EaeI







R0508S





200 units 3.000 U/ml Lot: 0351212 RECOMBINANT Store at -20°C Exp: 12/14

Recognition Site:

5'...Y G G C C R ... 3' 3′...RCCGG₄Y...5′

Single Letter Code: R = A or G. Y = C or T

Source: An E. coli strain that carries the cloned Eael gene from Enterobacter aerogenes (N. Brown)

New Reaction Buffer

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 NFBuffer 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 μ g of λ DNA in 1 hour at 37°C in a total reaction volume of 50 µl.

Diluent Compatibility: Diluent Buffer A 50 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

Quality Control Assays

Ligation: After 10-fold overdigestion with Eael. approximately 75% 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 ul reaction containing 1 µg of DNA and 15 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 30 units of enzyme with 1 ug sonicated 3H DNA (105 cpm/ μg) for 4 hours at 37°C in 50 μl reaction buffer released < 0.1% radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1 100% NEBuffer 2 100% NFBuffer 3 50% 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.

Heat Inactivation: 80°C for 20 minutes.

Note: Blocked by overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation.

Companion Products:

dam / dcm Competent E. coli

#C2925H 20 transformation reactions #C29251 24 transformation reactions

CERTIFICATE OF ANALYSIS

EaeI



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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 A 50 mM KCI. 10 mM Tris-HCI. 0.1 mM EDTA. 1 mM DTT, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

Quality Control Assays

Ligation: After 10-fold overdigestion with Eael, approximately 75% 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.

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