BceAI





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

R0623S



Exp: 11/14

25 units Lot: 0081211 1,000 U/ml Store at -20°C

Recognition Site:

5′... A C G G C (N)₁₂...3′ 3′... T G C C G (N)₁₄...5′

Source: An *E. coli* strain that carries the cloned BceAl gene from Bacillus cereus 1315 (C. Nkenfou)

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 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 37°C.

1X NEBuffer 3:

100 mM NaCl 50 mM Tris-HCI 10 mM MgCl_o 1 mM dithiothreitol pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of pBR322 DNA in 1 hour at 37°C in a total reaction volume of 50 μl.

Diluent Compatibility: Diluent Buffer A 50 mM KCI, 10 mM Tris-HCI, 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

Quality Control Assays

Ligation: After 5-fold overdigestion with BceAl, > 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 5 units of enzyme incubated for 16 hours resulted in no degradation of the DNA bands due to nonspecific nucleases. However, fragments produced by noncanonical cleavage due to star activity may be observed with 3 units of enzyme in similar conditions.

Exonuclease Activity: Incubation of 15 units of enzyme with 1 μg sonicated 3H DNA (105 cpm/μg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

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: Suitable for an extended or overnight digestion. Enzyme is active > 8 hours.

Heat Inactivation: 65°C for 20 minutes.

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

Conditions of low ionic strength, high enzyme concentration, glycerol concentration > 5% or pH > 8.0 may result in star activity.

To avoid star activity, do not use this enzyme in NEBuffer 1. NEBuffer 2 or NEBuffer 4.

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

CERTIFICATE OF ANALYSIS

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