EcoRV-HF™

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

R3195S 005121114111

R3195S

4,000 units	**20,000 U/ml**	**Lot: 0051211**
RECOMBINANT	**Store at –20°C**	**Exp: 11/14**

Recognition Site:

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

Note: EcoRV-HF™ has the same specificity as EcoRV (NEB #R0195), but it has been engineered for reduced star activity.

Source: An *E. coli* strain that carries the cloned and modified (D19A, E27A) EcoRV gene from the plasmid J62 pLG74 (L.I. Glatman)

Supplied in: 200 mM NaCl, 10 mM Tris-HCl (ph 7.4), 0.1 mM EDTA, 1 mM DTT, 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 B
300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 500 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

Quality Controls

Ligation: After 10-fold overdigestion with EcoRV- 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 with EcoRV-HF.

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 120 units of EcoRV-HF incubated for 16 hours at 37°C resulted in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Exonuclease Activity: Incubation of a 50 µl reaction containing 200 units of EcoRV-HF with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA (20^5 cpm/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of a 50 µl reaction containing 30 units of enzyme with 1 µg of φX174 RF I DNA for 4 hours at 37°C resulted in < 30% conversion to RFII as determined by agarose gel electrophoresis.

Blue/White Screening Assay: An appropriate vector is digested at a unique site within the lacZ$^\alpha$ gene with a 10-fold excess of enzyme. The vector DNA is then ligated, transformed, and plated onto 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, removal of even a single base gives rise to a white colony. Enzyme preparations must produce fewer than 3% white colonies to be Blue/White certified.

(see other side)

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	25%
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 0.50 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 65°C for 20 minutes.

Plasmid Cleavage: Number of units required to cleave 1 µg of supercoiled plasmid DNA in one hour: pBR322 = 2 units.

Notes: Cleaves to leave a ligatable blunt end in the tetracycline resistance gene of pBR322. EcoRV-HF can be used in conjunction with NEB's LITMUS™ Vectors to add sticky ends to blunt-ended fragments.

Cleavage of mammalian genomic DNA is impaired by some combinations of overlapping CpG methylation.

Companion Products Sold Separately:

EcoRV

#R0195S	4,000 units
#R0195L	20,000 units
#R0195T	4,000 units
#R0195M	20,000 units

EcoRV-HF™ RE-Mix™

#R5195S	200 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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