BglI

NEW ENGLAND BioLabs Inc.
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R0143S 042121014101

R0143S

2,000 units **10,000 U/ml** **Lot: 0421210**
RECOMBINANT **Store at –20°C** **Exp: 10/14**

Recognition Site:

5′. . . GCCNNNN▼NGGC . . . 3′
3′. . . CGGN▲NNNNCCG . . . 5′

Source: An *E. coli* strain that carries the cloned BglI gene from *Bacillus globigii* (ATCC 49760)

Supplied in: 200 mM NaCl, 20 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1.0 mM DTT, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 3.

Reaction Conditions: 1X NEBuffer 3. Incubate at 37°C.

1X NEBuffer 3:
100 mM NaCl
50 mM Tris-HCl
10 mM $MgCl_2$
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 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).

Quality Control Assays

Ligation: After 50-fold overdigestion with BglI, > 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 200 units of BglI 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 400 units of enzyme with 1 µg sonicated [^{3}H] DNA (10^5 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.2% radioactivity.

Endonuclease Activity: Incubation of 40 units of enzyme with 1 µg of ϕX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 30% conversion to RF II.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	50%
NEBuffer 2	75%
NEBuffer 3	**100%**
NEBuffer 4	50%

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: Suitable for an extended or overnight digestion. Enzyme is active > 8 hours.

Heat Inactivation: 40 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Notes: The various sticky ends produced by BglI cleavage can be used to reconstitute plasmid and phage genomes and to exchange wild-type and mutant DNA fragments. (Burger, K.J. and Schinzel, R. (1983) *Mol. Gen.* 189, 269–274).

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

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

U.S. Patent N0. 5,366,882

CERTIFICATE OF ANALYSIS

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