











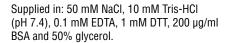
500 units Lot: 0161211 Exp: 11/14 10.000 U/ml Store at -20°C

Recognition Site:

5'... CAGNNN CTG...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 Iwoffii N (R. Morgan)

Now Recombinant



Reagents Supplied with Enzyme: 10X NFBuffer 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 μ g 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 ug/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 [3H] E. coli DNA (105 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 #C2925 24 transformation reactions

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

CERTIFICATE OF ANALYSIS

AlwNI



info@neb.com www.neb.com

1-800-632-7799

R0514S







NEB 4 37° dcm ₩

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5'... CAGNNN CTG...3' 3′... G T C,N N N G A C ... 5′

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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.

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