ApaLI

1-800-632-7799
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www.neb.com

R0507S 040120814081

R0507S

2,500 units **10,000 U/ml** **Lot: 0401208**

RECOMBINANT **Store at –20°C** **Exp: 8/14**

Recognition Site:

5′. . . G▼T G C A C . . . 3′
3′. . . C A C G T▲G . . . 5′

Source: An *E. coli* strain that carries the cloned ApaLI gene from *Acetobacter pasteurianus* (ATCC 12875)

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 4, 100X BSA.

Reaction Conditions: 1X NEBuffer 4, supplemented with 100 µg/ml BSA. Incubate at 37°C.

1X NEBuffer 4:
50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
1 mM dithiothreitol
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of λ DNA (Hind III digest) in 1 hour at 37°C in a total reaction volume of 50 µl.

Diluent Compatibility: Diluent Buffer A
50 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

Quality Control Assays

Ligation: After 10-fold overdigestion with ApaLI, > 95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5′ termini concentration of 1–2 µM) at 16°C. Of these ligated fragments, > 95% can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 200 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme.

Exonuclease Activity: Incubation of 200 units of enzyme with 1 µg sonicated ^{3}H DNA (10^{5} cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 100 units of enzyme with 1 µg M13mp19 DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 10% conversion to RF II.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	100%
NEBuffer 2	100%
NEBuffer 3	10%
NEBuffer 4	**100%**

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

Survival in a Reaction: Suitable for an extended or overnight digestion. Enzyme is active > 8 hours.

Heat Inactivation: No

Notes: Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation.

ApaLI does not cut M13 DNA. Reports of a single site in M13 DNA have been attributed to a sequencing error. The corrected sequence is GTGCTC, which can be cleaved by either BsiHKAI or Bsp1286I.

= Time-Saver™ Qualified (See www.neb.com for details).

U.S. Patent No. 5,616,484

CERTIFICATE OF ANALYSIS

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