SspI-HF™

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

R3132S 002121114111

R3132S

1,000 units	**20,000 U/ml**	**Lot: 0021211**
RECOMBINANT	**Store at –20°C**	**Exp: 11/14**

Recognition Site:

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

Note: SspI-HF™ has the same specificity as SspI (NEB# R0132), but it has been engineered for reduced star activity.

Source: An *E. coli* strain that carries the cloned and modified (Y98F) SspI gene from *Sphaerotilus* species (ATCC 13925)

Supplied in: 200 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 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 SspI-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 SspI-HF.

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 100 units of SspI-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 500 units of SspI-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.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	25%
NEBuffer 2	100%
NEBuffer 3	0%
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 0.5 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 65°C for 20 minutes.

Plasmid Cleavage: Number of units required to cleave 1 µg of supercoiled plasmid DNA in one hour: pUC19 = 5 units, pBR322 = 4 units.

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

(see other side)

CERTIFICATE OF ANALYSIS

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New icons (see www.neb.com for details)

= Time-Saver™ Qualified
= indicates that the enzyme has been engineered
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