NotI

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

R0189S 052120814081

R0189S

NEB3 BSA 37° Yes

500 units **10,000 U/ml** **Lot: 0521208**
RECOMBINANT **Store at –20°C** **Exp: 8/14**

Recognition Site:

5′. . . GC▼GGCCGC . . . 3′
3′. . . CGCCGG▲CG . . . 5′

Source: An *E. coli* strain that carries the cloned NotI gene from *Nocardia otitidis-caviarum* (ATCC 14630)

Also Available In High Fidelity (HF™) Format

Supplied in: 200 mM NaCl, 20 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 0.15% Triton X-100, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 3, 100X BSA.

Reaction Conditions: 1X NEBuffer 3, supplemented with 100 µg/ml BSA. Incubate at 37°C.

1X NEBuffer 3:
100 mM NaCl
50 mM Tris-HCl
10 mM $MgCl_2$
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 pBC4 DNA in 1 hour at 37°C in a total reaction volume of 50 µl.

Diluent Compatibility: Diluent Buffer C
250 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 0.15% Triton X-100, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Quality Control Assays

Ligation: After 10-fold overdigestion with NotI, > 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 200 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 (10^5 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity

Endonuclease Activity: Incubation of 200 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	0%
NEBuffer 2	50%
NEBuffer 3	**100%**
NEBuffer 4	25%

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 0.25 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 50 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Note: Cleavage of mammalian genomic DNA is blocked by CpG methylation.

Supercoiled plasmids may require up to 5-fold more NotI for complete digestion than linear DNAs.

(see other side)

CERTIFICATE OF ANALYSIS

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(see other side)

CERTIFICATE OF ANALYSIS

Companion Products:

NotI-HF™
#R3189S 500 units
#R3189L 2,500 units
#R3189M 2,500 units

NotI-HF™ RE-Mix™
#R5189S 25 reactions

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

U.S. Patent No. 5,371,006

Companion Products:

NotI-HF™
#R3189S 500 units
#R3189L 2,500 units
#R3189M 2,500 units

NotI-HF™ RE-Mix™
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