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Recognition Site:

5′... GGNCC...3′ 3′... CCNGG...5′

Source: An *E. coli* strain that carries the cloned Sau96I gene from *Staphylococcus aureus* PS96 (ATCC 49831)

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol.



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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 dithiothreitol 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 dithiothreitol, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

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Quality Control Assays

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> 95% can be recut.

1 unit of enzyme.

Ligation: After 10-fold overdigestion with Sau96I, > 95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5' termini concentration of $1-2 \ \mu$ 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 10 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 25 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 < 0.2% radioactivity.

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Enzyme Properties

Activity in NEBuffers:

 NEBuffer 1
 50%

 NEBuffer 2
 100%

 NEBuffer 3
 100%

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

Heat Inactivation: 80°C for 20 minutes.

Notes: Blocked by overlapping dcm methylation.

Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation.

Companion Products:

dam-/dcm- Competent E. coli#C2925H20 transformation reactions#C2925I24 transformation reactions

CERTIFICATE OF ANALYSIS

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