





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



R0124S



10.000 U/ml Lot: 0201208 2.000 units RECOMBINANT Store at -20°C Exp: 8/14

#### **Recognition Site:**

5′... G<sup>V</sup>C G C ... 3′ 3′... C G C<sub>4</sub>G ... 5′

**Source:** An *E. coli* strain that carries the cloned HinP1I gene from Haemophilus influenzae P. (S. Shen)

**New Reaction Buffer** 

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

Reaction Conditions: 1X NEBuffer 4. 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  $\mu$ g of  $\lambda$  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 dithiothreitol, 200 ug/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

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

#### **Quality Control Assays**

**Ligation:** After 10-fold overdigestion with HinP11. > 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 100 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 <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% NFBuffer 2 100% NEBuffer 3 100% NEBuffer 4 100%

**Quality Control Assays** 

> 95% can be recut.

1 unit of enzyme.

< 0.1% radioactivity.

**Enzyme Properties** 

**Activity in NEBuffers:** 

NEBuffer 1 100%

NFBuffer 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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for 4 hours at 37°C in 50 µl reaction buffer released

1-2 µM) at 16°C. Of these ligated fragments,

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

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

Notes: HinP1I is an isoschizomer of Hhal.

HinP1I produces a 5' extension, whereas Hhal produces a 3' extension. The 5' extension can be efficiently ligated into the Accl site of M13 and pUC cloning vectors.

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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# HinP1I



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