

Hpy99I



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R0615S 005121114111



R0615S



100 units Lot: **0051211** Exp: **11/14**
2,000 U/ml Store at **-20°C**

Recognition Site:

5'... CGWCG▼... 3'
3'...▲GCGWC ... 5'

Single Letter Code: W = A or T

Source: An *E. coli* strain that carries the cloned Hpy99I gene from *Helicobacter pylori* J99 (R.A. Alm)

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, 100X BSA.

Reaction Conditions: 1X NEBuffer 4, supplemented with 100 µg/ml BSA. 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
10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA,
1 mM dithiothreitol, 200 µg/ml BSA and
50% glycerol (pH 7.4 @ 25°C)

Quality Control Assays

Ligation: After 5-fold overdigestion with Hpy99I, > 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 20 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 µg sonicated ³H DNA (10⁵ cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released 0% radioactivity.

Enzyme Properties

Activity in NEBuffers:
NEBuffer 1 10%
NEBuffer 2 10%
NEBuffer 3 0%
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 1.0 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 65°C for 20 minutes.

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

Incubation times longer than 4 hours are not recommended.

U.S. Patent No. 6,280,992

CERTIFICATE OF ANALYSIS

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