NaeI

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R0190S 058121214121

R0190S

500 units **10,000 U/ml** **Lot: 0581212**
RECOMBINANT **Store at –20°C** **Exp: 12/14**

Recognition Site:

5′. . . G C C▼G G C . . . 3′
3′. . . C G G▲C C G . . . 5′

Source: An *E. coli* strain that carries the cloned NaeI gene from *Nocardia aerocolonigenes* (ATCC 23870)

New Reaction Buffer

Supplied in: 50 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 4.

Reaction Conditions: 1X NEBuffer 4. 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 pXba DNA in 1 hour at 37°C in a total reaction volume of 50 µl.

Diluent Compatibility: Diluent Buffer A
50 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

Quality Control Assays

Ligation: After 10-fold overdigestion with NaeI, approximately 75% 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, > 95% can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 100 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 50 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 < 1.0% radioactivity.

Endonuclease Activity: Incubation of 50 units of enzyme with 1 µg ϕX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 5% conversion to RF II.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	100%
NEBuffer 2	75%
NEBuffer 3	10%
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 0.50 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 40 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Notes: Demonstrates marked site preferences and cuts pBR322 DNA very slowly. NgoMIV, an isoschizomer of NaeI, exhibits less site preference.

Cleavage of mammalian genomic DNA is blocked by CpG methylation.

U.S. Patent No. 5,292,651

CERTIFICATE OF ANALYSIS

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Enzyme Properties
Activity in NEBuffers:
NEBuffer 1 100%
NEBuffer 2 75%
NEBuffer 3 10%
NEBuffer 4 100%
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