EagI-HF™

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

R3505S 005121114111

R3505S

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

Recognition Site:

5′... C▼GGCCG...3′
3′... GCCGG▲C...5′

Note: EagI-HF™ has the same specificity as EagI (NEB #R0505), but it has been engineered for reduced star activity.

Source: An *E. coli* strain that carries the cloned and modified (H43A) EagI gene from *Enterobacter agglomerans* (R. Morgan)

Supplied in: 500 mM KCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM dithiothreitol, 500 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 at 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of pXba 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 DTT, 500 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

Quality Controls

Ligation: After 20-fold overdigestion with EagI-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 EagI-HF incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of EagI-HF.

Exonuclease Activity: Incubation of 100 units of EagI-HF 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 20 units of EagI-HF with 1 µg ϕX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 20% conversion to RF II.

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.

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.

(see other side)

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

Survival in a Reaction: A minimum of 1.0 unit is required to digest 1 µg of substrate DNA in 16 hours.

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

Notes: Cleavage of mammalian genomic DNA is blocked by CpG methylation. For full EagI-HF activity, the pH of the reaction mix must be between 7.9 and 9.0 @ 25°C. Digestion at pH 7.4 yields 50% activity. When supplemented with BSA to 100 µg/ ml, 0.13 unit of EagI-HF will digest 1 µg of λ DNA in 16 hours. Under optimal reaction conditions, 10 units of EagI-HF are required to cleave one microgram of pBR322, pACYC184 or Adenovirus-2 DNA in one hour. To improve stability in storage, Triton X-100 has been added to the storage solution for EagI-HF.

The change in storage buffer for EagI-HF starting with Lot 0041007 (Lot 4, assay date 07/2010) has improved the activity of the enzyme in NEBuffers 2 and 3 from 50% and 10% to 100% and 100% respectively.

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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