

SacII



1-800-632-7799
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R0157S 059121114111

R0157S

2,000 units 20,000 U/ml Lot: 0591211

RECOMBINANT Store at -20°C Exp: 11/14

Recognition Site:

5'... CCGC[▼]GG... 3'
3'... GG[▲]CGCC... 5'

Source: A *Streptomyces lividans* strain that carries the cloned SacII gene from *Streptomyces achromogenes* (ATCC 12767)

Supplied in: 50 mM KCl, 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 Adenovirus-2 DNA 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 DTT, 200 µg/ml BSA and 50% glycerol
(pH 7.4 @ 25°C).

Quality Control Assays

Ligation: After 20-fold overdigestion with SacII, > 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 75 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 ³H DNA (10⁵ cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 50 units of enzyme with 1 µg pBR322 DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 20% conversion to RF II.

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

Enzyme Properties

Activity in NEBuffers

NEBuffer 1	25%
NEBuffer 2	75%
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.

Heat Inactivation: 50 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Notes: Certain SacII sites (e.g. one in the right arm of λ DNA) are resistant to cleavage. The reason particular SacII sites in λ DNA and φX174 DNA are cleaved at significantly lower rates than those found with other substrates is unclear at present.

Cleavage of mammalian genomic DNA is blocked by CpG methylation.

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

CERTIFICATE OF ANALYSIS

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