NciI

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

R0196S 015120914091

R0196S

2,000 units **20,000 U/ml** **Lot: 0151209**
RECOMBINANT **Store at –20°C** **Exp: 9/14**

Recognition Site:
5′... C C▼S G G ...3′
3′... G G S▲C C ...5′

Single Letter Code: S = C or G

Source: An *E. coli* strain that carries the cloned NciI gene from *Neisseria cinerea* (NRCC 31006)

Supplied in: 50 mM KCl, 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 DTT
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of λ 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 NciI, approximately 50% 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 20 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 ^{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	100%
NEBuffer 2	25%
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: No

Notes: NciI produces DNA fragments that have a single-base 5′ extension which are more difficult to ligate than blunt-ended fragments.

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

Conditions of high enzyme concentration, glycerol concentration > 5% or pH > 8.0 may result in star activity.

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

CERTIFICATE OF ANALYSIS

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