BclI

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

R0160S 020121014101

R0160S

NEB3 50° dam No

3,000 units **15,000 U/ml** **Lot: 0201210**

RECOMBINANT **Store at –20°C** **Exp: 10/14**

Recognition Site:

5′. . . T▼GATCA . . . 3′
3′. . . ACTAG▲T . . . 5′

Source: An *E. coli* strain that carries the cloned BclI gene from *Bacillus caldolyticus* (A. Atkinson)

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 50°C.

1X NEBuffer 3:
100 mM NaCl
50 mM Tris-HCl
10 mM $MgCl_2$
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 (*dam*⁻) in 1 hour at 50°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 50-fold overdigestion with BclI, > 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 40 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 150 units of enzyme with 1 µg sonicated ^3H DNA (10^5 cpm/µg) for 4 hours at 50°C in 50 µl reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 40 units of enzyme with 1 µg ϕX174 RF I DNA for 4 hours at 50°C in 50 µl reaction buffer resulted in < 50% conversion to RF II.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	50%
NEBuffer 2	100%
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: Intermediate activity. Suitable for extended digestion, but < 8 hours.

Heat Inactivation: No

Notes: Blocked by *dam* methylation.

Incubation at 37°C results in 50% activity. Cleaves to leave a 5′ GATC extension which can be ficiently ligated to DNA fragments generated by BamHI, BglII, MboI, Sau3AI, and BstYI.

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

#C2925H	20 transformation reactions
#C2925I	24 transformation reactions

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

CERTIFICATE OF ANALYSIS

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