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Certificate of Analysis

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Not I

Catalog No: 2295

Lot No: See Product Label

Package Size: See Product Label

Concentration: See Product Label

Source: *Nocardia otitidis-cavarum*

Storage Conditions: Store at -20°C

Recognition/Cut Site

5'GC↓GGCCGC-3'
3'-CGCCGG↑CG-5'

Unit Definition

One unit is the amount of enzyme required to completely digest 1 µg of Ad-2 DNA in 1 hour in a total reaction volume of 0.05 ml.

Reaction Temperature

37°C

Heat Inactivation

10 minutes at 65°C

Assay Conditions

50 mM Tris-HCl (pH 7.5 @ 37°C)
100 mM NaCl
10 mM MgCl₂
1 mM Dithiothreitol
100 µg/ml bovine serum albumin
1.0 µg of Ad-2 DNA
Incubation is at 37°C for 1 hour in a reaction volume of 50 µl

Storage Buffer

20 mM Tris-HCl (pH 7.5 @ 4°C)
100 mM KCl
0.1 mM EDTA
1 mM dithiothreitol
0.01% (v/v) Triton X-100
500 µg/ml bovine serum albumin
50% (v/v) Glycerol

Quality Control

Endonuclease: Incubation of 20, 35, and 50 units of Not I with 1 µg of Ad-2 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.

Nicking: Incubation of 20, 35, and 50 units of Not I with 1 µg of φX-174 DNA at 37°C for 5 hours (a 250-fold over-digestion) resulted in ≤10% conversion from RF I to RF II and ≤5% conversion to RF III, as determined by agarose gel electrophoresis. Reaction volume of 50 µl.

3'-Exonuclease: Incubation of 5, 10, 20, and 30 units of Not I with 5 pmol of lambda/Taq I fragments (3'-labeled using Klenow exo⁻ and [³H]dCTP), incubated for 1 hour at 37°C resulted in a ≤0.3 slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

5'-Exonuclease/5'-Phosphatase: Incubation of 5, 10, 20, and 30 units of Not I with 0.25 µg of [5'-³²P]lambda/Hae III fragments for 1 hour at 37°C resulted in a ≤0.3 slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

Ligation: Incubation of 2 units of Not I with 1 µg of Ad-2 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