BspCNI

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

R0624S 0021210 13101

R0624S

100 units **Lot: 0021210** **Exp: 10/13**
2,000 U/ml **Store at –20°C**

Recognition Site:

5′...C T C A G $(N)_{10}$▼...3′
3′...G A G T C $(N)_{8}$▲...5′

and

5′...C T C A G $(N)_{9}$▼...3′
3′...G A G T C $(N)_{7}$▲...5′

New Reaction Buffer

Note: The cleavage site of BspCNI varies. Two equally represented species of fragments are produced from BspCNI cleavage.

Source: *Bacillus* species (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 4, 100X BSA and 1600X S-adenosylmethionine (SAM) (32 mM)

Reaction Conditions: 1X NEBuffer 4, supplemented with 100 µg/ml BSA and SAM (20 µM). **Incubate at 25°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 µg of λ DNA in 1 hour at 25°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 dithiothreitol, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Quality Control Assays

Ligation: After 5-fold overdigestion with BspCNI, approximately 50% 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 8 units of enzyme incubated for 16 hours resulted in no degradation of the DNA bands due to nonspecific nucleases.

Exonuclease Activity: Incubation of 4 units of enzyme with 1 µg sonicated [^{3}H] DNA (10^5 cpm/µg) for 4 hours at 25°C in 50 µl reaction buffer released < 0.2% radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	100%
NEBuffer 2	75%
NEBuffer 3	10%
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: Not recommended for digest over 1 hour.

Heat Inactivation: 80°C for 20 minutes.

Note: Requires S-adenosylmethionine for optimal activity (supplied with enzyme).

Not sensitive to *dam, dcm* or mammalian CpG methylation.

Incubation at 37°C results in 75% activity.

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

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NEBuffer 1 100%
NEBuffer 2 75%
NEBuffer 3 10%
NEBuffer 4 100%
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