ApoI

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

R0566S 008120714071

R0566S

1,000 units **10,000 U/ml** **Lot: 0081207**

RECOMBINANT **Store at –20°C** **Exp: 7/14**

Recognition Site:

5′... R▼A A T T Y ...3′
3′... Y T T A A▲R ...5′

Single Letter Code: R = A or G, Y = C or T

Source: An *E. coli* strain that carries the cloned ApoI gene from *Arthrobacter protophormiae* (C. Polisson)

Supplied in: 100 mM NaCl, 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 3, 100X BSA.

Reaction Conditions: 1X NEBuffer 3, supplemented with 100 µg/ml BSA.
Incubate at 50°C

1X NEBuffer 3:
100 mM NaCl
50 mM Tris-HCl
10 $MgCl_2$
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 in 1 hour at 50°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 20-fold overdigestion with ApoI, > 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 ^3H DNA (10^5 cpm/µg) for 4 hours at 50°C in 50 µl reaction buffer released < 0.1% radioactivity.

Blue/White Screening Assay: This enzyme has been tested to determine the integrity of the DNA ends produced after digestion with an excess of enzyme. An appropriate vector is digested at a unique site within *lacZ*$^{\alpha}$ gene with a 10-fold excess of enzyme, ligated, transformed and plated on XGal/IPTG/Amp plates. Successful expression of β-galactosidase is a function of how intact its gene remains after cloning, an intact gene gives rise to a blue colony, an interrupted gene (i.e. degraded DNA end) gives rise to a white colony. Enzymes must produce fewer than 3% white colonies to be Blue/White Certified.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	10%
NEBuffer 2	75%
NEBuffer 3	**100%**
NEBuffer 4	75%

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: 80°C for 20 minutes.

Plasmid Cleavage: Number of units required to cleave 1 µg of supercoiled plasmid DNA in one hour: pUC19 = 1 unit.

Notes: Cleaves to leave 5′ AATT extension which can be ligated to DNA fragments generated by EcoRI digestion.

Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Incubation at 37°C results in 50% activity.

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

U.S. Patent No. 5,200,337

CERTIFICATE OF ANALYSIS

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