# **Hpy166II**





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

# **R0616S**



## **Recognition Site:**

5′... G T N N A C ... 3′ 3′... C A N N T G ... 5′

**Source:** An *E. coli* strain that carries the cloned Hpy166II gene from *Helicobacter pylori* J166 (M.J. Blaser)

More Units, New Storage Conditions

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

**Reagents Supplied with Enzyme:** 10X NFBuffer 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 dithiothreitol pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of pBR322 DNA in 1 hour at 37°C in total reaction volume of 50 µl.

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

### **Quality Control Assays**

Ligation: After 10-fold overdigestion with Hpy166II, > 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 200 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 500 units of enzyme with 1 μg sonicated [3H] 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 2 100% NEBuffer 3 50% 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 0.13 unit is required to digest 1  $\mu g$  of substrate DNA in 16 hours.

Heat Inactivation: 65°C for 20 minutes.

**Note:** Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation.

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

CERTIFICATE OF ANALYSIS

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