

SmaI



1-800-632-7799
info@neb.com
www.neb.com



R0141S 084120914091

R0141S



2,000 units **20,000 U/ml** **Lot: 0841209**
RECOMBINANT **Store at -20°C** **Exp: 9/14**

Recognition Site:

5' . . . CCCG[▼]GGG . . . 3'
3' . . . GGG[▲]CCC . . . 5'

Source: An *E. coli* strain that carries the cloned SmaI gene from *Serratia marcescens* (ATCC 49779)

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 25°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 25°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 10-fold overdigestion with SmaI, approximately 50% 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 40 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 80 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 φX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 15% 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^α* gene with a 5-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.

Enzymes Properties

Activity in NEBuffers:

NEBuffer 1	0%
NEBuffer 2	0%
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 0.13 unit is required to digest 1 µg of substrate DNA in 16 hours.

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

Notes: SmaI is an isoschizomer of XmaI.

(See other side)

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

SmaI produces blunt-ended fragments whereas XmaI produces a 5' extension. SmaI has a half-life of 15 minutes at 37°C. Incubation at 37°C results in 50% activity.


Cleavage of mammalian genomic DNA is blocked by CpG methylation.

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

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