dam Methytransferase

NEW ENGLAND BioLabs Inc.
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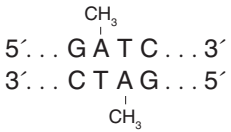
M0222S 015141116111

M0222S

RR SAM

500 units **8,000 U/ml** **Lot: 0151411**
RECOMBINANT **Store at –20°C** **Exp: 11/16**

Methylation Site:



Description: *dam* Methytransferase modifies the adenine residue (N^6) in the sequence above.

Source: An *E. coli* strain that carries plasmid pTP166 carrying the *dam* modification gene of *E. coli* (M. Marinus).

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 *dam* Methytransferase Reaction Buffer, 400X S-adenosylmethionine (32 mM).

Reaction Conditions: 1X *dam* Methytransferase Reaction Buffer, 80 µM S-adenosylmethionine. Incubate at 37°C.

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

Protection Assay Conditions: *dam* Methytransferase is incubated with 1 µg of λ DNA in 10 µl of 1X *dam* Methytransferase Reaction Buffer, supplemented with 80 µM S-adenosylmethionine, for 1 hour at 37°C followed by 15 minutes at 65°C. The extent of protection is determined by addition of 40 µl 1X NEBuffer 3 supplemented with 10 mM $MgCl_2$ and 10 units of MboI restriction endonuclease. Incubation at 37°C for 1 hour is followed by analysis on an agarose gel.

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

Quality Control Assays

16-Hour Incubation: Incubation of 60 units of *dam* Methytransferase with 1 µg of HindIII-digested λ DNA in 50 µl 1X NEBuffer 2 for 16 hours at 37°C resulted in no detectable contamination.

Exonuclease Activity: Incubation of 120 units of *dam* Methytransferase 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 < 0.1% of the total radioactivity.

Storage of SAM: S-adenosylmethionine (Sigma Catalog #A7007) 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.

CERTIFICATE OF ANALYSIS

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5′. . . G A T C . . . 3′
3′. . . C T A G . . . 5′

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