





100 units 2.000 U/ml Lot: 0241206

RECOMBINANT Store at -20°C Exp: 6/14

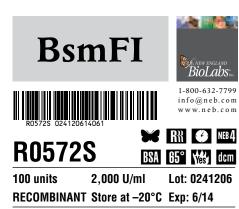
Recognition Site:

5[']... GGGAC(N)₁₀ \checkmark ... 3['] 3[']... CCCTG(N)₁₄ \land ... 5[']

Source: An *E. coli* strain that carries the cloned BsmFI gene from *Bacillus stearothermophilus* F (ER2683)

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

Now Recombinant



Recognition Site:

 $\begin{array}{c} 5^{\prime}\ldots \, G\,G\,G\,A\,C\,(\mathsf{N})_{10}^{\checkmark}\ldots \,3^{\prime}\\ 3^{\prime}\ldots \,C\,C\,C\,T\,G\,(\mathsf{N})_{14}^{}\ldots \,5^{\prime} \end{array}$

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Reagents Supplied with Enzyme: 10X NEBuffer 4, 100X BSA.

Reaction Conditions: 1X NEBuffer 4 supplemented with 100 µg/ml BSA. Incubate at 65°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 pBR322 DNA in 1 hour at 65°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 DTT, 200 μg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

Quality Control Assays

Ligation: After 5-fold overdigestion with BsmFI, > 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.

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16-Hour Incubation: A 50 µl reaction containing 1 µg of pBR322 DNA and 5 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 100 units of enzyme with 1 μ g sonicated ³H DNA (10⁵ cpm/ μ g) for 4 hours at 65°C in 50 μ I reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 10 units of enzyme with 1 μ g pUC19 plasmid DNA for 4 hours at 37°C in 50 μ l reaction buffer resulted in 10% conversion to RF II.

Enzyme Properties

Activity in NEBuffers

 NEBuffer 1
 25%

 NEBuffer 2
 50%

 NEBuffer 3
 50%

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

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Heat Inactivation: 80°C for 20 minutes.

Plasmid Cleavage: Number of units required to cleave 1 μ g of supercoiled plasmid DNA in one hour: 2 units.

Notes: BsmFI is an isoschizomer of Finl. Occasionally, BsmFI has been shown to cleave the sequence GGGAC(9/13). The exact frequency of this occurrence has yet to be determined.

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

Incubation at 37° results in 50% activity.

Incubation longer than 4 hours is not recommended.

Companion Products:

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

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

CERTIFICATE OF ANALYSIS

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