

dam
Methytransferase



1-800-632-7799
info@neb.com
www.neb.com



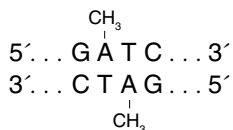
M0222S 014120614061

M0222S

RRR SAM

500 units 8,000 U/ml Lot: 0141206
RECOMBINANT Store at -20°C Exp: 6/14

Methylation Site:



Description: *dam* Methytransferase modifies the adenine residue (N⁶) in the sequence above.

dam
Methytransferase



1-800-632-7799
info@neb.com
www.neb.com



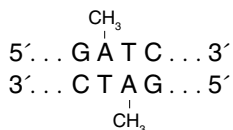
M0222S 014120614061

M0222S

RRR SAM

500 units 8,000 U/ml Lot: 0141206
RECOMBINANT Store at -20°C Exp: 6/14

Methylation Site:



Description: *dam* Methytransferase modifies the adenine residue (N⁶) 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

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 MgCl₂ 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.

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 MgCl₂ 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 50 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 100 units of *dam* Methytransferase 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 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:

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

CERTIFICATE OF ANALYSIS

Quality Control Assays

16-Hour Incubation: Incubation of 50 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 100 units of *dam* Methytransferase 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 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:

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

CERTIFICATE OF ANALYSIS