EcoRI-HF™

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

R3101S 0081301150111

R3101S

10,000 units **20,000 U/ml** **Lot: 0081301**
RECOMBINANT **Store at –20°C** **Exp: 1/15**

Recognition Site:

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

Note: EcoRI-HF™ has the same specificity as EcoRI (NEB #R0101), but it has been engineered for reduced star activity.

Source: An *E. coli* strain that carries the cloned and modified (K62E) EcoRI gene from *E. coli* RY13 (R.N. Yoshimori)

Supplied in: 300 mM NaCl, 10 mM KPO_4 (pH 7.5), 0.1 mM EDTA, 10 mM 2-mercaptoethanol, 0.15% Triton X-100, 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 DTT
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 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).

Quality Controls

Ligation: After 20-fold overdigestion with EcoRI-HF, > 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 100 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.

Endonuclease Activity: Incubation of 100 units of enzyme with 1 µg ϕX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 10% conversion to RF II.

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*$^\alpha$ 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	10%
NEBuffer 2	100%
NEBuffer 3	0%
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.

(see other side)

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

Heat Inactivation: 200 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Companion Products Sold Separately:

EcoRI

#R0101S	10,000 units
#R0101L	50,000 units
#R0101T	10,000 units
#R0101M	50,000 units

EcoRI-HF™ RE-Mix™

#R5101S	500 reactions

New icons (see www.neb.com for details)

= Time-Saver™ Qualified

= indicates that the enzyme has been engineered

= indicates that the enzyme has reduced star activity

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