

BstEII



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



R0162S 084121214121

R0162S



2,000 units 10,000 U/ml Lot: 0841212
RECOMBINANT Store at -20°C Exp: 12/14

Recognition Site:

5'...GGTNACC...3'
3'...CCANTGG...5'

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

New Reaction Conditions

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New Reaction Conditions

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50% glycerol.

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
50 mM Tris-HCl
10 mM MgCl₂
1 mM DTT
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of λ DNA in 1 hour at 60°C in a total reaction volume of 50 µl.

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)

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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 µM) 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 ³H DNA (10⁵ 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 µg φX174 RF I DNA for 4 hours at 60°C in 50 µl reaction buffer resulted in < 10% conversion to RF II.

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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 µg 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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