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BioLabs

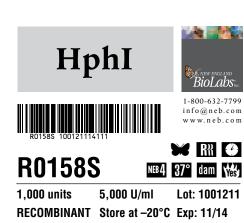
1,000 units 5,000 U/ml Lot: 1001211 RECOMBINANT Store at -20°C Exp: 11/14

Recognition Site:

5[']... GGTGA $(N)_8^{\checkmark}$... 3['] 3[']... CCACT $(N)_{7}_{\land}$... 5[']

Source: An *E. coli* strain that carries the cloned HphI gene from *Haemophilus parahaemolyticus* (ATCC 49700)

More Units



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More Units

Supplied in: 300 mM NaCl, 10 mM Tris-HCl (pH 7.5), 1.0 mM EDTA, 1 mM DTT, 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 @ 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 μ l.

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 Control Assays

Ligation: After 5-fold overdigestion with Hphl, approximately 50% of the DNA fragments can

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be ligated with T4 DNA Ligase (at a 5' termini concentration of 1–2 μ M) at 16°C. Of these ligated fragments, > 95% can be recut.

16-Hour Incubation: A 50 μ I reaction containing 1 μ g of DNA and 40 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 50 units of enzyme with 1 μ g sonicated ³H DNA (10⁵ cpm/ μ g) for 4 hours at 37°C in 50 μ l reaction buffer released < 0.2% radioactivity.

Enzyme Properties

Activity in NEBuffers:

 NEBuffer 1
 NR

 NEBuffer 2
 75%

 NEBuffer 3
 0%

 NEBuffer 4
 100%

 NEBuffer 1 is not recommended (NR) due to star activity.

 When using a buffer other than the optimal

(supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

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NEBuffer 1NRNEBuffer 275%NEBuffer 30%NEBuffer 4100%

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Survival in a Reaction: A minimum of 0.25 unit is required to digest 1 μg of substrate DNA in 16 hours.

Heat Inactivation: 40 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Notes: Blocked by overlapping dam methylation.

It has been suggested that HphI may cleave at N_g/N_g depending on the sequence between the recognition and cleavage sites Cho, S.-H. and Kang, C. (1990) *Mol. Cells* 1, 81–86.). Incubation of > 12 units for over 4 hours on ϕ X174 DNA results in additional cleavage products. This has not yet been shown to occur on other DNAs. Low pH and high glycerol concentration enhance this activity.

Companion Products:

dam-/dcm-Competent E. coli#C2925H20 transformation reactions#C2925I24 transformation reactions

■ Time-Saver[™] Qualified (See www.neb.com for details). U.S. Patent No. 5,731,185

CERTIFICATE OF ANALYSIS

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