

Apal



1-800-632-7799
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R0114S 045120814081

R0114S



5,000 units 50,000 U/ml Lot: 0451208

RECOMBINANT Store at -20°C Exp: 8/14

Recognition Site:

5'...GGGCCC...3'
3'...CCCGGG...5'

Source: An *E. coli* strain that carries the cloned Apal gene from *Acetobacter pasteurianus* sub. *pasteurianus* (ATCC 9432)

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 500 µg/ml BSA and 50% glycerol.

More Units, Higher Concentration

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

Reaction Conditions: 1X NEBuffer 4, supplemented with 100 µg/ml BSA.
Incubate at 25°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 pXba DNA in 1 hour at 25°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 Apal, > 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.

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16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 100 units of Apal 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 100 units of Apal with 1 µg sonicated ³H DNA (10⁵ cpm/µg) for 4 hours at 25°C in 50 µl reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 100 units of Apal with 1 µg φX174 RF I DNA for 4 hours at 25°C in 50 µl reaction buffer resulted in < 20% conversion to RF II.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	25%
NEBuffer 2	50%
NEBuffer 3	0%
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.

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Heat Inactivation: 200 units of Apal were inactivated by incubation at 65°C for 20 minutes.

Plasmid Cleavage: Number of units required to cleave 1 µg of supercoiled plasmid DNA in one hour: 1 unit.

Notes: Apal is an isoschizomer of Bsp120I, but yields a 3' extension.

Blocked by overlapping *dcm* methylation.

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

Apal is inhibited by salt concentrations above 50 mM.

Incubation at 37°C results in 100% activity, however the half-life of this enzyme at 37°C is only 30 minutes

Companion Products:

dam⁻/dcm⁻ Competent *E. coli*
#C2925H 20 transformation reactions
#C2925I 24 transformation reactions

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

CERTIFICATE OF ANALYSIS

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