NarI

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

R0191S 058121214121

R0191S

500 units **Lot: 0581212** **Exp: 12/14**
5,000 U/ml **Store at –20°C**

Recognition Site:

5′... GG▼CGCC...3′
3′... CCGC▲GG...5′

Source: An *E. coli* strain that carries the cloned NarI gene from *Norcardia argentinensis* (ATCC 31306)

More Units
New Reaction Buffer

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 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 dithiothreitol, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Quality Control Assays

Ligation: After 10-fold overdigestion with NarI, > 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 pXba DNA and 50 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 < 1.0% radioactivity.

Endonuclease Activity: Incubation of 50 units of enzyme with 1 µg Litmus 28i 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	75%
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: 200 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Plasmid Cleavage: Number of units required to cleave 1 µg of supercoiled plasmid DNA in one hour: pBR322 = 4 units pUC 19 = 20 units.

Notes: NarI is an isoschizomer of KasI. NarI produces a 2-base 5′ extension whereas KasI produces a 4-base 5′ extension. Demonstrates marked site preferences. On pBR322 the NarI site at 548 bp is cut very slowly.

Cleavage of mamalian genomic DNA is blocked by CpG methylation.

CERTIFICATE OF ANALYSIS

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