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BioLabs

1-800-632-7799

info@neb.com

www.neb.com

2,000 units Lot: 0221208 Exp: 8/14 RECOMBINANT 10,000 U/ml Store at -20°C

Recognition Site:

 $\begin{array}{c} 5^{\prime}...\,G \mathrel{\mathsf{R}} G \mathrel{\mathsf{C}} Y^{{}^{\bullet}}\!C \:...\,3^{\prime} \\ 3^{\prime}...\,C_{{}^{\bullet}}\!Y \mathrel{\mathsf{C}} G \mathrel{\mathsf{R}} G \:...\,5^{\prime} \end{array}$

Single Letter Code: R = A or G, Y = C or T

Source: An *E. coli* strain that carries the cloned BanII gene from *Bacillus aneurinolyticus* (IAM 1077)

Now Recombinant

Supplied in: 50 mM NaCl, 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 μ g of λ DNA in 1 hour at 37°C in a total reaction volume of 50 μ l.

Diluent Compatibility: Diluent Buffer A 50 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 200 μ g/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

<u>Quality Control Assays</u>

Ligation: After 10-fold overdigestion with BanII, > 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 μ I reaction containing 1 μ g of DNA and 50 units of enzyme incubated for 16 hours resulted in no degradation of the DNA bands due to nonspecific nucleases. However, fragments produced by noncanonical cleavage due to star activity may be observed with 10 units of enzyme in similar conditions.

Exonuclease Activity: Incubation of 50 units of enzyme with 1 μ g sonicated ³H DNA (10⁵ cpm/ μ g) for 4 hours at 37°C in 50 μ l reaction buffer released < 0.3% radioactivity.

Enzyme Properties

Activity in NEBuffers: NEBuffer 1 100%

NEBuffer 2 10% NEBuffer 3 50% 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: Intermediate activity. Suitable for extended digestion, but < 8 hours.

Heat Inactivation: 25 units of enzyme were inactivated by incubation at 80°C for 20 minutes.

Plasmid Cleavage: Number of units required to cleave 1 μ g of supercoiled plasmid DNA in one hour: 2 units.

Note: Not sensitive to *dam, dcm* or mammalian CpG methylation.

■ Time-Saver[™] Qualified (See www.neb.com for details).

CERTIFICATE OF ANALYSIS

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BanIIImage: BanII and the second secon

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