BstYI





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

R0523S



Recognition Site:

5'...RGATCY...3' 3'...YCTAGR...5'

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

Source: An *E. coli* strain that carries the cloned BstYl gene from *Bacillus stearothermophilus* Y406 (Z. Chen)

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

Reagents Supplied with Enzyme: 10X NFBuffer 2.

Reaction Conditions: 1X NEBuffer 2. Incubate at 60°C.

1X NEBuffer 2: 50 mM NaCl 10 mM Tris-HCl 10 mM MgCl₂ 1 mM dithiothreitol pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 μg of λ DNA in 1 hour at 60°C in a total reaction volume of 50 μ l.

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

Quality Control Assays

Ligation: After 100-fold overdigestion with BstYI, > 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 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 500 units of enzyme with 1 μ g sonicated ³H DNA (10⁵ cpm/ μ g) for 4 hours at 60°C in 50 μ l reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 10 units of enzyme with 1 μ g ϕ X174 RF I DNA for 4 hours at 60°C in 50 μ l reaction buffer resulted in < 10% conversion to RF II.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1 50% NEBuffer 2 **100%** NEBuffer 3 75% 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: 80°C for 20 minutes.

Notes: BstYl is an isoschizomer of Xholl.

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

Incubation at 37°C results in 30% activity.

= Time-Saver™ Qualified (See www.neb.com for details).

U.S. Patent No. 6,403,354

CERTIFICATE OF ANALYSIS

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