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## Certificate of Analysis

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## Hpa II

Catalog No: 2260

**Lot No:** See Product Label

**Package Size:** See Product Label

**Concentration:** See Product Label

**Source:** Haemophilus parainfluenzae

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

### Recognition/Cut Site

5'-C↓CGG-3'

3'-GGC↑C-5'

### Unit Definition

One unit is the amount of enzyme required to completely digest 1 µg of Lambda DNA in 1 hour at 37°C in a total reaction volume of 50 µl.

### Reaction Temperature

37°C

### Heat Inactivation

10 minutes at 65°C

### Assay Conditions

20 mM Tris-HCl (pH 7.4)

10 mM MgCl<sub>2</sub>

1 µg of Lambda DNA

Reaction volume 50 µl

### Storage Buffer

20 mM Tris-HCl (pH 7.4)

0.5 mM EDTA

1 mM DTT

500 µg/ml Bovine serum albumin

50% (v/v) Glycerol

### Quality Control

**Endonuclease:** Incubation of 20, 35, and 50 units of Hpa II with 1 µg of Lambda DNA at 37°C for 5 hours (a 250-fold over-digestion) resulted in the same sharp characteristic banding pattern as 4 U/µg in the standard 1 hours assay reaction, as determined by agarose gel electrophoresis. A second incubation using SV40 as the DNA substrate 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 Hpa II with 5 pmol of Lambda/Taq I fragments (3'-labeled with Klenow exo<sup>-</sup> and [<sup>3</sup>H]dGTP and [<sup>3</sup>H]dCTP), incubated for 1 hour at 37°C resulted in a ≤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 Hpa II with 0.25 µg of [5'-<sup>32</sup>P]Lambda/Hae III fragments for 1 hour at 37°C resulted in a ≤0.2% slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

**Ligation/Recut:** Incubation of 4 units of Hpa II with 1 µg of Lambda DNA at 37°C produces fragments that ligate with > 95% efficiency. The recut efficiency of these ligated fragments is 100%.