



500 units 5,000 U/ml Lot: 0471208 RECOMBINANT Store at -20°C Exp: 8/14

Recognition Site:

5′... T A C^VG T A ... 3′ 3′... A T G C A T ... 5′

Source: An E. coli strain that carries the cloned SnaBl gene from Sphaerotilus natans (ATCC 15291)

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% alvcerol.

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 ug of T7 DNA in 1 hour at 37°C in a total reaction volume of 50 ul.

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 SnaBI. > 95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5' termini concentration of $1-2 \mu$ M) at 16°C. Of these ligated fragments, > 95% can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of T7 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 µg ϕ X174 RF I DNA (10⁵ cpm/ ug) for 4 hours at 37°C in 50 ul reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 4 units of enzyme with 1 ug oX174 RF I DNA for 4 hours at 37° C in 50 µl reaction buffer resulted in < 20% conversion to RF II.

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 µg of substrate DNA in 16 hours.

Heat Inactivation: 80°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 pH > 8.0 may result in star activity.

= Time-Saver[™] Qualified (See www.neb.com for details). U.S. Patent No. 6.025.179

CERTIFICATE OF ANALYSIS

Enzyme Properties

Activity in NEBuffers:

VEBuffer 1	25%
VEBuffer 2	50%
IFDuffer 0	050/

NEBuffer 3 25%

NEBuffer 4 100%

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U.S. Patent No. 6 025 179

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ALC: NO.



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