EcoNI

NEW ENGLAND BioLabs Inc.
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www.neb.com

R0521S 027121014101

R0521S

NEB4 37° Yes

1,000 units **15,000 U/ml** **Lot: 0271210**
RECOMBINANT **Store at –20°C** **Exp: 10/14**

Recognition Site:

5′. . . C C T N N▼N N N A G G . . . 3′
3′. . . G G A N N N▲N N T C C . . . 5′

Source: An *E. coli* strain that carries the cloned EcoNI gene from *Escherichia coli* CDC A-193 (ATCC 12041)

Now Recombinant

Supplied in: 50 mM KCl, 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 4.

Reaction Conditions: 1X NEBuffer 4. 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 µg of λ 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

Ligation: After 2-fold overdigestion with EcoNI, > 75% 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% were recut. EcoNI leaves a single base 5′ extension, and these fragments are more difficult to ligate than blunt-ended fragments.

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 30 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 15 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 < 0.5% radioactivity.

Enzyme Properties

Activity in NEBuffers:

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

Heat Inactivation: 15 units of enzyme 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: pBR322 = 3 units.

Notes: EcoNI produces DNA fragments that have a single-base 5′ extension which are more difficult to ligate than blunt-ended fragments.

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

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

CERTIFICATE OF ANALYSIS

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Enzyme Properties
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NEBuffer 1 100%
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