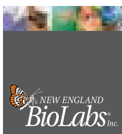
EcoRI



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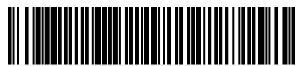


R0101S

10,000 units **20,000 U/ml** **Lot: 0331210**
RECOMBINANT **Store at –20°C** **Exp: 10/14**

Recognition Site:

5´. . . G▼A A T T C . . . 3´
3´. . . C T T A A▲G . . . 5´

Source: An *E. coli* strain that carries the cloned EcoRI gene from *E. coli* RY13 (R. N. Yoshimori)

Also Available In High Fidelity (HF™) Format

Supplied in: 300 mM NaCl, 10 mM KPO_4 (pH 7.5), 0.1 mM EDTA, 10 mM DTT, 0.15% Triton X-100, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer EcoRI.

Reaction Conditions: 1X NEBuffer EcoRI. Incubate at 37°C.

1X NEBuffer EcoRI:
50 mM NaCl
100 mM Tris-HCl
10 mM $MgCl_2$
0.025% Triton X-100
pH 7.5 @ 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 C
250 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 0.15% Triton X-100, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Heat Inactivation: 250 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Quality Control Assays

Ligation: After 100-fold overdigestion with EcoRI, > 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 100 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 [^{3}H] DNA (10^5 cpm/µg) for 4 hours at 37°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*^{α} 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	100%
NEBuffer 2	100%
NEBuffer 3	100%
NEBuffer 4	100%

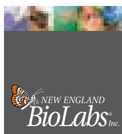
When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

Survival in a Reaction: A minimum of 0.13 unit is required to digest 1 µg of substrate DNA in 16 hours.

(see other side)

CERTIFICATE OF ANALYSIS

EcoRI



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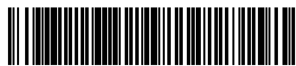

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(see other side)

CERTIFICATE OF ANALYSIS

Plasmid Cleavage: Number of units required to cleave 1 µg of supercoiled plasmid DNA in one hour: LITMUS = 4 units, pBR322 = 2.5 units, pUC19 = 2.5 units.

Notes: Cleavage of mammalian genomic DNA is impaired by overlapping CpG methylation.

Conditions of low ionic strength, high enzyme concentration, glycerol concentration > 5% or pH > 8.0 may result in star activity.

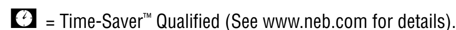
Companion Products Sold Separately:

EcoRI-HF™

#R3101S	10,000 units
#R3101L	50,000 units
#R3101T	10,000 units
#R3101M	50,000 units

EcoRI-HF™ RE-Mix™

#R5101S	500 reactions


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

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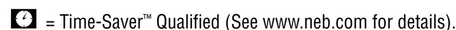
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Page 2 (R0101)