EagI

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

R0505S 046121114111

R0505S

NEB3 37° RR Yes

500 units **10,000 U/ml** **Lot: 0461211**

RECOMBINANT **Store at –20°C** **Exp: 11/14**

Recognition Site:

5′... C▼GGCCG...3′
3′... GCCGG▲C...5′

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

New "Supplied in:" Conditions
Also Available In High Fidelity (HF™) Format

Supplied in: 500 mM NaCl, 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 3.

Reaction Conditions: 1X NEBuffer 3. Incubate at 37°C.

1X NEBuffer 3:
100 mM NaCl
50 mM Tris-HCl
10 mM $MgCl_2$
1 mM dithiothreitol
pH 7.9 @ 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 C
250 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 0.15% Triton X-100, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Quality Control Assays

Ligation: After 100-fold overdigestion with EagI, > 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 200 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 ^3H 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*$^\alpha$ 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	25%
NEBuffer 3	**100%**
NEBuffer 4	10%

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

(See other side)

CERTIFICATE OF ANALYSIS

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(See other side)
CERTIFICATE OF ANALYSIS

Notes: EagI is an isoschizomer of XmaIII.

Cleavage of mammalian genomic DNA is blocked by CpG methylation.

For full EagI 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 will digest 1 µg of λ DNA in 16 hours.

Under optimal reaction conditions, 10 units of EagI 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.

Companion Products Sold Separately:

EagI-HF™	
#R3505S	500 Units
#R3505L	2.500 Units
#R3505M	2.500 Units

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

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