

SspI



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R0132S 034121114111



R0132S

1,000 units 5,000 U/ml Lot: 0341211

RECOMBINANT Store at -20°C Exp: 11/14

Recognition Site:

5'... AAT[▼]ATT... 3'
3'... TTA[▲]TAA... 5'

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

New Storage Conditions

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Supplied in: 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).

Reagents Supplied with Enzyme:

10X NEBuffer Ssp I

Reaction Conditions:

1X NEBuffer SspI
Incubate at 37°C.

1X NEBuffer SspI:

50 mM NaCl
100 mM Tris-HCl
10 mM MgCl₂
0.025% Triton X-100
pH 7.5 @ 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.

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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 SspI, > 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 30 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 500 units of enzyme with 1 µg sonicated ³H DNA (10⁵ cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

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Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	50%
NEBuffer 2	100%
NEBuffer 3	50%
NEBuffer 4	50%

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: 30 units of enzyme were inactivated by incubation at 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 = 2 units.

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

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

(see other side)

CERTIFICATE OF ANALYSIS

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Companion Products Sold Separately:

Sspl-HF™	
#R3132S	1,000 Units
#R3132L	5,000 Units
#R3132M	5,000 Units

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

U.S. Patent No. 5,516,678

