MspI Methyltransferase

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

M0215S 006120714071

M0215S

RRR SAM

100 units **5,000 U/ml** **Lot: 0061207**
RECOMBINANT **Store at –20°C** **Exp: 7/14**

Methylation Site:

5′. . . C^{CH_3}CGG . . . 3′
3′. . . GGCC^{CH_3} . . . 5′

Description: MspI Methyltransferase recognizes the same sequence as the HpaII Methyltransferase, but modifies the external cytosine residue (C^5) in the sequence above.

Source: An *E. coli* strain that carries the cloned MspI modification gene from *Moraxella* species (ATCC 49670)

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

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

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

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

Protection Assay Conditions: MspI Methyltransferase is incubated with 1 µg of λ DNA in 10 µl 1X MspI 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 MspI Methyltransferase is determined by the addition of 40 µl NEBuffer 2 supplemented with 10 mM $MgCl_2$ and 5 units of MspI restriction endonuclease. Incubation for MspI at 37°C 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 MspI restriction endonuclease.

Quality Control Assays

16-Hour Incubation: Incubation of 10 units of MspI Methyltransferase 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 10 units of MspI Methyltransferase with 1 µg sonicated [^{3}H] 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 < 0.05% of the total radioactivity.

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

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