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BioLabs



 100 units
 Lot: 0151211
 Exp: 11/14

 2,000 U/ml
 Store at -20°C

#### **Recognition Site:**

 $\begin{array}{c} 5^{\prime}\ldots \P_{g}(\mathsf{N}) \land \mathsf{C} (\mathsf{N})_{5} \: \mathsf{C} \: \mathsf{T} \: \mathsf{C} \: \mathsf{C} (\mathsf{N})_{10}^{\bullet}\ldots 3^{\prime} \\ 3^{\prime}\ldots \P_{12}(\mathsf{N}) \: \mathsf{T} \: \mathsf{G} (\mathsf{N})_{5} \: \mathsf{G} \: \mathsf{A} \: \mathsf{G} \: \mathsf{G} (\mathsf{N})_{7} \ldots 5^{\prime} \end{array}$ 

**Source:** Bacillus stearothermophilus 25B (Z. Chen)

Supplied in: 500 mM NaCl, 10 mM Tris-HCl (pH 7.6), 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100 and 50% glycerol.



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Supplied in: 500 mM NaCl, 10 mM Tris-HCl (pH 7.6), 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100 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  $\mu$ g of  $\lambda$  DNA in 1 hour at 37°C in a total reaction volume of 50  $\mu$ l.

**Diluent Compatibility:** Diluent Buffer B 300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol. (pH 7.4 @ 25°C)

# <u>Quality Control Assays</u>

**16-Hour Incubation:** A 50  $\mu$ I reaction containing 1  $\mu$ g of DNA and 2 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 (see note).

**Exonuclease Activity:** Incubation of 20 units of enzyme with 1  $\mu$ g sonicated <sup>3</sup>H DNA (10<sup>5</sup> cpm/ $\mu$ g) for 4 hours at 37°C in 50  $\mu$ l reaction buffer released < 0.1% radioactivity.

# Enzyme Properties

# Activity in NEBuffers:

 NEBuffer 1
 75%

 NEBuffer 2
 100%

 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.

**Survival in a Reaction:** Intermediate activity. Suitable for extended digestion, but < 8 hours.

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### Heat Inactivation: No

**Plasmid Cleavage**: Number of units required to cleave 1  $\mu$ g of supercoiled plasmid DNA in one hour: 2 units.

**Notes:** Addition of greater than 2 units of BsaXI in a 16 hour incubation is not recommended due to DNA binding.

BsaXI cleaves DNA substrates twice to excise its recognition site generating a 27 base-pair fragments with 3-base 3'overhangs.

Not sensitive to *dam, dcm* or mammalian CpG methylation.

■ Time-Saver<sup>™</sup> Qualified (See www.neb.com for details).

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