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Certificate of Analysis

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Apa I

Catalog No: 2015

Lot No: 2709015
Package Size: See Product Label
Concentration: 10 units/µl
Source: *Acetobacter pasteurianus*
Storage Conditions: Store at -20°C

Recognition/Cut Site

5'-GGGCC↓C-3'
3'-C↑CCGGG-5'

Unit Definition

One unit is the amount of enzyme required to completely digest 1 µg of Ad-2 DNA in 1 hour at 30°C.

Heat Inactivation

10 minutes at 65°C

Reaction Temperature

30ºC

Reaction Buffer

20 mM Tris-HCl (pH 7.5 @ 37°C)
50 mM potassium acetate
10 mM magnesium acetate
1 mM dithiothreitol (DTT)
100 µg/ml bovine serum albumin*
**supplied separately*

Assay Conditions

20 mM Tris-HCl (pH 7.5 @ 37°C)
50 mM potassium acetate
10 mM magnesium acetate
1 mM dithiothreitol (DTT)
100 µg/ml bovine serum albumin
1 µg Ad-2 DNA
Incubation time is 1 hour at 30°C
Reaction volume is 50 µl

Storage Buffer

50 mM Tris-HCl (pH 7.5)
50 mM KCl
0.1 mM EDTA
10 mM β-mercaptoethanol
200 µg/ml bovine serum albumin
0.1% Triton X-100™
50% glycerol

Quality Control

Non-specific Endonuclease: 20, 35, and 50 units of Apa I were incubated with Ad-2 DNA for 5 hours at 30°C and compared to a standard 4 Unit, 1 hour assay. A second assay was performed under the same conditions using lambda as the DNA.

3'-Exonuclease: 5, 10, 15, 20 and 30 units of Apa I were incubated with [^{3}H] labeled 3'-ends of Lambda/Taq I DNA fragments for 1 hour at 30°C.

5'-Exonuclease/5'-Phosphatase: 5, 10, 15, 20 and 30 units of Apa I were incubated with [γ – ^{32}P] labeled 5' – ends of Lambda/Hae III DNA fragments for 1 hour at 30°C.

Nicking: 20, 35, and 50 units of Apa I were incubated with pBR322 DNA for 5 hours.

Ligation/Recut: 4 units of Apa I per µg of Ad-2 DNA were incubated for 1 hour at 30°C. Resulting fragments were ligated with T4 DNA Ligase and recut with Apa I.