HpaI

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R0105S 047121014101

R0105S

500 units **5,000 U/ml** **Lot: 0471210**

RECOMBINANT **Store at –20°C** **Exp: 10/14**

Recognition Site:

5′. . . G T T▼A A C . . . 3′
3′. . . C A A▲T T G . . . 5′

Source: An *E. coli* strain that carries the cloned HpaI gene from *Haemophilus parainfluenzae* (ATCC 49669)

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 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 dithiothreitol
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 KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Quality Control Assays

Ligation: After 20-fold overdigestion with HpaI, > 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 50 units of enzyme incubated for 16 hours resulted in no degradation of the DNA bands due to nonspecific nucleases. However, fragments produced by noncanonical cleavage due to star activity may be observed with 10 units of enzyme in similar conditions.

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 < 0.1% radioactivity.

Endonuclease Activity: Incubation of 25 units of enzyme with 1 µg pUC19 DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 10% conversion from supercoiled to linear.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	25%
NEBuffer 2	25%
NEBuffer 3	10%
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 0.25 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: No

Notes: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation.

Conditions of high enzyme concentration, glycerol concentration > 5% or pH > 8.0 may result in star activity.

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

U.S. Patent No. 5,298,404 CERTIFICATE OF ANALYSIS

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