MmeI

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

R0637S 004120713071

R0637S

NEB4 SAM 37° Yes

100 units **2,000 U/ml** **Lot: 0041207**

RECOMBINANT **Store at –20°C** **Exp: 7/13**

Recognition Site:

5′...TCCRAC(N)$_{20}$▼...3′
3′...AGGYTG(N)$_{18}$▲...5′

Single Letter Code: R = A or G, Y = C or T

Source: An *E. coli* strain that carries the cloned MmeI gene from *Methylophilus methylotrophus*

Supplied in: 300 mM NaCl, 10 mM Tris-HCl (pH 7.4 @ 25°C), 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 4, 32 mM S-adenosylmethionine (SAM).

Reaction Conditions: 1X NEBuffer 4, supplemented with 50 µM S-adenosylmethionine (supplied). Incubate at 37°C.

1X NEBuffer 4:
50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
1 mM dithiothreitol
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of ϕX174 RF I DNA in 1 hour at 37°C in 50 µl of reaction buffer.

Diluent Compatibility: Diluent Buffer B
300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Quality Control Assays

Ligation: After digestion (no overdigestion) with MmeI, > 95% of the DNA fragments can be ligated with concentrated T4 DNA Ligase (at a 5′ termini concentration of 1–2 µM) at 16°C. Of these ligated fragments, none can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 2 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme.

Exonuclease Activity: Incubation of 100 units of enzyme with 1 µg sonicated ^{3}H DNA (10^5 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	NR
NEBuffer 2	NR
NEBuffer 3	NR
NEBuffer 4	**100%**

NEBuffers 1, 2 and 3 are **not** recommended (NR) due to star activity.

When using a buffer other than the optimal (supplied) NEBuffer, complete cleavage cannot be obtained.

Survival in a Reaction: A minimum of 1.0 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 2 units of enzyme were inactivated by incubation at 80°C for 20 minutes.

Notes On Use: Excess MmeI blocks cleavage. Reactions using MmeI should be done at or near stoichiometric concentrations.

Complete cleavage occurs within 15 minutes at 37°C. Significant cleavage occurs on ice and at 50°C.

Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation.

MmeI activity is inhibited by high ionic strength (> 200 mM).

Requires S-adenosylmethionine for optimal activity.

Potassium is necessary for optimal cleavage efficiency.

= Time-Saver™ Qualified (See www.neb.com for details).

U.S. Patent No. 7,115,407

CERTIFICATE OF ANALYSIS

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