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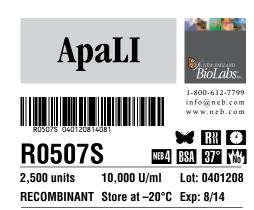
2,500 units 10,000 U/ml Lot: 0401208 RECOMBINANT Store at -20°C Exp: 8/14

Recognition Site:

5′... G^T G C A C ... 3′ 3′... C A C G T_AG ... 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 $\mu g/ml$ BSA and 50% glycerol.



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

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

Reaction Conditions: 1X NEBuffer 4, supplemented with 100 μ g/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 μ g of λ DNA (Hind III digest) in 1 hour at 37°C in a total reaction volume of 50 μ l.

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)

Reagents Supplied with Enzyme:

Reaction Conditions: 1X NEBuffer 4.

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50 mM KCI. 10 mM Tris-HCI. 0.1 mM EDTA.

Diluent Compatibility: Diluent Buffer A

1 mM dithiothreitol. 200 µg/ml BSA and

supplemented with 100 µg/ml BSA.

10X NEBuffer 4, 100X BSA.

50 mM potassium acetate

10 mM magnesium acetate

reaction volume of 50 µl.

50% glycerol (pH 7.4 @ 25°C)

Incubate at 37°C.

20 mM Tris-acetate

1 mM dithiothreitol

pH 7.9 @ 25°C

1X NEBuffer 4:

<u>Quality Control Assays</u>

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> 95% can be recut.

< 0.1% radioactivity.

conversion to RF II.

of enzyme.

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

16-Hour Incubation: A 50 ul reaction containing

1 µg of DNA and 200 units of enzyme incubated

for 16 hours resulted in the same pattern of DNA

Exonuclease Activity: Incubation of 200 units of

enzyme with 1 μ g sonicated ³H DNA (10⁵ cpm/ μ g)

Endonuclease Activity: Incubation of 100 units

of enzyme with 1 ug M13mp19 DNA for 4 hours

at 37°C in 50 μ l reaction buffer resulted in < 10%

for 4 hours at 37°C in 50 µl reaction buffer released

bands as a reaction incubated for 1 hour with 1 unit

 $1-2 \mu$ M) at 16°C. Of these ligated fragments,

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 $1-2 \ \mu$ M) at 16°C. Of these ligated fragments, > 95% can be recut.

16-Hour Incubation: A 50 μ I reaction containing 1 μ g 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 μ g sonicated ³H DNA (10⁵ cpm/ μ g) for 4 hours at 37°C in 50 μ l reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 100 units of enzyme with 1 μ g M13mp19 DNA for 4 hours at 37°C in 50 μ l 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.

Image: Saver[™] Qualified (See www.neb.com for details).

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

CERTIFICATE OF ANALYSIS

Enzyme Properties

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NEBuffer 3	10%
NEBuffer 4	100%

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Image: Content of the second seco

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