

ApeKI



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



R0643S 004120614061

R0643S



250 units 4,000 U/ml Lot: 0041206
RECOMBINANT Store at -20°C Exp: 6/14

Recognition Site:

5'... G[▼]C W G C ... 3'
3'... C G W C G ... 5'[▲]

Single Letter Code: W = A or T

Source: An *E. coli* strain that carries the cloned ApeKI gene from *Aeropyrum pernix* K1 (ATCC 700893)

Supplied in: 300 mM NaCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 3.

Reaction Conditions: 1X NEBuffer 3. Incubate at 75°C.

1X NEBuffer 3:
100 mM NaCl
50 mM Tris-HCl
10 mM MgCl₂
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 75°C in a total reaction volume of 50 µl.

Diluent Compatibility: Diluent Buffer B
300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Control Assays

Ligation: After 5-fold overdigestion with ApeKI, > 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, approximately > 95% can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of λ DNA and 9 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 20 units of enzyme with 1 µg sonicated ³H DNA (10⁵ cpm/µg) for 4 hours at 37°C* in 50 µl reaction buffer released < 0.1% radioactivity.

*This quality control was performed at 37°C to detect any *E. coli* contaminants which are not active at 75°C.

Enzyme Properties

Activity in NEBuffers:
NEBuffer 1 25%
NEBuffer 2 75%
NEBuffer 3 100%
NEBuffer 4 50%

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

Note: ApeKI is an isoschizomer of TseI.

ApeKI is a highly thermostable restriction enzyme that can survive temperatures as high as 95°C. At 95°C, the half-life of the enzyme is 20 minutes.

Incubation at 37°C results in 10% activity.

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

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