# **BstEII**





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

# **R0162S**

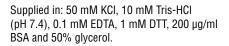


### **Recognition Site:**

5'... G T N A C C ... 3' 3'... C C A N T G G ... 5'

**Source:** An *E. coli* strain that carries the cloned BstEII gene from *Bacillus stearothermophilus* ET (N. Welker)

### **New Reaction Conditions**



Reagents Supplied with Enzyme: 10X NEBuffer 3, 100X BSA

Reaction Conditions: 1X NEBuffer 3, supplemented with 100 μg/ml BSA. Incubate at 60°C.

#### 1X NEBuffer 3: 100 mM NaCl

100 mM NaCl 50 mM Tris-HCl 10 mM MgCl<sub>2</sub> 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 60°C in a total reaction volume of 50  $\mu$ l.

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 BstEII, > 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 DNA and 50 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 50 units of enzyme with 1 μg sonicated <sup>3</sup>H DNA (10<sup>5</sup> cpm/μg) for 4 hours at 60°C in 50 μl reaction buffer released < 0.1% radioactivity.

**Endonuclease Activity:** Incubation of 25 units with 1  $\mu$ g  $\phi$ X174 RF I DNA for 4 hours at 60°C in 50  $\mu$ l reaction buffer resulted in < 10% conversion to RF II.

### **Enzyme Properties**

Activity in NEBuffers:

NEBuffer 1 50% NEBuffer 2 75% NEBuffer 3 **100%** NEBuffer 4 75%

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 1.00 unit is required to digest 1  $\mu$ g of substrate DNA in 16 hours.

Heat Inactivation: No

**Notes:** Of the over 3,000 known restriction endonucleases, very few produce extensions of more than 4 bases.

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

Incubation at 37°C results in 50% activity.

(see other side)

CERTIFICATE OF ANALYSIS

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Due to potential for star activity it is recommended that extended incubations be done at 37°C.

Conditions of low ionic strength, high enzyme concentration, glycerol concentration > 5%, or pH > 8.0 may result in star activity.

## Companion Products Sold Separately:

BstEII-HF™

#R3162S 2,000 units #R3162L 10,000 units #R3162M 10,000 units

BstEII-HF™ RE-Mix™

#R5162S 100 reactions

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

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