# **EcoNI**





## R0521S



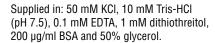
15,000 U/ml Lot: 0271210 1,000 units RECOMBINANT Store at -20°C Exp: 10/14

#### **Recognition Site:**

5'... C C T N N N N A G G ... 3' 3'... G G A 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**



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 ug of λ DNA in 1 hour at 37°C in a total reaction volume of 50 µl.

**Diluent Compatibility:** Diluent Buffer A 50 mM KCI, 10 mM Tris-HCI, 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

**EcoNI** 



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

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NEB 4 37° 1

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#### **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 3H DNA (105 cpm/ ug) for 4 hours at 37°C in 50 ul reaction buffer released < 0.5% radioactivity.

#### **Enzyme Properties Activity in NEBuffers:**

NFBuffer 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 ug 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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