

BfaI



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R0568S 016121014101

R0568S



500 units Lot: 0161210 Exp: 10/14
5,000 U/ml Store at -20°C (see below)

Recognition Site:

5'...CTAG...3'
3'...GATC...5'

Source: *Bacteroides fragilis* (VPI 3392)

**-70°C Storage Is Recommended For
Periods Greater Than 30 Days.
Avoid Freeze/Thaw Cycles.**

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Supplied in: 250 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 500 $\mu\text{g/ml}$ 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 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 $\mu\text{g/ml}$ BSA and
50% glycerol (pH 7.4 @ 25°C).

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Quality Control Assays

Ligation: After 5-fold overdigestion with BfaI, < 5% 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.

Note: This ligation efficiency was not increased by using a lower overdigestion. The relatively low ligation efficiency is due to the character of the overhang left by Bfa I and not due to any contaminant.

16-Hour Incubation: A 50 μl reaction containing 1 μg of DNA and 200 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 μg sonicated [^3H] DNA (10⁵ cpm/ μg) for 4 hours at 37°C in 50 μl reaction buffer released < 0.1% radioactivity.

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Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	75%
NEBuffer 2	50%
NEBuffer 3	10%
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: 50 units of enzyme were inactivated by incubation at 80°C for 20 minutes.

Notes: BfaI is an isoschizomer of Mael and Rmal.

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

BfaI has shown minimal cleavage of unpurified PCR products. Therefore, PCR products should be purified prior to Bfa I digestion in 1X NEBuffer 4.

Storage at -20°C is recommended for short periods.

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

CERTIFICATE OF ANALYSIS

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