SbfI-HF™

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

R3642S 001121114111

R3642S

500 units **20,000 U/ml** **Lot: 0011211**
RECOMBINANT **Store at –20°C** **Exp: 11/14**

Recognition Site:

5′. . . C C T G C A▼G G . . . 3′
3′. . . G G▲A C G T C C . . . 5′

Note: SbfI-HF™ has the same specificity as SbfI (NEB# R0642), but it has been engineered for reduced star activity.

Source: An *E. coli* strain that carries the cloned and modified (K251A) SbfI gene from *Streptomyces* species Bf-61 (S.K. Degtyarev.)

Supplied in: 200 mM NaCl, 10 mM Tris-HCl (ph 7.4), 0.1 mM EDTA, 1 mM DTT, 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 DTT
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 Controls

Ligation: After a 10-fold overdigestion with SbfI- HF, > 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 with SbfI-HF.

16-Hour Incubation: A 50 µl reaction containing 1 µg of λDNA and 15 units of SbfI-HF incubated for 16 hours at 37°C resulted in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Exonuclease Activity: Incubation of a 50 µl reaction containing 100 units of SbfI-HF with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA (20^5 cpm/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of a 50 µl reaction containing 15 units of SbfI-HF with 1 µg of pBR322 DNA for 4 hours at 37°C resulted in < 10% conversion to RFII as determined by agarose gel electrophoresis.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	50%
NEBuffer 2	25%
NEBuffer 3	0%
NEBuffer 4	**100%**

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

Heat Inactivation: 65°C for 20 minutes.

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

New icons (see www.neb.com for details)
= Time-Saver™ Qualified
= indicates that the enzyme has been engineered
= indicates that the enzyme has reduced star activity

CERTIFICATE OF ANALYSIS

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