SacI-HF™

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

R3156S 001120714071

R3156S

2,000 units	**20,000 U/ml**	**Lot: 0011207**
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′

Note: SacI-HF™ has the same specificity as SacI (NEB #R0156), but it has been engineered for reduced star activity.

Source: An *E.coli* strain that carries the cloned and modified (Q117H/R200A) SacI gene from *Streptomyces achromogenes* (ATCC 12767)

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 4, 100X BSA.

Reaction Conditions: 1X NEBuffer 4, supplemented with 100 µg/ml BSA. 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 (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 Controls

Ligation: After 20-fold overdigestion with SacI-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.

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 100 units of SacI-HF incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of SacI-HF.

Exonuclease Activity: Incubation of 100 units of SacI-HF with 1 µg sonicated [^{3}H] DNA (10^5 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.05% radioactivity.

Endonuclease Activity: Incubation of 60 units of SacI-HF with 1 µg ϕX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 5% conversion to RF II.

Blue/White Screening Assay: An appropriate vector is digested at a unique site within the lacZ$^\alpha$ gene with a 10-fold excess of SacI-HF. The vector DNA is then ligated, transformed, and plated onto 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, removal of even a single base gives rise to a white colony. Enzyme preparations 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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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-HF 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.

Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping 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

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