AlwNI

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

R0514S 016121114111

R0514S

NEB4 37° dcm Yes

500 units **Lot: 0161211** **Exp: 11/14**
10,000 U/ml **Store at –20°C**

Recognition Site:

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

Source: An *E. coli* strain that carries the cloned AlwNI gene from *Acinetobacter lwoffii* N (R. Morgan)

Now Recombinant

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

Quality Control Assays

Ligation: After 20-fold overdigestion with AlwNI, > 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 10 units of AlwNI incubated for 16 hours at 37°C resulted in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis

Exonuclease Activity: Incubation of a 50 µl reaction containing 1,000 units of AlwNI with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA (10^5 cpm/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	10%
NEBuffer 2	100%
NEBuffer 3	100%
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 0.13 unit is required to digest 1 µg of substrate DNA in 16 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: pUC19 = 2 units, pBR322 = 1 unit.

Note: Blocked by overlapping *dcm* methylation.

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

The high enzyme concentration described in the 16-hour incubation assay may result in star activity

Companion Products:
dam$^-$/dcm$^-$ Competent *E. coli*

#C2925H	20 transformation reactions
#C2925I	24 transformation reactions

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

CERTIFICATE OF ANALYSIS

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