SacI

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

R0156S 050120714071

R0156S

RR BSA NEB1 37° Yes1

2,000 units **20,000 U/ml** **Lot: 0501207**

RECOMBINANT **Store at –20°C** **Exp: 7/14**

Recognition Site:

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

Source: An *E.coli* strain that carries the cloned SacI gene from *Streptomyces achromogenes* (ATCC 12767)

Also Available In High-Fidelity (HF™) Format

Supplied in: 100 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 1, 100X BSA.

Reaction Conditions: 1X NEBuffer 1, supplemented with 100 µg/ml BSA. Incubate at 37°C.

1X NEBuffer 1:
10 mM Bis Tris Propane-HCl
10 mM $MgCl_2$
1 mM dithiothreitol
pH 7.0 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of λ DNA (HindIII digest) in 1 hour at 37°C in a total reaction volume of 50 µl.

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

Quality Control Assays

Ligation: After 20-fold overdigestion with SacI , > 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 100 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 100 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 60 units of enzyme with 1 µg ϕX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 10% conversion to RF II.

Blue/White Screening Assay: This enzyme has been tested to determine the integrity of the DNA ends produced after digestion with an excess of enzyme. An appropriate vector is digested at a unique site within *lacZ*$^\alpha$ gene with a 10-fold excess of enzyme, ligated, transformed and plated on XGal/IPTG/Amp plates. Successful expression of β-galactosidase is a function of how intact its gene remains after cloning, an intact gene gives rise to a blue colony, an interrupted gene (i.e. degraded DNA end) gives rise to a white colony. Enzymes must produce fewer than 3% white colonies to be Blue/White Certified.

Heat Inactivation: 20 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	**100%**
NEBuffer 2	50%
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.

(See other side)

CERTIFICATE OF ANALYSIS

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(See other side)
CERTIFICATE OF ANALYSIS

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

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

Notes: SacI is inhibited by salt concentrations above 50 mM. Mini-prep DNA containing residual salt may be resistant to cleavage. A 70% alcohol wash or dialysis can be used to remove the salt.

SacI is sensitive to cytosine methylation at GAGmCTC but not GAGCTmC and insensitive to adenine methylation at GmAGCTC.

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

Companion Products:

SacI-HF™

#R3156S	2,000 Units
#R3156L	10,000 Units
#R3156M	10,000 Units

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

U.S. Patent No. 5,532,153

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