HphI

1-800-632-7799
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R0158S 100121114111

R0158S

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

Recognition Site:

5′. . . G G T G A (N)$_8$▼. . . 3′
3′. . . C C A C T (N)$_7$▲. . . 5′

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

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 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 DTT, 500 µg/ml BSA and 50% glycerol. (pH 7.4 @ 25°C).

Quality Control Assays

Ligation: After 5-fold overdigestion with HphI, approximately 50% 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 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 µg sonicated ^{3}H DNA (10^5 cpm/µg) for 4 hours at 37°C in 50 µl 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.

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_9/N_8 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 ϕX174 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*

#C2925H	20 transformation reactions
#C2925I	24 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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