











500 units 10,000 U/ml Lot: 0131212 RECOMBINANT Store at -20°C Exp: 12/14

### **Recognition Site:**

5'... A CGT...3' 3'... TGCA...5'

**Source:** An *E. coli* strain that carries the cloned HpyCH4IV gene from *Helicobacter pylori* CH4 (S.A. Thompson)

Supplied in: 100 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200  $\mu$ g/ml BSA and 50% glycerol.

**Reagents Supplied with Enzyme:** 10X NEBuffer 1

**Reaction Conditions:** 1X NEBuffer 1. Incubate at 37°C.

1X NEBuffer 1:

10 mM Bis Tris Propane-HCI 10 mM MgCl<sub>2</sub> 1 mM dithiothreitol pH 7.0 @ 25°C

**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1  $\mu$ g of pUC19 DNA in 1 hour at 37°C in a total reaction volume of 50  $\mu$ l.

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 10-fold overdigestion with HpyCH4IV, > 95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5´ termini concentration of 1–2  $\mu$ M) at 16°C. Of these ligated fragments, > 95%can be recut.

**16-Hour Incubation:** A 50  $\mu$ l reaction containing 1  $\mu$ g of DNA and 30 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 µg sonicated <sup>3</sup>H DNA (10<sup>5</sup> 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 25% NEBuffer 3 10% NEBuffer 4 25%

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 0.25 unit is required to digest 1  $\mu g$  of substrate DNA in 16 hours.

**Heat Inactivation:** 100 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

**Notes:** Maell is an isoschizomer of HpyCH4IV.

Cleavage of mammalian genomic DNA is blocked by CpG methylation.

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

U.S. Patent No. 6,194,188

CERTIFICATE OF ANALYSIS

# HpyCH4IV



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**R0619S** 



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