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Certificate of Analysis

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Hind III

Catalog No: 2220

Lot No: See Product Label

Package Size: See Product Label

Concentration: See Product Label

Source: Haemophilus influenzae Rd

Storage Conditions: Store at -20°C

Recognition/Cut Site

5'-A↓AGCTT-3'

3'-TTCGA↑A-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

50 mM Tris-HCl (pH 8.0)
10 mM MgCl₂
50 mM NaCl
1 µg of Lambda DNA
Reaction volume 50 µl

Storage Buffer

10 mM Tris-HCl (pH 7.4)
200mM NaCl
0.5 mM EDTA
1 mM DTT
500 µg/ml BSA
5 µg/ml Poly-L-lysine
0.1% Triton X-100
50% (v/v) glycerol

Quality Control

Endonuclease: Incubation of 30, 60, and 90 units of Hind III with 1 µg of Lambda DNA at 37°C for 5 hours (a 450-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. A second incubation using pBR322 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.

Nicking: Incubation of 30, 60, and 90 units of Hind III with 1 µg of φX-174 DNA at 37°C for 5 hr resulted in ≤20% conversion of RF I to RF II and no conversion to RF III, as determined by agarose gel electrophoresis.

3'-Exonuclease: Incubation of 2, 5, 15, 45, and 60 units of Hind III with 5 pmoles 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/5'-Phosphatase: Incubation of 2, 5, 15, 45, and 60 units of Hind III 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.

Ligation/Recut: Incubation of 6 units of Hind III 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%.

For more information or to re-order, please visit www.chimerx.com