

NlaIII



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



R0125S 063121113111

R0125S



500 units 10,000 U/ml Lot: 0631211

RECOMBINANT Store at -70°C Exp: 11/13

Recognition Site:

5'...CATG...3'
3'...GTAC...5'

Source: An *E. coli* strain that carries the cloned NlaIII gene from *Neisseria lactamica* (NRCC 2118)

Supplied in: 200 mM KCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:

10X NEBuffer 4, 100X BSA.

Reaction Conditions:

1X NEBuffer 4, supplemented with 100 µg/ml BSA. Incubate at 37°C.

1X NEBuffer 4:

50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
1 mM DTT
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 15-fold overdigestion with NlaIII, > 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, > 95% can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 75 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 200 units of enzyme with 1 µg sonicated ³H DNA (10⁵ cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.15% radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	25%
NEBuffer 2	25%
NEBuffer 3	25%
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: 10 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Notes: Cleaves to leave a 3' CATG extension which can be ligated to DNA fragments generated by SphI.

Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Incubations longer than 3 hours are not recommended. NaCl and KCl inhibit activity, (NH₄)₂SO₄ does not.

For long term storage, store at -70°C. The half life of NlaIII is 6 months at -70°C.

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

CERTIFICATE OF ANALYSIS

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