EcoRI Methyltransferase

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

M0211S 011120714071

M0211S

RR SAM

10,000 units 40,000 U/ml Lot: 0111207
RECOMBINANT Store at –20°C Exp: 7/14

Methylation Site:

5′. . . G A A^{CH_3} T T C . . . 3′
3′. . . C T T A A G . . . 5′

Description: EcoRI Methyltransferase modifies the internal adenine residue (N^6) in the sequence above.

Source: An *E. coli* strain that carries the cloned EcoRI modification gene from *Escherichia coli* RY13 (R.N. Yoshimori)

Supplied in: 200 mM NaCl, 100 mM KPO_4 (pH 7.4), 0.1 mM EDTA, 10 mM 2–mercaptoethanol, 200 µg/ml BSA, and 50% glycerol.

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

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

1X EcoRI Methyltransferase Reaction Buffer:
50 mM NaCl
50 mM Tris-HCl
10 mM EDTA
pH 8.0 @ 25°C

Protection Assay Conditions: EcoRI Methyltransferase is incubated with 1 µg of λ DNA in 10 µl 1X EcoRI 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 EcoRI Methyltransferase is determined by the addition of 40 µl NEBuffer 2 and 5 units of EcoRI 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 EcoRI restriction endonuclease.

Quality Control Assays

16-Hour Incubation: A 50 µl reaction containing 1 µg of HindIII digested λ DNA and 1,500 units of EcoRI Methyltransferase incubated for 16 hours at 37°C in NEBuffer 2 resulted in no detectable degradation.

Exonuclease Activity: Incubation of 4,000 units of EcoRI 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 0.3% 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.

Note: EcoRI Methyltransferase is inhibited by $MgCl_2$.

Only 50% activity is retained at a concentration of 4 mM $MgCl_2$.

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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