



Lot: 0081206 1.000 units 5.000 U/ml RECOMBINANT Store at -20°C Exp: 6/14

**Recognition Site:** 

5<sup>°</sup>... G T C T C  $(N)_1^{\checkmark}$ ... 3<sup>°</sup> 3<sup>°</sup>... C A G A G  $(N)_5^{\sim}$ ... 5<sup>°</sup> **Source:** An *E. coli* strain that carries the cloned

BsmAl gene from *Bacillus stearothermophilus* A664 (Z. Chen)

### **New Reaction Buffer**



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5′... G T C T C (N), <sup>♥</sup>... 3′ 3'...5'

Source: An E. coli strain that carries the cloned BsmAl gene from *Bacillus stearothermophilus* A664 (Z. Chen)

Supplied in: 300 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 500 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme: 10X NEBuffer 4.

Reaction Conditions: 1X NEBuffer 4. Incubate at 55°C.

#### 1X NEBuffer 4:

50 mM potassium acetate 20 mM Tris-acetate 10 mM magnesium acetate 1 mM dithiothreitol pH 7.9 @ 25°CC

Unit Definition: One unit is defined as the amount of enzyme required to digest 1  $\mu$ g of  $\lambda$  DNA in 1 hour at 55°C in a total reaction volume of 50 µl.

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

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### **Quality Control Assays**

Ligation: After 10-fold overdigestion with BsmAl. > 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 ul reaction containing 1 µg of DNA and 10 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 200 units of enzyme with 1 µg sonicated <sup>3</sup>H DNA (10<sup>5</sup> cpm/µg) for 4 hours at 55°C in 50 µl reaction buffer released < 0.1% radioactivity.

## Enzyme Properties

**Activity in NEBuffers:** NEBuffer 1 50%

NFBuffer 2 100% NEBuffer 3 100% 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.

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

Heat Inactivation: 80°C for 20 minutes.

Notes: BsmAl is an isoschizomer of Alw26i.

Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation.

Incubation at 37°C results in 50% activity and at 65°C results in 10% activity.

Freshly diluted 10X NEBuffer is recommended for best results, as the enzyme is dependent of fresh DTT.

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

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CERTIFICATE OF ANALYSIS

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