
500 units $\quad 5,000 \mathrm{U} / \mathrm{ml} \quad$ Lot: 0471208

RECOMBINANT Store at $-20^{\circ} \mathrm{C}$ Exp: 8/14
Recognition Site:
$5^{\prime}$. . . T A C'G T A . . . 3'
$3^{\prime}$. . . A T GC A T . . . 5'
Source: An E. coli strain that carries the cloned SnaBI gene from Sphaerotilus natans (ATCC 15291)

Supplied in: $50 \mathrm{mM} \mathrm{KCl}, 10 \mathrm{mM}$ Tris- HCl ( pH 7.4 ) 0.1 mM EDTA, 1 mM DTT, $200 \mu \mathrm{~g} / \mathrm{ml}$ BSA and 50\% glycerol.

## Reagents Supplied with Enzyme:

10X NEBuffer 4, 100X BSA.
Reaction Conditions: 1X NEBuffer 4 supplemented with $100 \mu \mathrm{~g} / \mathrm{ml}$ BSA. Incubate at $37^{\circ} \mathrm{C}$.

## 1X NEBuffer 4:

50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
1 mM DTT
pH $7.9 @ 25^{\circ} \mathrm{C}$
Unit Definition: One unit is defined as the amount of enzyme required to digest $1 \mu \mathrm{~g}$ of T7 DNA in 1 hour at $37^{\circ} \mathrm{C}$ in a total reaction volume of $50 \mu \mathrm{l}$.

Diluent Compatibility: Diluent Buffer A $50 \mathrm{mM} \mathrm{KCl}, 10 \mathrm{mM}$ Tris-HCl, 0.1 mM EDTA, 1 mM DTT, $200 \mu \mathrm{~g} / \mathrm{ml}$ BSA and $50 \%$ glycerol (pH 7.4 @ $25^{\circ} \mathrm{C}$ )

Supplied in: $50 \mathrm{mM} \mathrm{KCl}, 10 \mathrm{mM}$ Tris-HCI ( pH 7.4 ), 0.1 mM EDTA, 1 mM DTT, $200 \mu \mathrm{~g} / \mathrm{ml}$ BSA and 50\% glycerol.

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## Quality Control Assays

Ligation: After 20-fold overdigestion with SnaBI, $>95 \%$ of the DNA fragments can be ligated with T4 DNA Ligase (at a 5 ' termini concentration of $1-2 \mu \mathrm{M})$ at $16^{\circ} \mathrm{C}$. Of these ligated fragments, $>95 \%$ can be recut.

16-Hour Incubation: A $50 \mu$ l reaction containing $1 \mu \mathrm{~g}$ of T 7 and 15 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. Digest containing > 15 units of enzyme produced star activity bands. For this reason, 16 hour incubations are not recommended (see note).

Exonuclease Activity: Incubation of 50 units of enzyme with $1 \mu \mathrm{~g} \phi \mathrm{X} 174$ RF I DNA ( $10^{5} \mathrm{cpm} /$ $\mu \mathrm{g}$ ) for 4 hours at $37^{\circ} \mathrm{C}$ in $50 \mu \mathrm{l}$ reaction buffer released $<0.1 \%$ radioactivity

Endonuclease Activity: Incubation of 4 units of enzyme with $1 \mu \mathrm{~g} \phi \mathrm{X} 174$ RF I DNA for 4 hours at $37^{\circ} \mathrm{C}$ in $50 \mu \mathrm{l}$ reaction buffer resulted in < 20\% conversion to RF II.

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## Enzyme Properties

Activity in NEBuffers:
NEBuffer 1 25\%
NEBuffer 2 50\%
NEBuffer 3 25\%
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 0.50 unit is required to digest $1 \mu \mathrm{~g}$ of substrate DNA in 16 hours.

Heat Inactivation: $80^{\circ} \mathrm{C}$ for 20 minutes.
Notes: Cleavage of mammalian genomic DNA is blocked by CpG methylation.
Incubations longer than 3 hours are not recommended.
Conditions of low ionic strength, high enzyme concentration, glycerol concentration >5\%, or $\mathrm{pH}>8.0$ may result in star activity.
$\boldsymbol{\square}=$ Time-Saver"w ${ }^{\text {rw }}$ Qualified (See www.neb.com for details) U.S. Patent No. 6,025,179

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## Enzyme Properties

Activity in NEBuffers:
NEBuffer $1 \quad 25 \%$
NEBuffer $250 \%$
NEBuffer 3 25\%
NEBuffer 4 100\%
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CERTIFICATE OF ANALYSIS

