

1,000 units Lot: 0051209 Exp: 9/14 RECOMBINANT 10,000 U/ml Store at -20°C

Recognition Site:

5′....CCRYGG....3′ 3′....GGYRCC....5′

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

Source: An *E. coli* strain that carries the cloned Btgl gene from *Bacillus thermoglucosidasius* (X. Pan)

Now Recombinant, New Storage Conditions



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Supplied in: 300 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme: 10X NEBuffer 3, 100X BSA.

Reaction Conditions: 1X NEBuffer 3, supplemented with 100 μ g/ml BSA. Incubate at 37°C.

1X NEBuffer 3:

100 mM NaCl 50 mM Tris-HCl 10 mM MgCl₂ 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 pBR322 DNA in 1 hour at 37°C in a total reaction volume of 50 μ l.

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

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Quality Control Assays

Ligation: After 50-fold overdigestion with Btgl, > 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 64 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 125 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 < 0.01% radioactivity.

Enzyme Properties

Activity in NEBuffers: NEBuffer 1 25% NEBuffer 2 50% 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.

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Activity in NEBuffers:

 NEBuffer 1
 25%

 NEBuffer 2
 50%

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

Heat Inactivation: 80°C for 20 minutes.

Notes: Dsal is an isoschizomer of Btgl.

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

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

CERTIFICATE OF ANALYSIS

Survival in a Reaction: A minimum of 0.5 unit is required to digest 1 μ g of substrate DNA in 16 hours.

Heat Inactivation: 80°C for 20 minutes.

Notes: Dsal is an isoschizomer of Btgl.

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