

500 units 5,000 U/ml Lot: 0101211 RECOMBINANT Store at –20°C Exp: 11/14

Recognition Site:

5[']... Y A C^VGT R ... 3['] 3[']... R T G_AC A Y ... 5['] Single Letter Code: R = A or G, Y = C or T

Source: An *E. coli* strain that carries the cloned BsaAl gene from *Bacillus stearothermophilus* G668 (Z. Chen)

New Reaction Buffer

Supplied in: 250 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 0.15% Triton X-100, 200 µg/ml BSA 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 dithiothreitol pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 μ g of λ DNA in 1 hour at 37°C in a total reaction volume of 50 μ l.

Diluent Compatibility: Diluent Buffer C 250 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 0.15% Triton X-100, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Quality Control Assays

Ligation: After 2-fold overdigestion with BsaAl, > 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 μ I reaction containing 1 μ g of λ DNA and 25 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 μ g sonicated ³H DNA (10⁵ cpm/ μ g) for 4 hours at 37°C in 50 μ l reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 10 units of enzyme with 1 μ g pUC19 plasmid DNA for 4 hours at 37°C in 50 μ l reaction buffer resulted in < 20% conversion to a nicked form.

Enzyme Properties

Activity in NEBuffers: NEBuffer 1 100% NEBuffer 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.

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

Heat Inactivation: No.

Plasmid Cleavage: Number of units required to cleave 1 μ g of supercoiled plasmid DNA in one hour: 20 units.

Note: Cleavage of mammalian genomic DNA is blocked by CpG methylation.

■ Time-Saver[™] Qualified (See www.neb.com for details).

CERTIFICATE OF ANALYSIS

Enzyme Properties

Activity in NEBuffers:

VLDUIIEI I	100 /0
VEBuffer 2	100%
VEBuffer 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.

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

Heat Inactivation: No.

Plasmid Cleavage: Number of units required to cleave 1 μ g of supercoiled plasmid DNA in one hour: 20 units.

Note: Cleavage of mammalian genomic DNA is blocked by CpG methylation.

■ Time-Saver[™] Qualified (See www.neb.com for details).



SUU units 5,000 U/ml Lot: 010121 RECOMBINANT Store at -20°C Exp: 11/14

Recognition Site:

5′... Y A C^VGT R ... 3′ 3′... R T G CA Y ... 5′

Single Letter Code: R = A or G, Y = C or T

Source: An *E. coli* strain that carries the cloned BsaAl gene from *Bacillus stearothermophilus* G668 (Z. Chen)

New Reaction Buffer

Supplied in: 250 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 0.15% Triton X-100, 200 µg/ml BSA 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 dithiothreitol pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 μ g of λ DNA in 1 hour at 37°C in a total reaction volume of 50 μ l.

Diluent Compatibility: Diluent Buffer C 250 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 0.15% Triton X-100, 200 μ g/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Quality Control Assays

Ligation: After 2-fold overdigestion with BsaAI, > 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 μ I reaction containing 1 μ g of λ DNA and 25 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 μ g sonicated ³H DNA (10⁵ cpm/ μ g) for 4 hours at 37°C in 50 μ l reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 10 units of enzyme with 1 μ g pUC19 plasmid DNA for 4 hours at 37°C in 50 μ l reaction buffer resulted in < 20% conversion to a nicked form.