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Certificate of Analysis

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Spe I

Catalog No: 2398

Lot No: See Product Label

Package Size: See Product Label

Concentration: See Product Label

Source: Sphaerotilus species

Storage Conditions: Store at -20°C

Recognition/Cut Site

5'-A↓CTAGT-3'
3'-TGATC↑A-5'

Unit Definition

One unit is the amount of enzyme required to completely digest 1 µg of Adeno-2 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

20 mM Tris-HCl (pH 7.4)

5 mM MgCl₂

50 mM KCl

1 µg of Ad-2 DNA

Incubation is at 37°C for 1 hour in a reaction volume of 50 µl

Storage Buffer

50mM Tris-HCl (pH 7.5)

200 mM NaCl

0.1 mM EDTA

0.2% (v/v) Triton X-100

1 mM dithiothreitol

500 µg/ml Bovine Serum Albumin

50% (v/v) Glycerol

Quality Control

Endonuclease: Incubation of 10, 20, and 30 units of Spe I with 1 µg of Ad-2 DNA at 37°C for 5 hours (a 150-fold over-digestion) resulted in the same sharp characteristic banding pattern as the standard assay reaction, as determined by agarose gel electrophoresis. Reaction volume of 50 µl.

Nicking: Incubation of 4, 10, and 20 units of Spe I with 1 µg of φX-174 DNA at 37°C for 5 hours (a 100-fold over-digestion) resulted in ≤60% conversion of RF I to RF II and ≤10% conversion to RF III, as determined by agarose gel electrophoresis. Reaction volume 50 µl.

3'-Exonuclease: Incubation of 2, 5, 10, and 20 units of Spe 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 2, 5, 10, and 20 units of Spe I with 0.25 µg of 5'-ends of [5'-³²P]lambda/HaeIII fragments for 1 hour at 37°C resulted in a less than ≤0.2 slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

Ligation: Incubation of 4 units of Spe 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