SfiI

NEW ENGLAND BioLabs Inc.
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R0123S 026120614061

R0123S

3,000 units **20,000 U/ml** **Lot: 0261206**
RECOMBINANT **Store at –20°C** **Exp: 6/14**

Recognition Site:

5′. . . GGCCNNNN▼NGGCC . . . 3′
3′. . . CCGGN▲NNNNCCGG . . . 5′

Source: An *E. coli* strain that carries the cloned SfiI gene from *Streptomyces fimbriatus* (ATCC 15051)

New Reaction Buffer

Supplied in: 300 mM NaCl, 20 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 5 mM 2-mercaptoethanol, 0.15% Triton X-100, 200 µg/ml BSA and 50% glycerol.

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

Reaction Conditions: 1X NEBuffer 4, supplemented with 100 µg/ml BSA.
Incubate at 50°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 Adenovirus-2 DNA in 1 hour at 50°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 DTT, 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 SfiI, > 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 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 200 units of enzyme with 1 µg sonicated ^{3}H DNA (10^{5} cpm/µg) for 4 hours at 50°C in 50 µl reaction buffer released < 0.05% radioactivity.

Endonuclease Activity: Incubation of 100 units of enzyme with 1 µg ϕX174 RF I DNA for 4 hours at 50°C in 50 µl reaction buffer resulted in < 10% conversion to RF II.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	0%
NEBuffer 2	**100%**
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: A minimum of 0.25 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: No

Notes: Impaired by overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation.

SfiI requires two copies of its recognition sequence for cleavage to occur.

Incubation at 37°C results in 10% activity.

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

U.S. Patent No. 5,637,476

CERTIFICATE OF ANALYSIS

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