







R0640S



100 units 5,000 U/ml Lot: 0091211 RECOMBINANT Store at -20°C Exp: 11/13

Recognition Site:

5′... C^TATG... 3′ 3′... GTA₄C...5′

Source: An *E. coli* strain that carries the cloned CviAII gene from *Chlorella virus* PBCV-1

Supplied in: 250 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 0.15% Triton X-100, 200 μ g/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme: 10X NEBuffer 4. 100X BSA

Reaction Conditions: 1X NEBuffer 4, supplemented with 100 ug/ml BSA. Incubate at 25°C.

1X NEBuffer 4:

50 mM potassium acetate 20 mM Tris acetate 10 mM magnesium acetate 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 pUC19 DNA in 1 hour at 25°C in a total reaction volume of 50 µl.

Diluent Compatibility: Diluent Buffer C 250 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 0.15% Triton X-100, 200 μg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Quality Control Assays

Ligation: After 10-fold overdigestion with CviAII, > 95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5' termini concentration of 1–2 μ M) at 16°C. Of these ligated fragments, > 95% can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 15 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 20 units of enzyme with 1 μ g sonicated [³H] DNA (10⁵ cpm/ μ g) for 4 hours at 25°C in 50 μ l reaction buffer released < 0.1% radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1 75% NEBuffer 2 25% 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: A minimum of 1 unit is required to digest 1 μ g of substrate DNA in 16 hours.

Heat Inactivation: 60 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Note: CviAII is a neoschizomer of NIaIII.

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

Incubation at 37°C results in 20% activity.

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

CERTIFICATE OF ANALYSIS

CviAII



1-800-632-7799 in fo@neb.com www.neb.com

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