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## Certificate of Analysis

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## Hae III

Catalog No: 2180

**Lot No:** See Product Label

**Package Size:** See Product Label

**Concentration:** See Product Label

**Source:** Haemophilus aegyptius

**Storage Conditions:** Store at -20°C

### Recognition/Cut Site

5'-GG↓CC-3'

3'-CC↑GG-5'

### Unit Definition

One unit is the amount of enzyme required to completely digest 1 µg of lambda DNA in 1 hour in a total reaction volume of 50 µl.

### Reaction Temperature

37°C

### Heat Inactivation

10 minutes at 80°C

### Assay Conditions

50 mM Tris-HCl (pH 8.0)

10 mM MgCl<sub>2</sub>

50 mM NaCl

1 µg of lambda DNA

Incubation is at 37°C for 1 hour in a reaction volume of 50 µl.

### Storage Buffer

50 mM Tris-HCl (pH 7.5)

100 mM KCl

0.5 mM dithiothreitol

500 µg/ml bovine serum albumin

50% (v/v) glycerol

### Quality Control

**Non-specific Endonuclease:** Incubation of 50, 100, and 150 units of Hae III with 1 µg of φX-174 DNA at 37°C for 5 hours (a 750-fold over-digestion) resulted in the same sharp characteristic banding pattern as 4 U/µg in the standard 1 hour assay reaction, as determined by agarose gel electrophoresis.

**3'-Exonuclease:** Incubation of 5, 10, 15, 20, and 30 units of Hae III and 5 pmole of 3'-ends of lambda/Taq I fragments (3'-labelled with Klenow exo<sup>-</sup> and [<sup>3</sup>H]dCTP), incubated for 1 hour at 37°C resulted in ≤0.2 slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

**5'-Exonuclease/5'-Phosphatase:** Incubation of 5, 10, 15, 20, and 30 units of Hae III with 0.25 µg of 5'-ends of [5'-<sup>32</sup>P]lambda/Hae III fragments for 1 hour at 37°C resulted in ≤0.2 slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

**Ligation/Recut:** Incubation of 4 units of Hae III with 1 µg of Lambda DNA at 37°C produces fragments that ligate with ≥95% efficiency. The recut efficiency of the ligated fragments was 100%.