

AhdI



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



R0584S 010120814081

R0584S



1,000 units 5,000 U/ml Lot: 0101208

RECOMBINANT Store at -20°C Exp: 8/14

Recognition Site:

5'...GACNNN ∇ NGTC...3'
3'...CTGNN \blacktriangle NNNCAG...5'

Source: An *E. coli* strain that carries the cloned AhdI gene from *Aeromonas hydrophila* (C. Polissou)

Supplied in: 10 mM Tris-HCl, 50 mM NaCl, (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50% glycerol.

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Supplied in: 10 mM Tris-HCl, 50 mM NaCl, (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 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 DTT
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg 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 DTT, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

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Quality Control Assays

Ligation: After 10-fold overdigestion with AhdI, < 5% of the DNA fragments can be ligated. Of these ligated fragments, > 95% can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 25 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 ³H DNA (10⁵ cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.05 % radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	25%
NEBuffer 2	75%
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.

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Survival in a Reaction: Suitable for an extended or overnight digestion. Enzyme is active > 8 hours.

Heat Inactivation: 100 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Plasmid Cleavage: Number of units required to cleave 1 µg of supercoiled plasmid DNA in one hour: 1 unit.

Note: AhdI is an isoschizomer of Eam1105I.

AhdI produces DNA fragments that have a single-base 3' extension which are more difficult to ligate than blunt-ended fragments. More efficient ligation can be achieved by using the Quick Ligation Kit (NEB #M2200).

Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping 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).

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS