NotI-HF™

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

R3189S 003120814081

R3189S

500 units	**20,000 U/ml**	**Lot: 0031208**
RECOMBINANT	**Store at –20°C**	**Exp: 8/14**

Recognition Site:

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

Note: NotI-HF™ has the same specificity as NotI (NEB #R0189), but it has been engineered for reduced star activity.

Source: An *E. coli* strain that carries the cloned and modified (K150A) NotI gene from *Nocardia otitidis-caviarum* (ATCC 14630)

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

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 pBC4 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 Controls

Ligation: After 10-fold overdigestion with NotI-HF, > 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 NotI-HF incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of NotI-HF.

Exonuclease Activity: Incubation of 200 units of NotI-HF 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.

Endonuclease Activity: Incubation of 200 units of NotI-HF 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	25%
NEBuffer 2	100%
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 0.13 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.

(see other side)

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

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

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

Companion Products:

NotI
#R0189S 500 units
#R0189L 2,500 units
#R0189M 2,500 units

NotI-HF™ RE-Mix™
#R5189S 25 reactions

New icons (see www.neb.com for details)
= Time-Saver™ Qualified
= indicates that the enzyme has been engineered
= indicates that the enzyme has reduced star activity

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

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