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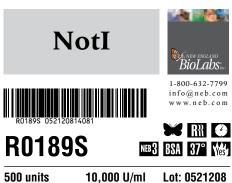
500 units 10,000 U/ml Lot: 0521208 RECOMBINANT Store at -20°C Exp: 8/14

Recognition Site:

5′...GC^{*}GGCCGC...3′ 3′...CGCCGGCG...5′

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

> Also Available In High Fidelity (HF™) Format



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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-HCI 10 mM MgCl 1 mM DTT pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 ug 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).

Supplied in: 200 mM NaCl, 20 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 0.15% Triton

Reaction Conditions: 1X NEBuffer 3, supplemented

100 mM NaCl 50 mM Tris-HCI 10 mM MgCl_a 1 mM DTT pH 7.9 @ 25°C

Diluent Compatibility: Diluent Buffer C

Quality Control Assays

Ligation: After 10-fold overdigestion with Notl. > 95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5' termini concentration of 1-2 μ M) at 16°C. Of these ligated fragments, > 95% can be recut.

16-Hour Incubation: A 50 ul 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 μ g sonicated ³H DNA (10⁵ cpm/ μ g) 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 ug oX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 5%conversion to RF II.

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Endonuclease Activity: Incubation of 200 units

at 37°C in 50 µl reaction buffer resulted in < 5%

of enzyme with 1 ug ϕ X174 RF I DNA for 4 hours

for 4 hours at 37°C in 50 µl reaction buffer released

bands as a reaction incubated for 1 hour with 1 unit

Quality Control Assays

be recut.

of enzyme.

< 0.1% radioactivity

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 Notl for complete digestion than linear DNAs.

(see other side)

CERTIFICATE OF ANALYSIS

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1X NEBuffer 3:

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

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

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 #R3189S
 500 units

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 2,500 units

 #R3189M
 2,500 units

NotI-HF[™] RE-Mix[™] #R5189S 25 reactions

Image: Barrier And Antiparties (See www.neb.com for details).

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