## **BssKI**







Exp: 2/14

BioLabs

1-800-632-7799

info@neb.com

www.neb.com

RX NEB3

Exp: 2/14

BSA 60° dcm 🗱

## **R0592S**

250 units

10,000 U/ml





Store at -20°C

#### **Recognition Site:**

5′... CCNGG...3′ 3′... GGNCC...5′

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

#### **Now Recombinant**

Lot: 0141202

Store at -20°C

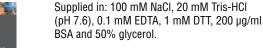
**BssKI** 

R0592S

250 units

10.000 U/ml

**Recognition Site:** 



## Reagents Supplied with Enzyme: 10X NEBuffer 3. 100X BSA.

Reaction Conditions: 1X NEBuffer 3, supplemented with 100  $\mu$ g/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  $\lambda$  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)

# Supplied in: 100 mM NaCl, 20 mM Tris-HCl (pH 7.6), 0.1 mM EDTA, 1 mM DTT, 200 $\mu$ g/ml BSA and 50% glycerol.

### Reagents Supplied with Enzyme:

10X NEBuffer 3, 100X BSA.

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

#### 1X NEBuffer 3:

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  $\lambda$  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  $\mu$ g/ml BSA, and 50% glycerol (pH 7.4 @ 25°C)

#### **Quality Control Assays**

**Ligation:** After 10-fold overdigestion with BssKI, > 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  $\mu$ l reaction containing 1  $\mu$ g of DNA and 36 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction produced in one hour with one unit of enzyme.

**Exonuclease Activity:** Incubation of 20 units for 4 hours at 60°C in 50 µl assay buffer with 1 µg sonicated [³H] DNA (10⁵ cpm/µg) released < 0.5% of the radioactivity.

#### **Enzyme Properties**

#### **Activity in NEBuffers:**

 NEBuffer 1
 0%

 NEBuffer 2
 50%

 NEBuffer 3
 100%

 NEBuffer 4
 50%

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

Heat Inactivation: 80°C for 20 minutes.

**Notes:** BssKI is an isoschizomer of ScrFI but leaves a 5-base 5'extension.

Blocked *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation.

Incubation at 37°C results in 10% activity.

#### **Companion Products:**

dam-/dcm- Competent E. coli

#C2925H 20 transformation reactions #C2925I 24 transformation reactions

CERTIFICATE OF ANALYSIS

#### **Quality Control Assays**

**Ligation:** After 10-fold overdigestion with BssKI, > 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 36 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction produced in one hour with one unit of enzyme.

**Exonuclease Activity:** Incubation of 20 units for 4 hours at  $60^{\circ}$ C in 50 µl assay buffer with 1 µg sonicated [ $^{3}$ H] DNA ( $10^{5}$  cpm/µg) released < 0.5% of the radioactivity.

### **Enzyme Properties**

#### **Activity in NEBuffers:**

NEBuffer 1 0% NEBuffer 2 50% NEBuffer 3 **100%** NEBuffer 4 50%

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

**Heat Inactivation:** 80°C for 20 minutes.

**Notes:** BssKI is an isoschizomer of ScrFI but leaves a 5-base 5'extension.

Blocked *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation.

Incubation at 37°C results in 10% activity.

#### **Companion Products:**

dam<sup>-</sup>/dcm<sup>-</sup> Competent *E. coli* 

#C2925H 20 transformation reactions #C2925I 24 transformation reactions

**Source:** An *E. coli* strain that carries the cloned BssKI gene from *Bacillus stearothermophilus* TBI

5′... CCNGG... 3′

3′...GGNCC<sub>1</sub>...5′

(Z. Chen)

Now Recombinant

CERTIFICATE OF ANALYSIS