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Certificate of Analysis

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Hha I

Catalog No: 2190

Lot No: See Product Label

Package Size: See Product Label

Concentration: See Product Label

Source: *Arthrobacter luteus*

Storage Conditions: Store at -20°C

Recognition/Cut Site

5'-GCG↓C-3'

3'-C↑GCG-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.

Heat Inactivation

10 minutes at 65°C

Assay Conditions

50 mM Tris-HCl (pH 8.0)

10 mM MgCl₂

50 mM NaCl

1 µg of Lambda DNA

Reaction volume of 50 µl

Storage Buffer

20 mM Tris-HCl (pH 7.5)

100 mM KCl

0.5 mM DTT

500 µg/ml Bovine Serum Albumin 0.1%

(w/v) Triton X-100

50% Glycerol

Quality Control

Non-Specific Endonuclease: Incubation of 20, 35, and 50 units of Dpn I 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 hour assay reaction, as determined by agarose gel electrophoresis. Reaction volume of 50 µl. A second incubation using SV40 DNA 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 Hha I with 5 pmol of Lambda/Taq I fragments (3'-labeled with Klenow exo⁻ and [³H]dCTP), incubated for 1 hour at 37°C resulted in a ≤0.1 slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

5'-Exonuclease: Incubation of 5, 10, 15, 20, 30 units of Hha I with 0.25 µg of [5'-³²P]Lambda/Hae III fragments for 1 hour at 37°C resulted in a ≤0.1 slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

Functional Assay: Incubation of 4 units of Acc I with 1 µg of Lambda DNA at 37°C produces fragments that ligate with ≥95% efficiency. The recut efficiency of these ligated fragments is ≥95%.