HgaI

1-800-632-7799
info@neb.com
www.neb.com

R0154S 028120714071

R0154S

100 units **2,000 U/ml** **Lot: 0281207**

RECOMBINANT **Store at –20°C Exp: 7/14**

Recognition Site:

5′... G A C G C $(N)_5$▼... 3′
3′... C T G C G$(N)_{10}$▲... 5′

Source: An *E. coli* strain that carries the cloned HgaI gene from *Haemophilus gallinarum* (ATCC 14385)

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 1.

Reaction Conditions: 1X NEBuffer 1. Incubate at 37°C.

1X NEBuffer 1:
10 mM Bis Tris Propane-HCl
10 mM $MgCl_2$
1 mM dithiothreitol
pH 7.0 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of ϕX174 RF I 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 2-fold overdigestion with HgaI, > 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 5 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 15 units of enzyme with 1 µg sonicated ^3H 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	**100%**
NEBuffer 2	75%
NEBuffer 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 1.00 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 150 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Notes: Cleaves single-stranded DNA slowly.

Of the over 3,000 known restriction endonucleases, HgaI is one of the few that produces extensions of more than 4 bases.

Overdigestions with > 5 units of HgaI per µg of DNA and incubations > 1 hour are not recommended.

Some inhibition of HgaI activity was observed in reaction mixtures containing greater than 5% glycerol. It is also observed that HgaI loses activity in the absence of substrate and is essentially inactive after one hour at 37°C.

Cleavage of mammalian genomic DNA is blocked by CpG methylation.

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

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

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