BsrFI

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

R0562S 0081211114111

R0562S

1,000 units **10,000 U/ml** **Lot: 0081211**
RECOMBINANT **Store at –20°C** **Exp: 11/14**

Recognition Site:

5′. . . R▼C C G G Y . . . 3′
3′. . . Y G G C C▲R . . . 5′

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

Source: An *E. coli* strain that carries the cloned BsrFI gene from *Bacillus stearothermophilus* CPW16 (Z. Chen)

More Units, New Reaction Buffer

Supplied in: 250 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 0.15% Triton X-100, 200 µg/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 pBR322 DNA in 1 hour at 37°C in a total reaction volume of 50 µl.

Diluent Compatibility: Diluent Buffer C
250 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 0.15% Triton X-100, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Quality Control Assays

Ligation: After 10-fold overdigestion with BsrFI, > 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 and10 units of enzyme incubated for 16 hours resulted in no degradation of the DNA bands due to nonspecific nucleases. However, fragments produced by noncanonical cleavage due to star activity may be observed with 1 unit of enzyme in similar conditions.

Exonuclease Activity: Incubation of 10 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 < 0.1% radioactivity.

Endonuclease Activity: Incubation of 15 units of enzyme with 1 µg ϕX174 RF I DNA for 4 hours at 37°C in a 50 µl reaction buffer resulted in a < 20% conversion to RF II.

Survival in a Reaction: A minimum of 0.13 unit is required to digest 1 µg of substrate DNA in 16 hours.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	10%
NEBuffer 2	100%
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.

Heat Inactivation: No

Notes: BsrFI is an isoschizomer of Cfr101. BsrFI can remain bound to DNA after cutting and alter migration rate of DNA during electrophoresis. To disrupt binding, add SDS to a final concentration of 0.5% or purity DNA before electrophoresis.

Cleavage of mammalian genomic DNA is blocked by CpG methylation.

Conditions of low ionic strength, high enzyme concentration, glycerol concentration > 5% or pH > 8.0 may result in star activity.

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

U.S. Patent No. 6,066,487

CERTIFICATE OF ANALYSIS

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