EcoP15I

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

R0646S 012120813081

R0646S

500 units **10,000 U/ml** **Lot: 0121208**

RECOMBINANT Store at –20°C Exp: 8/13

Recognition Site:

5´...C A G C A G $(N)_{25}$▼... 3´
3´...G T C G T C $(N)_{27}$▲... 5´

Source: An *E. coli* strain that carries the cloned EcoP15I *res-mod* genes from plasmid pSHI180 (D.N. Rao)

Supplied in: 100 mM $NaCl_2$, 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, 10X ATP

Reaction Conditions: 1X NEBuffer 3, supplemented with 100 µg/ml BSA and **1 mM ATP**. Incubate at 37°C.

1X NEBuffer 3:
100 mM NaCl
50 mM Tris-HCl
10 mM $MgCl_2$
1 mM dithiothreitol
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount enzyme required to digest 1 µg of pUC19 DNA 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

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 50 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 50 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 < 0.05% radioactivity.

Endonuclease Activity: Incubation of 25 units of enzyme with 1 µg ϕX174 RF I DNA for 4 ours at 37°C in 50 µl reaction buffer resulted in 5% conversion to RF II.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	75%
NEBuffer 2	100%
NEBuffer 3	**100%**
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: A minimum of 1 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 65°C for 20 minutes.

Notes: ATP is required for DNA cleavage. Efficient cleavage requires the presence of two inversely oriented recognition sites. A head to head orientation is preferred. The "head" of the sequence is defined as the dG at the 3´ end of the CAGCAG strand (top strand). Cleavage efficiently is also affected by the distance between the two sites.

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

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

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