NmeAIII

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

R0711S 002121214121

R0711S

NEB4 SAM 37° Yes

250 units **2,000 U/ml** **Lot: 0021212**

RECOMBINANT Store at –20°C Exp: 12/14

Recognition Site:

5′... GCCGAG $(N)_{20\text{-}21}$▼...3′
3′... CGGCTC $(N)_{18\text{-}19}$▲...5′

Source: An *E.coli* strain that carries the cloned NmeAIII gene from *Neisseria meningitidis* 2491 (Achtman, M.)

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

Reagents Supplied with Enzyme:
10X NEBuffer 4, 400X S-adenosylmethionine (32 mM).

Reaction Conditions: 1X NEBuffer 4, 80 µM S-adenosylmethionine. 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 a total reaction volume of 50 µl.

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 a 5-fold overdigestion with NmeAIII, > 95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5′ termini concentration of 1–2 µM) at 16°C. Of these ligated fragments, < 5% can be recut.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	10%
NEBuffer 2	5%
NEBuffer 3	0%
NEBuffer 4	**100%**

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

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

Heat Inactivation: 80°C for 20 minutes.

Notes: Overdigestion with > 5 units of NmeAIII per µg of DNA and incubations > 1 hour are not recommended.

Requires S-adenosylmethionine for optimal activity (supplied with enzyme)

NmeAIII requires two copies of its recognition sequence for cleavage to occur. Thus, the single NmeAIII site in pUC19 is resistant to cleavage. A 10-fold overdigestion cuts less than half of the DNA.

The cleavage point may shift one base pair depending on the DNA sequence context between the recognition site and the position of cleavage. For a given sequence, generally one cut site will predominate. For details, see www.neb.com.

Significant cleavage occurs on ice and at 25°C.

* NmeAIII produces a stable partial digestion pattern even with excess enzyme. 1 unit is defined as the amount of enzyme required to produce this stable partial digestion pattern.

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

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U.S. Patent Pending

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