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Certificate of Analysis

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Hinc II

Catalog No: 2200

Lot No: See Product Label
Package Size: See Product Label
Concentration: See Product Label
Source: Haemophilus influenzae Rc
Storage Conditions: Store at -20°C

Recognition/Cut Site

5'-GTPy↓PuAC-3'
3'-CAPu↑PyTG-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

37ºC

Heat Inactivation

10 minutes at 65ºC

Assay Conditions

20mM Tris-HCl (pH 7.4)
50mM KCl
5 mM $MgCl_2$
1 µg of lambda DNA
Incubation is at 37°C for 1 hour in a reaction volume of 50 µl

Storage Buffer

10mM Tris-HCl (pH 7.4)
200mM NaCl
0.1mM EDTA
1 mM dithiothreitol
500 µg/ml bovine serum albumin
5 µg/ml poly-L-lysine
0.1% Triton X-100
50% (v/v) Glycerol

Quality Control

Non-specific Endonuclease: Incubation of 30, 60, and 90 units of Hinc II 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.

3'-Exonuclease: Incubation of 2, 5, 15, 45, and 60 units of Hinc II and 5 pmole of 3'-ends of lambda/Taq I fragments (3'-labelled with Klenow exo$^-$ and [^{3}H]dCTP), incubated for 1 hour at 37°C resulted in ≤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 Hinc II with 0.25 µg of 5'-ends of [5'-^{32}P]lambda/HaeIII fragments for 1 hour at 37°C resulted in ≤0.1 slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

Ligation/Recut: Incubation of 6 units of Hinc II with 1 µg of SV40 DNA at 37°C produces fragments that ligate with ≥ 65% efficiency. The recut efficiency of these ligated fragments is 100%.