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Certificate of Analysis

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Sph I

Catalog No: 2400

Lot No: See Product Label

Package Size: See Product Label

Concentration: See Product Label

Source: *Streptomyces phaeochromogenes*

Storage Conditions: Store at -20°C

Recognition/Cut Site

5'-GCATG↓C-3'
3'-C↑GTACG-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

37°C

Heat Inactivation

10 minutes at 65°C

Assay Conditions

50 mM Tris-HCl (pH 7.4)
6 mM MgCl₂
50 mM NaCl
50 mM KCl
1 µg of lambda DNA
Incubation is at 37°C for 1 hour in a reaction volume of 50 µl

Storage Buffer

50 mM Tris-HCl (pH 7.5)
1 mM EDTA
100 mM KCl
5 mM β-mercaptoethanol
0.1% Triton X-100
0.1 mM PMSF
50% (v/v) Glycerol
500 µg/ml Bovine Serum Albumin

Quality Control

Endonuclease: Incubation of 20, 35, and 50 units of Sph 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. A second incubation using pBR322 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 Sph I with 1 µg of φX-174 DNA at 37°C for 5 hours (a 250-fold over-digestion) resulted in <40% conversion of RF I to RF II and <5% conversion to RF III. Results determined by agarose gel electrophoresis. Reaction volume of 50 µl.

3'-Exonuclease: Incubation of 5, 10, 15, 20, and 30 units of Sph I and 5 pmoles of 3'-ends of lambda/Taq I fragments (3'-labelled with Klenow exo⁻ and [³H]dCTP), incubated for 1 hour at 37°C resulted in ≤0.2 slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

5'-Exonuclease/5'-Phosphatase: Incubation of 5, 10, 15, 20, and 30 units of Sph I with 0.25 µg 5'-ends of [5'-³²P]lambda/Hae III fragments for 1 hour at 37°C resulted in ≤0.2 slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

Ligation/Recut: Incubation of 4 units of Sph 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%

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