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BioLabs

10



1,000 U/ml 200 units Lot: 0141210

RECOMBINANT Store at -20°C Exp: 10/14

Recognition Site:

5′... GGN^VNCC...3′ 3′... C C N N G G ... 5′

Source: An E. coli strain that carries the cloned NIaIV gene from *Neisseria lactamica* (NRCC 2118)

Supplied in: 200 mM KCI, 10 mM Tris-HCI (pH 7.5), 0.1 mM EDTA, 1 mM dithiothreitol, 500 µg/ml BSA and 50% glycerol.



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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 dithiothreitol pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 ug of pBR322 DNA in 1 hour at 37°C in a total reaction volume of 50 µl.

Diluent Compatibility: Diluent Buffer B 300 mM NaCl. 10 mM Tris-HCl. 0.1 mM EDTA. 1 mM dithiothreitol, 500 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

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Quality Control Assays

Ligation: After 10-fold overdigestion with NIalV. > 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 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 ug sonicated [3H 1DNA (105 cpm/ µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

Heat Inactivation: 10 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

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Enzyme Properties

Activity in NEBuffers: NEBuffer 1 10%

NEBuffer 2 10% NFBuffer 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.

Notes: Blocked by overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation.

NaCl and KCl inhibit activity.

Companion Products:

dam-/dcm- Competent E. coli #C2925H 20 transformation reactions #C2925 24 transformation reactions

CERTIFICATE OF ANALYSIS

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1X NEBuffer 4: 50 mM potassium acetate 20 mM Tris-acetate 10 mM magnesium acetate

1 mM dithiothreitol pH 7.9 @ 25°C

amount of enzyme required to digest 1 µg of pBR322 DNA in 1 hour at 37°C in a total reaction volume of 50 µl.