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Certificate of Analysis

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Nsi I

Catalog No: 2298

Lot No: See Product Label
Package Size: See Product Label
Concentration: See Product Label
Source: Neisseria sicca
Storage Conditions: Store at -20°C

Recognition/Cut Site

5'-ATGCA↓T-3'
3'-T↑ACGTA-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 65ºC

Assay Conditions

50 mM Tris-HCl (pH 8.0)
10 mM $MgCl_2$
100 mM NaCl
1 μg of Lambda DNA
Reaction volume 50 µl

Storage Buffer

50 mM Tris-HCl (pH 7.5)
200 mM NaCl
0.1 mM EDTA
1.0 mMDTT
500 μg/ml Bovine Serum Albumin
50% Glycerol

Quality Control

Endonuclease: Incubation of 20, 35, and 50 units of Nsi 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.

Nicking: Incubation of 20, 35, and 50 units of Nsi I with 1 μg of ΦX-174 RF DNA at 37°C for 5 hr (a 250-fold over-digestion) resulted in ≤10% conversion of RF I to RF II and no conversion to RF III, as determined by agarose gel electrophoresis.

3'-Exonuclease: Incubation of 5, 10, 15, 20, and 30 units of Nsi I with 5 pmol of Lambda/Taq I fragments (3'-labeled with Klenow exo^- and [^{3}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: Incubation of 5,10,15,20, and 30 units of Nsi I with 0.25 µg of [5'-^{32}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.

Functional Assay: Incubation of 4 units of Nsi 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 100%.