released < 0.3% of the total radioactivity.
Endonuclease Activity: Incubation of 15 units with 1 µg of λ HindIII DNA fragments in a 50 µl NEBuffer 2 for 16 hours at 37°C resulted in no degradation of the DNA as determined by visualization of clear and sharp bands following gel electrophoresis.
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