**Methylation Site:**

\[
\begin{align*}
\text{CH}_3 & \quad 5' \ldots \text{G} \text{A} \text{T} \text{C} \ldots 3' \\
\text{CH}_3 & \quad 3' \ldots \text{C} \text{T} \text{A} \text{G} \ldots 5'
\end{align*}
\]

**Description:** 
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).

**Supply:** 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.

**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 μl 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.

**Exonuclease Activity:** Incubation of 100 units of dam Methytransferase with 1 μg sonicated ³²H DNA (10⁶ 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:**