

Nt.AlwI



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R0627S 001120714071

R0627S



500 units **10,000 U/ml** **Lot: 0011207**
RECOMBINANT **Store at -20°C** **Exp: 7/14**

Recognition Site:

5'... GGATC NNNNN'... 3'
3'... CCTAG NNNNN'... 5'

Description: Nt.AlwI is a nicking endonuclease that cleaves only one strand of DNA on a double-stranded DNA substrate. It is an engineered derivative of AlwI which catalyzes a single strand break 4 bases beyond the 3' end of the recognition sequence on the top strand.

Source: An *E. coli* strain containing a chimeric gene encoding the DNA recognition domain of AlwI and the cleavage/dimerization domain of N.BstNBI.

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 2.

Reaction Conditions: 1X NEBuffer 2.
Incubate at 37°C.

1X NEBuffer 2:

50 mM NaCl
10 mM Tris-HCl
10 mM MgCl₂
1 mM DTT
pH 7.9 @ 25°C

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

Unit Definition: One unit is defined as the amount of enzyme required to convert 1 µg of supercoiled pUC101 DNA (*dam-/dcm-*) to open circular form 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

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 50 units of enzyme incubated for 16 hours showed no degradation of DNA fragments.

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	100%
NEBuffer 3	25%
NEBuffer 4	50%

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 0.13 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 20 units of enzyme were inactivated by incubation at 80°C for 20 minutes.

Note: Nt.AlwI cleavage is blocked by *dam* methylation.

The nomenclature of this enzyme has been changed.

References:

1. Song, Q. et al. (2010). *Anal. Chem.* [Epub ahead of print].
2. Zhang, P. et al. (2010) *Protein Expr. Purif.* 69, 226–234. [Epub 2009 Sep 9].

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