BslI

NEW ENGLAND BioLabs Inc.
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www.neb.com

R0555S 011120814081

R0555S

NEB3 55° Yes dcm

1,000 units **10,000 U/ml** **Lot: 0111208**

RECOMBINANT **Store at –20°C** **Exp: 8/14**

Recognition Site:

5′. . . CCNNNNN▼NNGG . . . 3′
3′. . . GGNN▲NNNNNCC . . . 5′

Source: An *E. coli* strain that carries the cloned BslI gene from *Bacillus* species (D. Cowan, University College London)

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.

Reaction Conditions: 1X NEBuffer 3.
Incubate at 55°C.

1X NEBuffer 3:
100 mM NaCl
50 mM Tris-HCl
10 $MgCl_2$
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 55°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).

Quality Control Assays

Ligation: After 10-fold overdigestion with BslI, > 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 (10^5 cpm/µg) for 4 hours at 55°C in 50 µl reaction buffer released < 0.1% radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	10%
NEBuffer 2	50%
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 0.13 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 100 units of enzyme were inactivated by incubation at 80°C for 20 minutes.

Plasmid Cleavage: Number of units required to cleave 1 µg of supercoiled plasmid DNA in one hour: pBR322 = 2 units, pUC 19 = 1 unit.

Notes: Blocked by some combinations of overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation.

Incubation at 37°C results in 30% activity.

Companion Products:
dam⁻/dcm⁻ Competent *E. coli*

#C2925H	20 transformation reactions
#C2925I	24 transformation reactions

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

U.S. Patent No. 5,866,398

CERTIFICATE OF ANALYSIS

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Reagents Supplied with Enzyme: 10X NEBuffer 3.
Reaction Conditions: 1X NEBuffer 3. Incubate at 55°C.
1X NEBuffer 3: 100 mM NaCl, 50 mM Tris-HCl, 10 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 55°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).
Quality Control Assays
Ligation: After 10-fold overdigestion with BslI, > 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.
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Exonuclease Activity: Incubation of 50 units of enzyme with 1 µg sonicated 3H DNA (10^5 cpm/µg) for 4 hours at 55°C in 50 µl reaction buffer released < 0.1% radioactivity.
Enzyme Properties
Activity in NEBuffers:
NEBuffer 1 10%
NEBuffer 2 50%
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 0.13 unit is required to digest 1 µg of substrate DNA in 16 hours.
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CERTIFICATE OF ANALYSIS