AlwI

NEW ENGLAND BioLabs Inc.
1-800-632-7799
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www.neb.com

R0513S 0181207140?1

R0513S

NEB4 37° dam Yes

500 units **5,000 U/ml** **Lot: 0181207**
RECOMBINANT **Store at –20°C** **Exp: 7/14**

Recognition Site:

5′. . . G G A T C $(N)_4$▼. . . 3′
3′. . . C C T A G $(N)_5$▲. . . 5′

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

More Units

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 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 dithiothreitol
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of λ DNA (*dam*⁻) in 1 hour at 37°C in 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 2-fold overdigestion with AlwI, approximately 50% 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 25 units of enzyme with 1 µg sonicated [^{3}H] DNA (10^5 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

Enzyme Properties

Activity in NEBuffers

NEBuffer 1	50%
NEBuffer 2	100%
NEBuffer 3	10%
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: Intermediate activity. Suitable for extended digestion, but < 8 hours.

Heat Inactivation: 25 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Notes: AlwI produces DNA fragments that have a single-base 5′ extension which are more difficult to ligate than blunt-ended fragments.

Blocked by *dam* methylation.

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

CERTIFICATE OF ANALYSIS

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