

HhaI



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R0139S 020121014101

R0139S



2,000 units **20,000 U/ml** **Lot: 0201210**
RECOMBINANT Store at **-20°C** Exp: **10/14**

Recognition Site:

5'... GCGC... 3'
3'... C[▲]GCG... 5'

Source: An *E. coli* strain that carries the cloned HhaI gene from *Haemophilus haemolyticus* (ATCC 10014)

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.

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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 4, 100X BSA.

Reaction Conditions: 1X NEBuffer 4, supplemented with 100 µg/ml BSA. Incubate at 37°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 λ DNA in 1 hour at 37°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)

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Quality Control Assays

Ligation: After 10-fold overdigestion with HhaI, > 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 100 units of enzyme with 1 µg sonicated ³H DNA (10⁵ cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.3% radioactivity.

Enzymes Properties

Activity in NEBuffers:

NEBuffer 1 75%
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.

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Survival in a Reaction: A minimum of 0.25 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 14 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Notes: HinP1 I is an isoschizomer of HhaI.

HhaI produces a 3' extension whereas HinP1 I produces a 5' extension. Cleaves single-stranded DNA at about 50% the rate of double-stranded DNA.

Cleavage of mammalian genomic DNA is blocked by CpG methylation.

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

CERTIFICATE OF ANALYSIS

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