

AluI Methyltransferase



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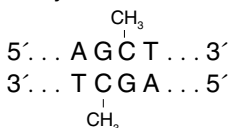
M0220S 007120414041

M0220S



100 units **5,000 U/ml** **Lot: 0071204**
RECOMBINANT **Store at -20°C** **Exp: 4/14**

Methylation Site:



Description: AluI Methyltransferase modifies the cytosine residue (C⁵) in the sequence above.

Source: An *E. coli* strain that carries the cloned AluI modification gene from *Arthrobacter luteus* (ATCC 21606).

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:

10X AluI Methyltransferase Reaction Buffer,
400X S-adenosylmethionine (32 mM).

Reaction Conditions: 1X AluI Methyltransferase Reaction Buffer, 80 µM S-adenosylmethionine.

1X AluI Methyltransferase Reaction Buffer:

50 mM Tris-HCl
10 mM EDTA
5 mM 2-mercaptoethanol
pH 7.5 @ 25°C

Protection Assay Conditions: AluI Methyltransferase is incubated with 1 µg of λ DNA in 10 µl 1X AluI 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 AluI Methyltransferase is determined by the addition of 40 µl NEBuffer 1 supplemented with 10 mM MgCl₂ and 10 units of AluI restriction endonuclease. Incubation at 37°C for 30 minutes 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 AluI restriction endonuclease.

Quality Control Assays

16-Hour Incubation: Incubation of 40 units of Methyltransferase with 1 µg λ DNA in 50 µl 1X NEBuffer 2 at 37°C resulted in no detectable endonuclease contamination.

Exonuclease Activity: Incubation of 40 units of AluI Methyltransferase 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 dithiothreitol] released 0.2% of the total radioactivity.

Storage of SAM: S-adenosylmethionine 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.

Companion Product:

S-adenosylmethionine (SAM)
#B9003S 0.5 ml

CERTIFICATE OF ANALYSIS

AluI Methyltransferase



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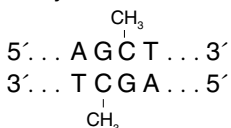
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